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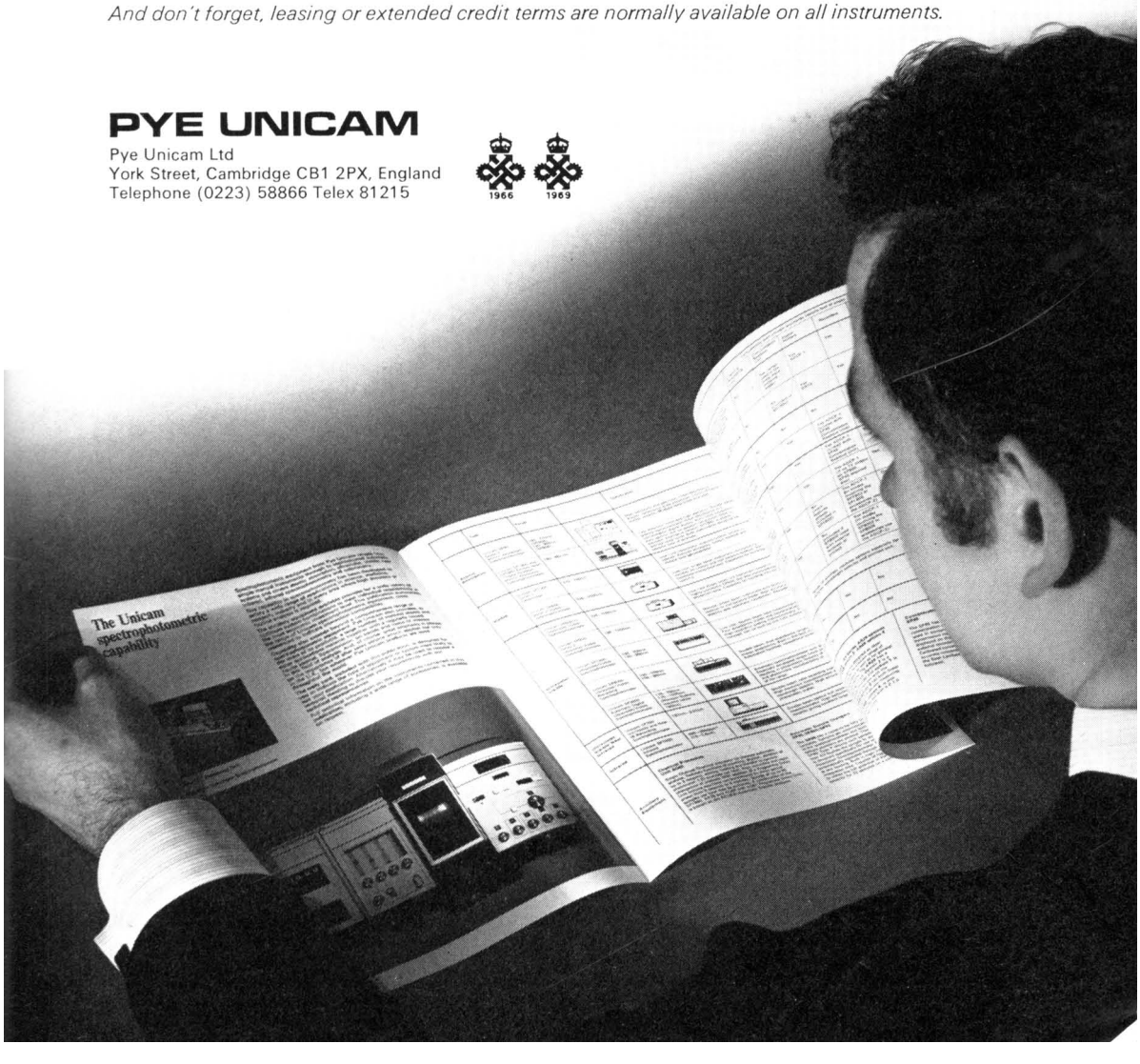

Summaries of Papers in this Issue

A Rapid Neutron-activation Method for the Simultaneous Determination of Arsenic and Antimony in Rocks

A simple, rapid, radiochemical neutron-activation method for the determination of arsenic and antimony in rocks is described. After fusion with alkali and separation of the insoluble hydroxides, arsenic and antimony are determined in the filtrate and in the hydroxide fraction, respectively, by a single precipitation of the sulphides after acidifying each fraction with hydrochloric acid. Chemical yields are determined by re-activation. The precision of the method is high for both elements, as demonstrated by the analysis of some standard rocks, and the sensitivity is adequate for most geochemical studies.

E. STEINNES

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Analyst, 1972, **97**, 241–244.

The Simultaneous Determination of Six Metals in Aluminium Alloys by Atomic-fluorescence Spectrophotometry

The rapid determination of copper, iron, magnesium, manganese, nickel and zinc in aluminium alloys by using a six-channel atomic-fluorescence spectrophotometer is reported. Simultaneous determination of all six metals at a single dilution is possible with this technique. The results compare favourably with the published values for the same alloys. The method was shown to permit sensitive and accurate analysis of these samples.

R. M. DAGNALL, G. F. KIRKBRIGHT, T. S. WEST and R. WOOD

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Analyst, 1972, **97**, 245–249.

Solvent Effects in the Determination of Cyclopentanone by Infrared Spectroscopy

The variation in frequency and intensity of each component of the Fermi resonance doublet in the carbonyl region of cyclopentanone is reported for twelve solvents. In mixed solvents the absorbance of the peak maximum is approximately the mean of that of the components when either the intensity or the amount of Fermi resonance is different for the components of the mixture. For solvents that form hydrogen bonds with cyclopentanone significant absorbance values cannot be obtained because of overlapping features caused by Fermi resonance and by different hydrogen-bonded species. The significance of the results for analytical purposes is summarised.

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Analyst, 1972, **97**, 250–253.

Residues of Prophylactics in Animal Products
Part II. The Determination of Amprolium Added to Eggs and Poultry Meat

The A.O.A.C. fluorimetric procedure for the determination of amprolium in feedstuffs has been applied to the determination of amprolium added to eggs and poultry meat at levels between 0·1 and 1·0 mg kg^{-1}. With suitable variations in the sample weight and volumes of reagents, the method was found to give reasonably satisfactory results.

I. C. COHEN, S. CRISP and J. NORCUP

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Analyst, 1972, **97**, 254–257.

The Determination of Nicotinic Acid by Fluorimetric Densitometry

A method is reported for the rapid determination of added nicotinic acid in meat. Nicotinic acid is converted on silica plates into a fluorescent compound by ultraviolet irradiation. After chromatographic separation from nicotinamide, the concentration is measured by means of a densitometer.

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Analyst, 1972, **97**, 258-259.

Determination of Quaternary Ammonium Salts in Lead Nitrate - Copper(II) Nitrate Solution

A method is described for determining the quaternary ammonium surfactants cetyltrimethylammonium bromide, tetradecyltrimethylammonium bromide and octadecyltrimethylammonium bromide by titration with sodium tetraphenylboron solution at pH 3, with methyl yellow as indicator. The error is within ± 1 per cent. for the determination of the above quaternary ammonium surfactants either alone or in the presence of lead nitrate or copper(II) nitrate, or both.

K. C. NARASIMHAM, Miss S. VASUNDARA and H. V. K. UDUPA

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Analyst, 1972, **97**, 260–262.

An Improved Test for Cocaine, Methaqualone and Methadone with a Modified Cobalt(II) Thiocyanate Reagent

The classical cobalt(II) thiocyanate reagent has been rendered more sensitive and selective so as to provide a convenient field test for cocaine, methaqualone and methadone, substances that fail to give clear positive responses in the widely used Marquis procedure.

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Analyst, 1972, **97**, 263–265.

An Automated Procedure for the Determination of Sulphur in Plant Tissue

An automated turbidimetric procedure for the determination of sulphur in plant tissue is described. A double-probe system is used so that the system is washed with a buffer solution on the wash cycle. This ensures a satisfactory return to the base-line. The values obtained are compared with those obtained by the standard gravimetric procedure.

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Analyst, 1972, **97**, 266-270.

THE ANALYST

A Rapid Neutron-activation Method for the Simultaneous Determination of Arsenic and Antimony in Rocks

BY E. STEINNES

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A simple, rapid, radiochemical neutron-activation method for the determination of arsenic and antimony in rocks is described. After fusion with alkali and separation of the insoluble hydroxides, arsenic and antimony are determined in the filtrate and in the hydroxide fraction, respectively, by a single precipitation of the sulphides after acidifying each fraction with hydrochloric acid. Chemical yields are determined by re-activation. The precision of the method is high for both elements, as demonstrated by the analysis of some standard rocks, and the sensitivity is adequate for most geochemical studies.

NEUTRON-ACTIVATION analysis appears at present to be the most useful technique for the determination of arsenic and antimony in silicate rocks.[1] Several investigators[2–6] have described radiochemical separation schemes for the simultaneous determination of a number of elements, including arsenic and antimony, by neutron activation. Methods based on two or more separation techniques, such as distillation, precipitation, solvent extraction or ion exchange, were used for the isolation of the two elements from complex mixtures as well as for their mutual separation.

After the advent of high-resolution solid-state detectors, the use of purely instrumental activation analysis has also been attempted for the determination of these elements. Gordon *et al.*[7] demonstrated that abundances of antimony higher than about 1 p.p.m. could be determined in standard silicate rocks by using the 1691-keV γ-ray of antimony-124. Brunfelt and Steinnes[8] showed that activation with epithermal neutrons facilitated the determination of antimony in the same standard rocks down to a concentration of about 0·1 p.p.m. via the 564-keV line of antimony-122 as well as the 602-keV line of antimony-124. Epithermal activation is advantageous also for arsenic,[9] but the high sodium-24 activity induced in most silicate rocks prohibits the determination of arsenic concentrations below a few parts per million. An additional difficulty is that the 559-keV line of arsenic-76 is difficult to resolve from the 564-keV line of antimony-122, even with the most sensitive detectors available.

The method described in this paper takes advantage of two important chemical properties of arsenic and antimony—after fusion with alkali, arsenic is found quantitatively in the water-soluble fraction of the fusion "cake", while most of the antimony is precipitated with the hydroxides; and both elements can be precipitated as sulphides from solutions in concentrated hydrochloric acid, whereas most other elements are not precipitated under the same conditions.

For these reasons, a very simple method can be used to obtain fractions of sufficient radiochemical purity for the determination of arsenic-76 and antimony-122. After fusion with alkali, the soluble and insoluble fractions are separated. Both fractions are acidified with hydrochloric acid, and arsenic and antimony are precipitated as sulphides in their respective fractions. The samples thus obtained are of sufficient radiochemical purity to be measured with a γ-ray spectrometer based on a sodium iodide detector.

EXPERIMENTAL

APPARATUS—

A 400-channel γ-spectrometer with a well-type, 3 × 3-inch, sodium iodide (thallium) crystal was used for the activity measurements.

REAGENTS—

The reagents used were of analytical-reagent grade.

Arsenic carrier solution—Prepare a stock solution by dissolving arsenic(III) oxide in M ammonia solution to give a solution containing 5 mg ml^{-1} of arsenic.

Arsenic standard solution—Dilute one volume of the arsenic carrier solution to one hundred volumes with M ammonia solution.

Antimony carrier solution—Prepare a stock solution by dissolving antimony(III) chloride in 3 M hydrochloric acid to give a solution containing 5 mg ml^{-1} of antimony.

Antimony standard solution—Prepare a stock solution by dissolving antimony metal in a few millilitres of aqua regia and diluting the mixture with a solution of 0·1 M citric acid and M hydrochloric acid to give a concentration of 50 μg ml^{-1} of antimony.

IRRADIATION—

Finely crushed rock samples, each of about 100 mg, were accurately weighed and wrapped in aluminium foil. Standards of arsenic and antimony were prepared by evaporating 100 μl of the standard solution on separate 3 × 3-cm sheets of aluminium foil. In the case of antimony, the aliquot should be neutralised with ammonia solution before evaporation. The arsenic and antimony contents of the aluminium foil were found to be negligible. Samples and standards were irradiated for 20 hours in the JEEP-II reactor (Kjeller, Norway) at a thermal neutron flux of about $1·5 \times 10^{13}$ neutrons cm^{-2} s^{-1}. The irradiated samples were stored for 4 days before starting the analyses so as to allow the short-lived activities to decay.

RADIOCHEMICAL PROCEDURE—

Transfer 1·00 ml of arsenic and antimony carrier solutions with a pipette into a nickel crucible containing enough sodium hydroxide solution to make the resulting mixture alkaline, and carefully evaporate the mixture to dryness under a heating lamp. Unwrap the aluminium foil and pour the rock sample quantitatively into the crucible, add 2 g of sodium hydroxide pellets and 0·5 g of sodium peroxide and fuse the mixture by heating it with an electrothermal heater. Release the fusion "cake" with 10 ml of water and transfer it to a 50-ml centrifuge tube. Centrifuge, decant the clear solution, wash the centrifuged solid with 10 ml of water, and add the washings to the previous clear solution in a beaker. Add water to make a volume of 75 ml, then add 75 ml of concentrated hydrochloric acid. Add about 100 mg of thioacetamide, and heat the mixture until precipitation of sulphide occurs. Filter the precipitate on to a membrane filter and wash it several times with 6 M hydrochloric acid. Dissolve the precipitate in a small volume of aqua regia, transfer the solution to a counting vial and dilute it with water to a volume of about 5 ml.

Dissolve the washed precipitate containing antimony with 10 ml of 3 M hydrochloric acid and centrifuge the solution in order to remove any undissolved matter present. Transfer the solution to a beaker, add about 100 mg of thioacetamide and heat. If precipitation of sulphide does not occur, add a few millilitres of water. Filter the precipitate on to a membrane filter and wash it several times with 2 M hydrochloric acid. Dissolve the precipitate in 5 ml of concentrated hydrochloric acid and transfer the solution to a counting vial. Dissolve the aluminium foils containing the standards in 6 M hydrochloric acid in separate 100-ml calibrated flasks, dilute to the mark with 6 M hydrochloric acid, and transfer 5·00 ml of the solution to counting vials.

ACTIVITY MEASUREMENTS—

The samples and standards were counted inside the well of the scintillation crystal, and the determinations were based on the photopeaks corresponding to the 559-keV γ-ray of arsenic-76 and the 564-keV γ-ray of antimony-122, respectively, the latter containing also a small contribution from the 602-keV γ-ray of antimony-124. Peak areas were calculated according to the method of Covell.[10] The arsenic fraction was counted at the end of the radiochemical separation, while the measurements on the antimony fraction were postponed until 7 days after the irradiation in order to eliminate possible interference from the 511-keV line of copper-64 carried down with the antimony precipitate. The radiochemical purity was checked by re-counting the fractions after 3 days.

DETERMINATION OF CHEMICAL YIELDS—

After the γ-activity measurements had been completed, the chemical yields were determined by re-activation.

Arsenic—The samples were diluted to 100 ml with water. Aliquots of 1·00 ml were sealed in polythene tubes and irradiated for 10 minutes at a thermal neutron flux of about $1{\cdot}5 \times 10^{13}$ neutrons cm^{-2} s^{-1}, together with aliquots of the arsenic carrier solution that were treated in the same way. After a delay of 1 day the activity of the 559-keV γ-ray of arsenic-76 was recorded without the tubes being opened.

Antimony—The samples were treated in the same way as described under *Arsenic*, except that 0·4 M citric acid solution was used for the dilution. The measurements were based on the 564-keV γ-ray of antimony-122.

RESULTS AND DISCUSSION

The present method was applied to a series of U.S. Geological Survey standard rocks, and the results are given in Table 1 together with previous neutron-activation data for the same rocks. The precision of the method evident from duplicate analyses appears to be satisfactory for both elements even at very low concentrations.

TABLE I

ABUNDANCES OF ARSENIC AND ANTIMONY IN SOME U.S. GEOLOGICAL SURVEY STANDARD ROCKS

	Arsenic, p.p.m.		Antimony, p.p.m.			
				Brunfelt and Steinnes		
Rock	Present work (single values in parentheses)	Literature values	Present work (single values in parentheses)	Epi-thermal neutron activation[8]	Radio-chemical separation method[11,12]	Other literature values
Andesite AGV-1	1·07 (1·09, 1·04)	1·0[5] 0·68[6]	4·07 (3·97, 4·16)	4·18	4·35	4·12[6] 4·6[15]
Basalt BCR-1 ..	0·67 (0·68, 0·66)	1·0[5] 0·58[6]	0·63 (0·62, 0·64)	0·52	0·58	0·93[6]
Dunite DTS-1 ..	0·040 (0·038, 0·041)	0·03[6]	0·47 (0·44, 0·49)	—	0·50	0·44[6]
Granite G-2 ..	0·26 (0·28, 0·24)	0·33[5] 0·2[6]	0·054 (0·048, 0·060)	<0·07	0·063	0·12[6] 0·040[15]
Granodiorite GSP-1 ..	0·093 (0·090, 0·095)	0·092[6]	3·20 (3·29, 3·11)	3·01	3·09	3·0[6] 3·35[15]
Periodotite PCC-1 ..	0·057 (0·060, 0·054)	0·05[6]	1·40 (1·36, 1·43)	—	1·39	1·56[6] 1·37[15]
Diabase W-1 ..	2·22 (2·23, 2·20)	1·7[4] 1·43[6] 2·38[13] 2·79[14]	0·94 (0·86, 1·02)	0·89	0·90	0·98[4] 1·2[6] 1·03[13] 1·20[14] 1·12[15]

The results in the literature for arsenic in diabase W-1 have quite a large scatter. The present value of 2·22 p.p.m. is fairly close to the mean value of the previous data. For the other six standard rocks, the present arsenic values are in agreement with the values obtained in previous work.[5,6]

Antimony has previously been determined in this laboratory by using non-destructive analysis with epithermal neutrons[8] as well as by using a radiochemical neutron-activation method based on extraction of antimony(V) chloride with diisopropyl ether as the principal separation step.[11] The present values are in good agreement with the results obtained in the previous investigation. The agreement with other results in the literature also appears to be reasonably good.

The chemical yields observed in this work were 91 $\pm$ 4 per cent. for arsenic and 50 to 72 per cent. for antimony. These results indicate that the determination of chemical yield for arsenic can be omitted in the routine application of the method. The yield for antimony

can be increased by carrying out the sulphide precipitation with hydrochloric acid of lower concentration, but this would probably result in greater contamination by copper-64 and possibly other radionuclides also.

The present method requires considerably less manual effort than previously published procedures for these elements, and should be attractive for the simultaneous study of both elements as well as for investigations on the individual elements. In spite of its simplicity, the method gives samples that are sufficiently pure to enable counting to be carried out with a γ-ray spectrometer equipped with a sodium iodide detector. No activities interfering with the arsenic determination were inferred from the decay measurements and, for antimony, only copper-64 is likely to interfere. This interference is easily eliminated by counting with a germanium (lithium) detector system, or by waiting for a further 3 days before counting the antimony fraction as indicated above.

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The Simultaneous Determination of Six Metals in Aluminium Alloys by Atomic-fluorescence Spectrophotometry

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The rapid determination of copper, iron, magnesium, manganese, nickel and zinc in aluminium alloys by using a six-channel atomic-fluorescence spectrophotometer is reported. Simultaneous determination of all six metals at a single dilution is possible with this technique. The results compare favourably with the published values for the same alloys. The method was shown to permit sensitive and accurate analysis of these samples.

The determination of trace amounts of elements in aluminium alloys by atomic-absorption spectrophotometry has been reported by many workers.[1–9] Some of the determinations, particularly that of magnesium, are known to suffer from pronounced chemical interferences, mainly from silicon and the host aluminium matrix. Methods of suppressing these interferences by the use of strontium,[10] strontium and sulphate ion,[11] calcium and sulphate ion,[2] nickel[12] and 8-hydroxyquinoline solution[3] have been recommended.

In this paper a method is reported for the rapid sequential determination of copper, iron, magnesium, manganese, nickel and zinc in aluminium alloys by using a commercially available six-channel atomic-fluorescence spectrophotometer; the results obtained are compared with those published by the British Chemical Standards[13] for the same alloys. The instrumental sensitivity and precision obtained in the determination of each metal in the presence of both strontium and aluminium by atomic-fluorescence spectrophotometry have also been examined.

Experimental

Apparatus—

A prototype multi-channel atomic-fluorescence spectrophotometer (Technicon Corporation, Model AFS-6) was used. In this instrument, the design of which is based on the system originally proposed by Mitchell and Johansson,[14] pulse-modulated hollow-cathode lamp sources are used to stimulate atomic-fluorescence radiation sequentially from atoms produced by nebulisation of the sample into a pre-mixed air - acetylene flame. The fluorescence radiation is collected from the flame with a wide-aperture mirror system and passed through appropriate interference filters to a single multiplier photo-tube detector. The characteristics of the filters used and the processing of the fluorescence signals by a multiplexed signal processing system and digital read-out have been described previously.[15]

Nebuliser and flame assembly—

The spectrophotometer includes an indirect nebuliser with pre-mix chamber similar to the Technicon Flame IV nebuliser, which is used with a circular carbon burner head that has thirty holes, each 0·036 inch in diameter. The optimum nebuliser uptake rate with the nitrogen-shielded air - acetylene flame was 3·8 ml min^{-1}.

Reagents—

A 1000 $\mu g\ ml^{-1}$ aluminium stock solution was prepared by dissolving 1 g of pure aluminium metal foil (Hopkin and Williams Ltd.) in 20 ml of a concentrated hydrochloric acid - nitric acid mixture (3 + 1 v/v); the solution was cooled and diluted to 1 litre with distilled water.

Solutions of copper, iron, magnesium, manganese, nickel and zinc (1000 μg ml^{-1}) and a 10 000 μg ml^{-1} solution of strontium, were prepared from analytical-reagent grade salts of the metals. These solutions were diluted to prepare solutions containing all six analyte metals for use as calibration standards; each of these solutions was also made to contain 100 μg ml^{-1} of aluminium and 500 μg ml^{-1} of strontium.

PREPARATION OF SAMPLES—

Each aluminium alloy (0·1 g) was dissolved in about 10 ml of concentrated hydrochloric acid in a platinum dish. The solution was evaporated to dryness and about 2 ml of 40 per cent. hydrofluoric acid solution were added, the solution being again evaporated to dryness. This procedure removed any silicon present in the alloy as the volatile fluoride. The residue was dissolved in 10 ml of a concentrated hydrochloric - nitric acid mixture (3 + 1 v/v) and the solution was then diluted to 100 ml with distilled water. Ten-millilitre aliquots of these concentrated solutions, together with 5 ml of the 10 000 μg ml^{-1} strontium solution, were diluted to 100 ml with distilled water. It was possible to determine all six analyte metals in the solution obtained without any need for further dilution, except in one instance (magnesium, 10·57 per cent. of which was present in the alloy).

PROCEDURE—

The optimum instrumental amplification for each channel (the "Set Level" cycle) is obtained when aspirating the standards containing strontium and aluminium and the six analyte metals at the upper concentration limit still on the linear portion of the calibration graph for each metal (see Table I). This solution of standards was also used to calibrate the instrument in preparation for the aluminium alloy analyses ("Calibrate" cycle). The blank value is obtained by aspirating a solution containing 100 μg ml^{-1} of aluminium and 500 μg ml^{-1} of strontium, and determining the emission from the solution ("Set Zero" cycle). In this way the blank reading for each channel is automatically subtracted from the measured atomic-fluoresence emission. The three cycles take approximately 3 minutes to complete. Subsequent analyses are carried out by aspirating the solution to be analysed and determining the fluorescence intensity ("Measure" cycle). The fluorescence intensity for each channel is automatically compared with that determined from the calibration standard solution, and the analytical value for each channel is displayed on a digital printer. The complete "Measure" cycle lasts for approximately 16 s.

TABLE I

DETECTION AND UPPER CONCENTRATION LIMIT OF THE ATOMIC-FLUORESCENCE SPECTROPHOTOMETRIC DETERMINATIONS

Metal	Wavelength/nm	Detection limit*/μg ml^{-1}	Upper concentration limit/μg ml^{-1}
Copper	324·8	0·03	10
Iron	248·3	0·1	10
Magnesium	285·2	0·008	5
Manganese	279·5	0·06	4
Nickel	232·0	0·07	4
Zinc	213·9	0·008	6

* Solutions also contained 100 μg ml^{-1} of aluminium and 500 μg ml^{-1} of strontium.

RESULTS

INSTRUMENTAL SENSITIVITY AND LINEAR RANGE—

The experimental parameters (height of observation of radiation, flow-rates of air, acetylene and shielding gas, and the nebuliser uptake rate) were optimised to produce the best over-all detection limits for the six metals. The fluorescence radiation was viewed between 5 and 8 mm above the primary reaction cones.

The detection limit for each metal, ascertained by selecting the point on the calibration graph at which the relative standard deviation of ten successive readings was 50 per cent., was determined by using the calibration standard solutions containing 100 μg ml^{-1} of aluminium and 500 μg ml^{-1} of strontium. The detection limits determined in this way at

TABLE II

EFFECT OF VARIOUS CONCENTRATIONS OF RELEASING AGENT ON THE MAGNESIUM ATOMIC-FLUORESCENCE SIGNAL

Solution		Signal	Relative standard deviation, per cent.
1 μg ml^{-1} of Mg		1·00	1·2
1 μg ml^{-1} of Mg + 100 μg ml^{-1} of Al		0·84	1·3
1 μg ml^{-1} of Mg + 100 μg ml^{-1} of Al	+ 100 μg ml^{-1} of Sr	0·97	1·4
	+ 250 μg ml^{-1} of Sr	1·00	2·3
	+ 500 μg ml^{-1} of Sr	1·01	3·0
	+ 750 μg ml^{-1} of Sr	1·00	3·5
	+ 1000 μg ml^{-1} of Sr	1·01	3·5
	+ 1500 μg ml^{-1} of Sr	1·01	4·8
	+ 3000 μg ml^{-1} of Sr	1·01	4·8

the optimum fluorescence line for each metal are shown in Table I; the upper concentration limit for each metal in the standard solutions for which a linear calibration graph was prepared is also shown in Table I.

PRECISION AND RELEASING AGENT CONCENTRATION—

Of the six analyte metals examined, the magnesium atomic-fluorescence spectrophotometric determination is the most seriously affected by chemical interferences from aluminium. This interference can be overcome by using strontium as a releasing agent.[10] The optimum concentration of strontium to be used was found by determining the atomic-fluorescence signal from a 1 μg ml^{-1} magnesium solution and the signals from 1 μg ml^{-1} magnesium solutions containing 100 μg ml^{-1} of aluminium together with various concentrations of strontium. The atomic-fluorescence signals determined are shown in Table II, together with the relative standard deviations of ten successive measurements for each solution.

At a strontium concentration of 500 μg ml^{-1} complete release of magnesium from the aluminium matrix occurs, and the thermal emission from the strontium is insufficient to produce enough shot noise on the photomultiplier tube to give fluorescence signals with a large coefficient of variation.

Table III shows the precision results obtained at different concentrations of the calibration standards for each metal investigated; the relative standard deviation was calculated from ten successive measurements for each solution.

TABLE III

PRECISION OF THE ATOMIC-FLUORESCENCE SIGNALS

Concentration/μg ml^{-1}	Relative standard deviation, per cent.					
	Cu	Fe	Mg	Mn	Ni	Zn
10·0	0·7	1·3	0·4	0·5	0·7	0·5
7·5	0·7	1·2	0·8	1·0	1·0	1·1
5·0	1·1	2·7	1·3	1·6	1·1	0·9
2·5	1·9	2·5	1·1	2·5	2·0	1·6
1·0	2·1	4·3	1·2	2·1	3·7	0·8
0·75	8·3	6·1	2·6	3·8	6·5	2·6
0·5	13·2	14·7	1·2	4·7	5·6	6·0
0·25	7·2	17·5	1·5	11·3	14·8	2·7
0·1	10·8	50·0	4·0	29·9	33·7	3·5

STANDARD ANALYSES—

Each metal in the solutions prepared from the alloys under optimum conditions was determined with the six-channel atomic-fluorescence spectrophotometer, and the results were compared with those published by the British Chemical Standards,[13] or reported by Alcan Research Limited (private communication). The results obtained are shown in Table IV.

TABLE IV

DETERMINATION OF METALS IN ALUMINIUM ALLOYS

		Element in alloy, per cent.					
Alloy	Technique	Cu	Fe	Mg	Mn	Ni	Zn
181/1	B.C.S.	3·99	0·36	1·42	0·10	2·04	0·02
	AFS-6	3·97	0·33	1·40	0·10	2·10	0·02
182/2	B.C.S.	0·045	0·47	0·075	0·21	0·05	0·10
	AFS-6	0·04	0·48	0·08	0·21	*	0·10
216/2	B.C.S.	4·56	0·28	0·75	0·71	0·17	0·20
	AFS-6	4·53	0·29	0·74	0·68	0·21	0·20
262	B.C.S.	0·03	0·19	10·57	0·06	—	0·05
	AFS-6	0·03	0·23	10·01	0·07	—	0·05
263/1	B.C.S.	0·09	0·35	4·92	0·36	—	0·05
	AFS-6	0·09	0·32	4·90	0·35	—	0·04
268	B.C.S.	1·34	0·39	0·56	0·22	0·03	0·05
	AFS-6	1·33	0·36	0·54	0·23	*	0·05
300	B.C.S.	1·28	0·30	2·76	0·41	—	5·98
	AFS-6	1·28	0·27	2·70	0·34	—	5·95
65C	AR	—	0·34	2·01	—	—	5·94
	AFS-6	—	0·29	1·98	—	—	5·97
66C	AR	—	0·33	1·94	0·11	—	6·04
	AFS-6	—	0·28	1·94	0·10	—	5·91
81C	AR	—	0·29	2·04	0·30	—	6·02
	AFS-6	—	0·30	1·98	0·31	—	5·81
113C	AR	—	0·29	2·2	0·27	—	6·0
	AFS-6	—	0·26	1·95	0·26	—	5·96
135C	AR	—	0·40	1·99	—	—	6·0
	AFS-6	—	0·39	1·91	—	—	5·98

B.C.S.: published analyses (British Chemical Standards[13]).
AR: Alcan Research Limited (private communication).
AFS-6: analyses carried out by atomic-fluoresence spectrophotometry.

* Concentration too low to be determined by atomic-fluorescence spectrophotometry.

CONCLUSION

The analytical results for the atomic-fluorescence determinations of each of the six analyte metals in the samples taken were shown to be the same, within experimental error, as the published values. The results for all six analyte metals were obtained virtually simultaneously for each alloy, which resulted in a considerable saving of operator time compared with any equivalent single-channel flame-spectrophotometric determination. The atomic-fluorescence spectrophotometer yields a result for each of the six analyte metals in a measuring cycle of 16 s. This time must be compared with the several minutes required to produce six results by a single-channel method (atomic-absorption or flame-emission spectrophotometry), which does not allow for the time required to change lamps and monochromator wavelength and slit settings. The fluorescence spectrophotometer was sufficiently sensitive to enable accurate and precise determinations to be made for each metal in all the alloys, with the exception of two nickel determinations. The maximum concentration of aluminium determined in each solution was 100 μg ml^{-1}; the precision and accuracy of the results indicated that, at this concentration, no analytical discrepancies arose from scattered radiation from particulate material in the flame.

We are grateful to Technicon Instrument Corporation for the loan of the atomic-fluorescence spectrophotometer used in this work, and for the grant of financial support to one of us (R. W.). We also thank Alcan Research Limited, Banbury, Oxfordshire, for the provision of some of the alloy samples.

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Solvent Effects in the Determination of Cyclopentanone by Infrared Spectroscopy

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The variation in frequency and intensity of each component of the Fermi resonance doublet in the carbonyl region of cyclopentanone is reported for twelve solvents. In mixed solvents the absorbance of the peak maximum is approximately the mean of that of the components when either the intensity or the amount of Fermi resonance is different for the components of the mixture. For solvents that form hydrogen bonds with cyclopentanone significant absorbance values cannot be obtained because of overlapping features caused by Fermi resonance and by different hydrogen-bonded species. The significance of the results for analytical purposes is summarised.

IT is well known that the carbonyl stretching vibration, which is usually near 1740 cm^{-1} in five-membered ring ketones, occurs as a doublet near 1730 to 1750 cm^{-1} in cyclopentanone. This splitting has been attributed[1–3] to Fermi resonance, the coincidental perturbation of two accidental degenerate vibrational levels that have the same symmetry. The higher wavenumber component, ν_1, is considered to be largely the carbonyl stretching mode and the lower wavenumber component, ν_2, largely an overtone (or combination) of low-frequency modes involving C–H bending. The measurable parameters in a Fermi resonance doublet are the wavenumber separation, Δ, and the ratio of the molar absorption coefficients ($R = \epsilon_2/\epsilon_1$). Both parameters may be solvent-dependent, and from this dependence the Fermi coupling coefficient[4] between ν_1 and ν_2 has been calculated. The analytical significance of these phenomena is that the frequency and intensity of a carbonyl band are parameters for identification and quantitative determination of the carbonyl compound under examination. Because there are occasions when analysis is required in solvents or solvent mixtures other than the solvent system for which reference data exists, it is important to assess the effect of solvent variation on these parameters.

EXPERIMENTAL

APPARATUS—

Absorbance and frequency measurements were made on solutions of cyclopentanone at a 0·073-mm path length by using a Grubb-Parsons Spectromaster infrared instrument for which the wavenumber scale was calibrated with water vapour[5] obtained by single-beam operation. The accuracy of the transmission scale was confirmed by using high-speed rotating sectors of standard transmission values.

REAGENTS—

Cyclopentanone—A sample supplied by Koch-Light Laboratories Ltd. was found to yield a single peak on a Phillips PV4000 chromatograph fitted with a 3-m column of polyethylene glycol 20M loaded to 5 per cent. on Chromosorb G.

Solvents—All solvents used were more than 98 per cent. pure as determined by gas chromatography.

Solutions—Cyclopentanone (25, 50, 75 or 100 μl) was added from a Hamilton microsyringe to the various solvents or solvent mixtures and each solution was made up to 5 ml in a calibrated flask.

Results and discussion

In this work, on the basis of a preliminary survey, twelve solvents that have a marked effect on the Fermi resonance doublet of cyclopentanone were chosen. The frequency, ν, and molar absorption coefficient, ϵ, of each component of the doublet were measured and are listed together with the values of $\Delta = \nu_1 - \nu_2$ and $R = \epsilon_2/\epsilon_1$ in Table I. If the value of Δ is large, normal infrared activity considerations apply and ϵ_1 is large while ϵ_2 is small; if Δ is small, then the intensity is shared by Fermi resonance. On a simple basis, solvents are expected to shift ν_1 by various amounts but will have no effect on ν_2. Bellamy and Williams[6] plotted ϵ_2/ϵ_1 for ethylene carbonate against ν_1 values in various solvents and showed that initially R increases slowly as ν_1 decreases but, as ν_1 approaches ν_2, R then increases rapidly because of the onset of Fermi resonance. In this work, Table I reveals that no such correlation exists between R and ν_1. The assignment of ν_1 to the mode largely associated with the carbonyl stretching vibration is based on the proximity of the frequency of ν_1 to that in deuterated cyclopentanone, in which only a single band is observed in this region.[3] However, the observations that ν_1 and ν_2 follow similar solvent shifts and that R is close to unity in most solvents suggest that ν_1 and ν_2 contain similar contributions from the components of the resonance interaction.

Table I

Wavenumber values and absorption coefficients of Fermi resonance doublets of the carbonyl band of cyclopentanone in various solvents

Solvent	ν_1/ cm^{-1}	ν_2/ cm^{-1}	Δ/ cm^{-1}	ϵ_1/ m^2 mol^{-1}	ϵ_2/ m^2 mol^{-1}	R
Acetonitrile	1743	1731	12	55·8	55·0	0·99
Bromoform	1737	1724	13	50·4	75·2	1·49
Chloroform	1741	1728	13	49·9	68·6	1·37
Diethyl ether	1759	1737	22	28·6	81·3	2·84
Dioxan	1741	1726	15	105·7	36·4	0·34
Ethanol	1745	1729	16	51·2*	73·2*	1·43*
Methyl iodide	1745	1721	24	74·8	39·9	0·53
Methylene bromide	1741	1727	14	55·2	60·2	1·09
Methylene iodide	1739	1724	15	40·2	58·7	1·66
Nitromethane	1743	1730	13	47·6	60·6	1·27
Pyridine	1742	1730	12	73·0	50·5	0·69
1,1,2,2-Tetrachloroethane	1742	1727	15	47·7	78·5	1·65

* Subject to errors because of hydrogen bonding.

All wavenumber values are considered to be accurate to ± 2 cm^{-1}; all absorption coefficient values are considered to be accurate to ± 2 m^2 mol^{-1} and precise to $\pm 0{\cdot}2$ m^2 mol^{-1}.

In order to investigate the influence of solvent and solvent mixtures on the Fermi resonance doublet, three pairs of solvents were chosen and graphs for the Beer - Lambert law were constructed for each solvent and for a 1 + 1 mixture of the two solvents. The absorbance measured was that of the peak of the strongest band in the carbonyl region. These measurements were designed to determine—

(*i*) whether the Fermi resonance interaction depends on the concentration in each of a series of solvents;

(*ii*) the values of the molar absorption coefficient in a 1 + 1 mixture of solvents for which the molar absorption coefficients are significantly different in each component of the pair;

(*iii*) whether with a solvent pair in which the Fermi resonance interaction is strong in one solvent and weak in the other the amount of interaction and hence the molar absorption coefficient occupy a mean value between the two; and

(*iv*) whether with a solvent pair in which hydrogen-bonding interaction is strong in one solvent and weak in the other the amount of bonding and hence the molar absorption coefficient occupy a mean value between the two.

1,1,2,2-TETRACHLOROETHANE - METHYLENE BROMIDE—

The basis of this choice of solvents is that they are miscible; the wavenumber values of the peak maxima (ν_2 in each instance) are nearly coincident but the molar absorption coefficients of cyclopentanone in the two solvents are significantly different.

The Beer - Lambert law graph is shown in Fig. 1. It was found that this law is obeyed in all three instances and that the molar absorption coefficient, as measured by the slope of the line, is the mean of the value in the individual solvents.

METHYLENE IODIDE - PYRIDINE—

This combination of solvents was chosen because the value of R was high for methylene iodide and low for pyridine. The graph shown in Fig. 2 (ν_1) indicates that both solvent systems follow the Beer - Lambert law and that the 1 + 1 mixture of solvents also leads to a value of the slope that is the mean of the values obtained in the separate solvents. It would thus appear that the amount of Fermi resonance interaction can be adjusted by varying the composition of a suitable binary mixture of solvents.

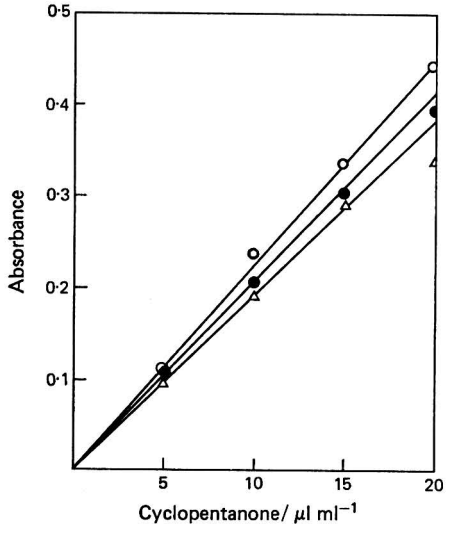


Fig. 1. Absorbance of carbonyl band of cyclopentanone in symmetrical tetrachloroethane (△), methylene bromide (O) and a 1 + 1 mixture (●)

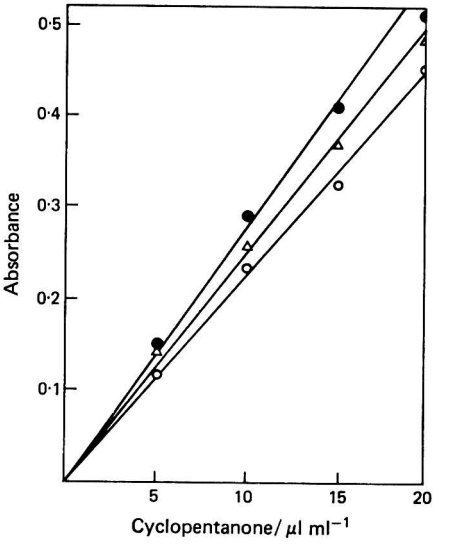
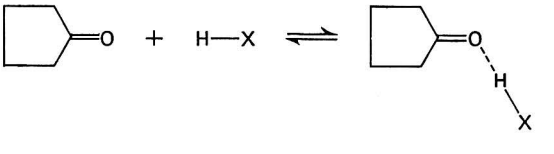


Fig. 2. Absorbance of carbonyl band of cyclopentanone in methylene iodide (O), pyridine (●) and a 1 + 1 mixture (△)

CHLOROFORM - ETHANOL—

This combination of solvents was selected because it is known that chloroform bonds weakly and that ethanol bonds strongly to carbonyl compounds. These solvents are, therefore, likely to influence the frequency and intensity of a carbonyl band by different amounts, particularly when Fermi resonance occurs. When the peak absorbance of the strongest band in the carbonyl region is plotted against concentration there is an apparent deviation from the Beer - Lambert law, which is particularly marked in ethanol, less marked in chloroform and present to an intermediate extent in the 1 + 1 mixture (Fig. 3).

The deviation from this law is apparent only in the sense that the law is being applied to a compound that takes part in the following reaction—



The concentration term used in the Beer - Lambert law should correspond to either the unbonded or the bonded form in the above equation. In the absence of Fermi resonance (for example, in acetone or cyclooctanone), separate bands are observed for each of the forms. In this work, overlapping Fermi resonance doublets prevent the observation of the different bonded forms and it is therefore not feasible to apply the Beer - Lambert law rigorously to each species in solution.

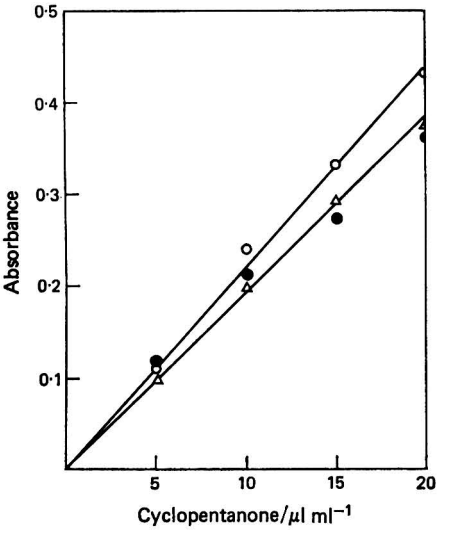


Fig. 3. Absorbance of carbonyl band of cyclopentanone in chloroform (○), ethanol (●) and a 1 + 1 mixture (△)

Conclusions

The significance of these results for analytical work that involves measurement of the peak absorbance of the doublet in the carbonyl region of the infrared spectrum of cyclopentanone can be summarised as follows. Straight-line graphs for the Beer - Lambert law were obtained by using various solvents. The slope of the graph for the Beer - Lambert law (and hence the molar absorption coefficient) varied with the solvent, and for a 1 + 1 mixture of solvents was a mean of the value in each solvent, unless hydrogen bonding between one or both solvents and cyclopentanone occurred.

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Residues of Prophylactics in Animal Products

Part II.* The Determination of Amprolium Added to Eggs and Poultry Meat

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The A.O.A.C. fluorimetric procedure for the determination of amprolium in feedstuffs has been applied to the determination of amprolium added to eggs and poultry meat at levels between 0·1 and 1·0 mg kg^{-1}. With suitable variations in the sample weight and volumes of reagents, the method was found to give reasonably satisfactory results.

AMPROLIUM [1-(4-amino-2-propyl-5-pyrimidinylmethyl)-2-picolinium chloride hydrochloride] is a widely used coccidiostat that is incorporated into poultry feeds or added to the drinking water. In order to be able to assess the amounts of amprolium that could occur in eggs or meat derived from poultry treated with this compound, a method capable of determining residues of amprolium above the level of about 0·1 mg kg^{-1} was required. An electron-capture gas-chromatographic procedure for the determination of residues of sulphaquinoxaline in poultry and eggs has recently been proposed,[1] which is based on a derivative of 2-amino-quinoxaline. Amprolium, however, is much more chemically stable than sulphaquinoxaline and a similar analytical approach proved to be unsatisfactory. Amprolium has been determined in poultry feeds colorimetrically,[2–5] photofluorimetrically[6] and by thin-layer chromatography.[7] Because of the high sensitivity of the photofluorimetric method, it was decided to try to modify this procedure so that it could be applied to the determination of amprolium in eggs and poultry meat. The method described below has been devised for this purpose and was found to be suitable for the determination of amprolium at concentrations of greater than 0·1 mg kg^{-1}.

EXPERIMENTAL

Recovery experiments were carried out on samples of eggs and poultry meat by spiking them with 50 μl of the appropriate stock solution to give samples containing 0·1, 0·5 or 1·0 mg kg^{-1} of amprolium. (The weakest stock solution had to be discarded after 2 weeks as decomposition was observed.) To assess the variation in extracting samples from different birds, meats from three sources, designated A, B and C, were analysed separately.

The effect of the concentration of trichloroacetic acid was investigated up to 40 per cent. Acid of 5 per cent. concentration was found to be satisfactory for meat samples while 10 per cent. acid was preferable for eggs. Lower extraction recoveries were obtained when chloroform, ethyl acetate, methanol or methanol - water (2 + 1 v/v) were used instead of trichloroacetic acid.

All samples were analysed in duplicate by developing two 5-ml aliquots from each 50 ml of extract solution, and unspiked samples were also examined to assess the effects of co-extractives. It was verified that fluorescence varies linearly with amprolium concentration over the range 0 to 1·0 mg kg^{-1}. The fluorimeter used in this work was a Baird-Atomic SP1-Fluorispec fitted with 5 × 5-mm silica cells.

Thiamine may be present in eggs and poultry meat and it may be oxidised to thiochrome, which fluoresces at 420 nm. This does not seriously interfere with the analysis of samples containing 0·5 mg kg^{-1} of amprolium (Fig. 1) but leads to large errors with samples containing 0·1 mg kg^{-1} of amprolium (Fig. 2). Thiamine can be removed by treating the sample with silver nitrate solution if necessary. Buquinolate, decoquinate, methyl benzoquate and diaveridine did not interfere when present at a level of 5·0 mg kg^{-1}.

* For particulars of Part I of this series, see reference list, p. 257.

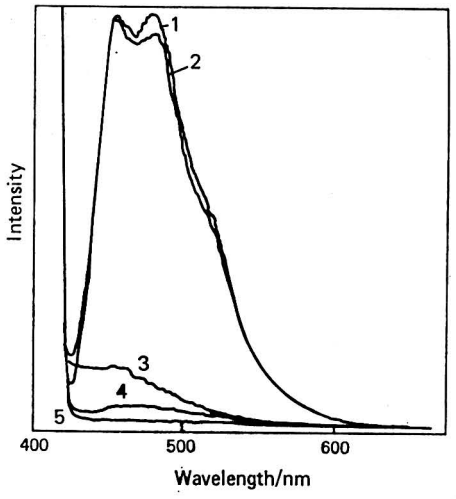

Fig. 1. Fluorescence of poultry samples containing 0·5 mg kg^{-1} of added amprolium. 1, Spiked sample, not treated with silver nitrate solution; 2, spiked sample, treated with silver nitrate solution; 3, unspiked sample, not treated with silver nitrate solution; 4, unspiked sample, treated with silver nitrate solution; and 5, reagent blank

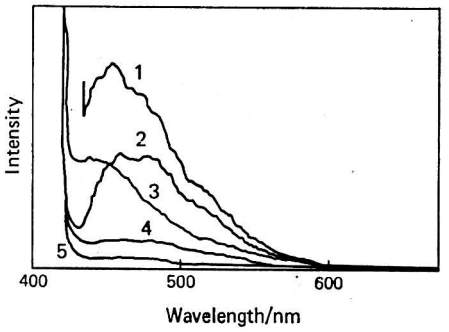

Fig. 2. Fluorescence of egg samples containing 0·1 mg kg^{-1} of added amprolium. Curves 1 to 5, as in Fig. 1

REAGENTS—

Analytical-reagent grade materials should be used when available.
Light petroleum, boiling range 60 *to* 80 °C.
Trichloroacetic acid solution, 5 *and* 10 *per cent. w/v.*
Sodium hydroxide solution, 30 *per cent. w/v.*
Silver nitrate solution, 5 *per cent. w/v.*
Potassium hexacyanoferrate(III) solution, 2 *per cent. w/v*—Freshly prepared.
*Pentanol.**
Amprolium solution, 40, 20, 4 *and* 2 *mg* l^{-1}.

PROCEDURE—

Extraction of poultry meat—Extract 20 g of minced poultry meat with light petroleum in a Soxhlet apparatus for 1 hour. Discard the extract and transfer the Soxhlet thimble to a beaker. Triturate the thimble and its contents with 25 ml of 5 per cent. w/v trichloroacetic acid solution and decant the resulting mixture through a Buchner funnel fitted with a Whatman No. 1 filter-paper. Repeat the trituration with a further 25 ml of 5 per cent. w/v trichloroacetic acid solution, transfer the mixture to the Buchner funnel and filter under suction, ensuring maximum extraction of the pulp by applying pressure with a bottle stopper. Adjust the volume of the combined extracts to 50 ml with 5 per cent. w/v trichloroacetic acid solution.

Extraction from egg samples—Transfer the contents of six eggs to a 100-ml beaker and blend them by stirring briskly. Transfer about 20 g of the mixture to a tared 100-ml beaker and weigh the beaker and its contents. Add 20 ml of 10 per cent. w/v trichloroacetic acid solution, stir the mixture well and filter it under suction through a Whatman No. 1 filter-paper. Wash the residue on the filter-paper with a further volume of 10 per cent. w/v trichloroacetic acid solution and adjust the volume of the combined extracts to 50 ml with 10 per cent. w/v trichloroacetic acid solution.

Development of fluorophore—Transfer with a pipette 5 ml of the trichloroacetic acid solution used to prepare the extracts into a 15-ml centrifuge tube for use as a reagent blank. Transfer

* A mixture consisting principally of 3-methylbutan-1-ol with a smaller proportion of 2-methylbutan-1-ol.

5-ml aliquots of the extract solution with a pipette into two 15-ml centrifuge tubes to act as duplicate sub-samples. Prepare a standard solution from each of the amprolium stock solutions by adding 5 μl of stock solution to 5 ml of trichloroacetic acid solution in a 15-ml centrifuge tube. Treat all the solutions as follows. Add 2·0 ml of sodium hydroxide solution followed by 0·2 ml of silver nitrate solution, shake the mixture and allow it to stand for exactly 2 minutes. Add 1 ml of potassium hexacyanoferrate(III) solution, shake the mixture and allow it to stand for exactly 3 minutes. Then add 1·5 ml of pentanol over the aqueous layer. Shake the mixture well for 1 minute, centrifuge it and remove about 1 ml of the organic phase for fluorescence measurement. (The addition of silver nitrate solution can be omitted without serious errors occurring for samples containing 0·5 mg kg^{-1} or more of amprolium.)

Measurement of fluorescence—Excite the solution at 405 nm and measure the fluorescence at 480 nm. The concentration of amprolium in the original sample is

$$\left(\frac{R_x - R_B}{R_s - R_B}\right) \times 2{\cdot}5\ S \text{ mg kg}^{-1}$$

where R_x is the fluorescence of the sample solution, R_s is the fluorescence of the standard solution, R_B is the fluorescence of the reagent blank and S μg ml^{-1} is the concentration of amprolium in the standard solution.

Results

The results of recovery experiments on samples of egg and poultry meat are given in Table I. The results for individual birds are presented in Table II. The reproducibility of the fluorophore development and its measurement were assessed by comparing a series of standard solutions; these results are given in Table III.

Table I

Recovery of amprolium from spiked samples of egg and poultry meat

Sample	Amprolium added/ mg kg^{-1}	Mean amprolium recovered, per cent.	Mean spread between duplicates, per cent.	Standard deviation, per cent.	Number of determinations
Eggs	0·1	68	6·0	±18	11
	0·5	72	2·5	± 6	10
	1·0	83	5·5	± 6	8
Chicken	0·1	62	4·7	±14	10
	0·5	56	5·0	± 7	17
	1·0	54	3·4	± 5	17

Table II

Comparison of the recoveries of amprolium from spiked meat samples of different origins

Bird	Amprolium added/ mg kg^{-1}	Mean amprolium recovered, per cent.	Mean spread between duplicates, per cent.	Standard deviation, per cent.	Number of determinations
A	0·1	59	6·6	±15	5
B	0·1	62	2·5	±15	6
C	0·1	63	5·0	±13	5
A	0·5	56	6·0	± 6	8
B	0·5	49	3·0	± 7	5
C	0·5	63	6·0	± 7	4
A	1·0	52	5·0	± 8	6
B	1·0	53	2·1	± 3	6
C	1·0	56	3·0	± 4	5

The determination of amprolium in a sample depends on two reactions and measurements, assuming the reagent blank is low, so the standard deviation in the method can be expected to be about twice the values recorded in Table III *plus* the variation in the extraction stage. For samples containing 0·1 mg kg^{-1} of amprolium, the blank from unspiked meat becomes

significant and estimates of recovery can vary by as much as three times the standard deviation in Table III *plus* the variation in the extraction stage. In a collaborative study[2] of six commercial feed samples containing 125 mg kg^{-1} of amprolium, the standard deviations ranged from 4·2 to 6·3 per cent. Because of the lower concentrations studied in this work, together with more formidable clean-up problems and the previous considerations, the standard deviations of the recovery experiments are considered to be reasonable.

TABLE III

VARIATION IN DEVELOPMENT AND MEASUREMENT OF FLUOROPHORE

Mass of amprolium/μg	Relative standard deviation in measured peak, per cent.	Number of determinations
0·2	±6·1	8
1·0	±2·5	7
2·0	±3·1	8

DISCUSSION

The percentage of amprolium recovered from poultry-meat samples is lower than is usually desirable in residue work. It was established that no amprolium was removed by the preliminary extraction with light petroleum. When amprolium was added to the acidified chicken extract it was quantitatively recovered, showing that there was no interference by co-extractives in the fluorophore development or measurement. Therefore, the unrecovered amprolium is either decomposed within the chicken tissue (this is thought to be unlikely as no variation was found when the meat was left for different periods between spiking and extraction) or irreversibly absorbed in the chicken substrate. Further extraction with trichloroacetic acid failed to remove any additional amprolium and no other suitable polar solvent has been found.

The sensitivity of the method is limited by the detection limit of the spectrofluorimeter and by the effect of co-extractives. On the instrument used in this work, a peak of thirty divisions represents 0·1 mg kg^{-1} of amprolium in the sample and so the maximum sensitivity is about 0·03 mg kg^{-1}. Co-extractives give rise to a background reading corresponding to 0·03 mg kg^{-1} of amprolium in poultry meat and 0·02 mg kg^{-1} in eggs. As the history of the samples was unknown, it cannot be proved whether or not this background arose from their treatment with amprolium.

CONCLUSION

The method described is applicable to samples of eggs and poultry meat containing 0·1 to 1·0 mg kg^{-1} of amprolium. The results are less reliable at the lower concentration.

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NOTE—Reference 1 constitutes Part I of this series.

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The Determination of Nicotinic Acid by Fluorimetric Densitometry

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A method is reported for the rapid determination of added nicotinic acid in meat. Nicotinic acid is converted on silica plates into a fluorescent compound by ultraviolet irradiation. After chromatographic separation from nicotinamide, the concentration is measured by means of a densitometer.

The use of nicotinic acid for the preservation of the red colour of raw minced meat is allowed in The Netherlands up to a concentration of 0·015 per cent. In order to determine this concentration we have used thin-layer chromatography on silica mixed with a fluorescence indicator that makes the spots visible by fluorescence quenching under ultraviolet light at a wavelength of 254 nm. The determination was carried out spectrophotometrically at 263 nm after extraction of the spots with ethanol (laboratory method of Keuringsdienst van Waren, unpublished).

By use of this method it was found that, after ultraviolet irradiation at a wavelength of 254 nm, fluorescence at 365 nm was induced. With a Vitatron densitometer - fluorimeter it is easy to detect and determine small amounts of the additive so that the time-consuming and unreliable extraction procedure can be omitted.

Experimental

Apparatus and materials—

Vitatron flying-spot densitometer, TLD 100.

Filters—A filter that operated at a wavelength of 365 nm was used as the primary filter and we selected one that operated at 435 nm as the secondary filter. The light source was a mercury lamp and the recorder was a Vitatron UR402.

Philips Blacklight lamp HPW, 125 *W*—The maximum emission of this lamp occurred at a wavelength of 365·5 nm.

Pleuger Chromatolux 44 *U* 100 *lamp*—The maximum emission of this lamp occurred at a wavelength of 253·7 nm.

Merck DC ready-made Kieselgel plates (*without fluorescence indicator*)—These were 20 × 20-cm plates with a layer thickness of 0·25 mm.

Standard nicotinic acid solution—This solution consisted of 20·0 mg of nicotinic acid in 1 litre of 96 per cent. ethanol.

Chromatographic solvent mixture—The solvent mixture used was benzene - acetone - methanol - acetic acid (56 + 44 + 16 + 4).

Determination of the irradiation time—

Amounts of 100 to 2000 ng of nicotinic acid (*i.e.*, 5 to 100 μl of standard nicotinic acid solution) were applied to the plates. The plates were developed for half an hour in an equilibrated tank, dried for half an hour at 105 °C and irradiated with the Chromatolux lamp at a distance of 6 cm for different lengths of time. Measurements of the fluorescence were made with the densitometer. The results of these measurements are given in Tables I and II.

From the tables it is clear that a reaction takes place which is almost completed in 25 minutes, so that half an hour must be sufficient for maximum fluorescence to occur. The meter readings show good proportionality with the amounts used.

Procedure—

A 15-g sample of minced meat is weighed into a 150-ml beaker and 50 ml of 96 per cent. ethanol are added. The beaker, covered with a watch-glass, is heated for half an hour on a boiling water bath. The ethanolic extract is then filtered through cotton-wool into a 100-ml calibrated flask. The residue is washed three times with 20, 20 and 15 ml of ethanol. After dilution to the calibration mark, 20 μl of the solution are spotted on to a thin-layer plate.

TABLE I

MEASUREMENT OF FLUORESCENCE IN COUNTS

15 counts ≡ 1 cm² of peak area

Time of irradiation/minutes	2	5	10	15	20	25	30	60
Amount of nicotinic acid added/ng—								
100	2	5	6	8	9	10	10	9
200	8	12	13	17	19	20	19	18
400	7	15	27	33	36	39	38	37
600	9	18	36	46	48	57	58	54
800	13	29	48	63	65	76	77	71
1000	22	41	64	80	85	86	96	90
1500	31	60	95	117	128	138	141	133
2000	43	81	124	156	160	166	166	158

Instrument settings: level, F; zero, 7; damping, 2; diaphragm, 0·25 nm; mode, linear II +, primary filter, 365 nm; secondary filter, 435 nm; amplitude, 14 (7 mm); paper velocity, 6 (4 cm min^{-1}); integrator, 7 (60 counts min^{-1} cm^{-1}); span, 8; and scanning speed, 3 cm min^{-1}.

On the same plate amounts of standard solution equivalent to 400 and 600 ng of nicotinic acid are also applied. The plate is developed for half an hour and dried for another half hour at 105 °C. It is next irradiated for half an hour with the Chromatolux lamp at a distance of 6 cm. The spots are then made visible under the Blacklight lamp and the plate is marked so that the measurements with the densitometer can be made in one run. The result is obtained by comparing the fluorescence of the sample with those of the standards.

Under the conditions described the R_F value of nicotinic acid is 0·35, while that of nicotinamide is 0·45. The amplitude of the flying spot of the densitometer should be adjusted so that only nicotinic acid is measured.

TABLE II

MEASUREMENT OF FLUORESCENCE IN COUNTS

Conditions as in Table I, but with span equal to 10

Time of irradiation/minutes	2	5	10	15	20	25	30	60
Amount of nicotinic acid added/ng—								
100	6	9	16	21	22	22	22	20
200	18	26	41	40	43	42	43	40
400	18	39	60	76	87	84	83	79
600	19	47	72	108	113	126	126	120
800	26	67	110	144	154	168	164	158
1000	51	90	142	180	190	212	210	201
1500	64	139	215	—	—	—	—	—
2000	92	172	—	—	—	—	—	—

RESULTS AND DISCUSSION

To 15-g samples of minced meat were added 750, 1500, 2250, 3000 and 3750 μg of nicotinic acid. The recoveries were 98·0, 99·3, 97·8, 101·8 and 96·3 per cent., respectively. No nicotinic acid was found in a blank sample of the meat. The sensitivity of the method for nicotinamide is about 1½ times as great as that for nicotinic acid. Among chemically similar compounds, benzoic acid does not show the induced fluorescence, but the herbicides paraquat and diquat do, with a higher sensitivity than that for nicotinic acid (two and five times greater, respectively). Ascorbic acid shows some fluorescence before irradiation, but in the concentration used in practice the ultraviolet light destroys this compound.

Better separation from nicotinamide is achieved by use of a solvent mixture of propanol - 10 per cent. ammonia solution (95 + 5), but the time required (3 hours) is much longer.[1]

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Determination of Quaternary Ammonium Salts in Lead Nitrate - Copper(II) Nitrate Solution

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A method is described for determining the quaternary ammonium surfactants cetyltrimethylammonium bromide, tetradecyltrimethylammonium bromide and octadecyltrimethylammonium bromide by titration with sodium tetraphenylboron solution at pH 3, with methyl yellow as indicator. The error is within ±1 per cent. for the determination of the above quaternary ammonium surfactants either alone or in the presence of lead nitrate or copper(II) nitrate, or both.

During the preparation of graphite-substrate lead dioxide anodes, a small addition of the quaternary ammonium surfactant cetyltrimethylammonium bromide (CTAB) to lead nitrate - copper(II) nitrate solution gave a smooth and adherent deposit free from pin-holes.[1] Quaternary ammonium surfactants are normally determined[2] by absorptiometric, titrimetric and gravimetric methods.

Most of the titrimetric methods reported involve two-phase titrations in which chloroform is used as the organic layer. They are based on the titration of the quaternary ammonium surfactants with an anionic surfactant, *e.g.*, sodium lauryl sulphate, in the presence of an indicator such as bromophenol blue[3,4] or methylene blue.[3,5] The two-phase titration with sodium lauryl sulphate as titrant and with bromophenol blue as indicator could not be applied to the determination of CTAB in the presence of lead nitrate - copper(II) nitrate solution as the results obtained were erratic. The use of sodium tetraphenylboron as titrant in the presence of methyl orange or bromophenol blue was reported by Cross.[6]

The single-phase titration with sodium tetraphenylboron, with methyl orange as indicator, has been reported by Uno, Miyajima and Tsukatani.[7] The use of potassium cadmium iodide as a titrant instead of sodium tetraphenylboron has also been reported.[8] Other indicators,[2] *viz.*, methyl red, fluorescein, bromophenol blue, methyl orange and Congo red, gave satisfactory results with pure CTAB solution but the end-point in the presence of lead and copper(II) nitrates was less sharp wih the first three indicators, whereas the last two gave lower and higher values, respectively. However, at concentrations of CTAB below 0·0025 M it is not possible to detect the end-point with any of these indicators.

Methyl yellow has also been tried as an indicator in the determination of surface-active substances inolving two-phase titrations.[9–12] Boden[13] used tetraphenylboron as a titrant with a cationic indicator to determine quaternary pyridinium halides of high molecular weight. Attempts to evolve a suitable titrimetric procedure for the determination of quaternary ammonium surfactants in the presence of lead and copper(II) nitrates led to the development of a single-phase titration with sodium tetraphenylboron, with methyl yellow as indicator. This method is similar to that reported by Uno *et al.*,[7] in which methyl orange is replaced with methyl yellow, and is the subject of the present paper.

Experimental

Reagents—

Sodium tetraphenylboron solutions, 0·0005 to 0·01 M—These were prepared by accurately weighing the pure substance (E. Merck, general reagent) and dissolving it in distilled water. The pH of the solutions was adjusted to 9 with 0·04 per cent. sodium hydroxide solution. (The solutions were stable for 1 week and the freshly prepared solutions must be kept overnight before use.) The sodium tetraphenylboron used was standardised according to the method described by Cross[6] and the purity was found to be not less than 99 per cent.

Cetyltrimethylammonium bromide solution, 0·0005 to 0·01 M—These were prepared by dissolving the pure substance (BDH) in hot distilled water.

Methyl yellow (p-*dimethylaminoazobenzene) indicator*—A 0·05 per cent. w/v solution in ethanol was used.

Acetic acid - sodium acetate buffer solution (pH 3·2)—Ten millilitres of buffer solution contained 9·5 ml of 0·2 M acetic acid and 0·5 ml of 0·2 M sodium acetate.

PROCEDURE—

CTAB solution (10 ml) was transferred by pipette into an Erlenmeyer flask. Buffer solution (1 ml) was added, followed, if necessary, by a further volume of buffer solution to adjust the pH to 3·0. (The volume of buffer solution needed varied from 1 to 4 ml for concentrations of CTAB from 0·0005 to 0·01 M.) Two drops of methyl yellow indicator solution were then added and the solution was titrated against standard sodium tetraphenylboron solution. The colour of the solution changed from orange to pink at concentrations of CTAB below 0·005 M and yellow to pink at concentrations above 0·005 M.

To 10 ml of CTAB solution, 3·5 g of solid lead nitrate (E. Merck, analytical reagent) were added, the solution was heated to 50 °C and, after cooling to 45 °C, was titrated against sodium tetraphenylboron by using the above procedure. This procedure was repeated in the presence of 35 g l^{-1} of copper(II) nitrate, which was used either alone or together with the lead nitrate (350 g l^{-1}). The colour introduced by the copper(II) nitrate did not interfere with the colour change at the end-point.

Other surfactants of the quaternary ammonium group, *viz.*, tetradecyltrimethylammonium bromide and octadecyltrimethylammonium bromide, can also be determined by the same procedure.

The results are presented in Tables I to IV.

TABLE I

DETERMINATION OF PURE CTAB SOLUTION BY TITRATION AGAINST SODIUM TETRAPHENYLBORON WITH METHYL YELLOW AS INDICATOR

CTAB taken/ g l^{-1}	CTAB (g l^{-1}) determined against 0·01 M $NaB(C_6H_5)_4$	Error, per cent.	0·005 M $NaB(C_6H_5)_4$	Error, per cent.	0·0025 M $NaB(C_6H_5)_4$	Error, per cent.	0·001 M $NaB(C_6H_5)_4$	Error, per cent.
3·605	3·572	−0·9	—	—	—	—	—	—
1·788	1·784	−0·2	1·791	+0·3	—	—	—	—
0·8848	0·8930	+0·9	0·8867	+0·2	0·8860	+0·1	—	—
0·3560	—	—	0·3563	+0·1	0·3580	+0·5	0·3567	+0·2
0·1752*	—	—	0·1737	−0·8	0·1745	−0·4	0·1748	−0·2

* The end-point is not sharp with 0·0005 M sodium tetraphenylboron.

TABLE II

DETERMINATION OF CTAB IN THE PRESENCE OF EITHER 350 g l^{-1} OF LEAD NITRATE OR 35 g l^{-1} OF COPPER(II) NITRATE BY TITRATION AGAINST SODIUM TETRAPHENYLBORON WITH METHYL YELLOW AS INDICATOR

CTAB taken/g l^{-1}		CTAB (g l^{-1}) determined against $NaB(C_6H_5)_4$ solutions: In presence of $Pb(NO_3)_2$	Error, per cent.	In presence of $Cu(NO_3)_2$	Error, per cent.
3·605	0·01 M $NaB(C_6H_5)_4$	3·620	+0·4	3·620	+0·4
1·775		1·777	+0·1	1·787	+0·6
0·9020		0·9047	+0·3	0·9047	+0·3
0·3576*		0·3620	+1·0	0·3620	+1·0
0·1788		0·1791	+0·2	0·1810	+1·5
3·605	0·005 M $NaB(C_6H_5)_4$	—	—	—	—
1·775		1·791	+1·0	1·791	+1·0
0·9020		0·8960	−0·6	0·8959	−0·6
0·3576*		0·3565	−0·3	0·3563	−0·3
0·1788*		0·1782	−0·3	0·1783	−0·3
3·605	0·0025 M $NaB(C_6H_5)_4$	—	—	—	—
1·775		—	—	—	—
0·9020		0·8950	−0·8	0·8950	−0·8
0·3576*		0·3580	+0·1	0·3604	+0·8
0·1788		0·1790	+0·1	0·1786	−0·1

* The end-point is not sharp with 0·001 and 0·0005 M sodium tetraphenylboron.

Table III

Determination of CTAB in the presence of 350 g l^{-1} of lead nitrate and 35 g l^{-1} of copper(II) nitrate by titration against sodium tetraphenylboron with methyl yellow as indicator

CTAB taken/ g l^{-1}	CTAB (g l^{-1}) determined against 0·01 M $NaB(C_6H_5)_4$	Error, per cent.	0·005 M $NaB(C_6H_5)_4$	Error, per cent.	0·0025 M $NaB(C_6H_5)_4$	Error, per cent.
3·629	3·598	−0·8	—	—	—	—
1·775	1·799	+1·0	1·791	+0·9	—	—
0·9020	0·9087	+0·7	0·8989	−0·4	0·8980	−0·45
0·3576	0·3593	+0·5	0·3586	+0·3	0·3566	−0·3
0·1788	0·1791	+0·2	0·1783	−0·3	0·1778	−0·6

Table IV

Determination of tetradecyltrimethyl- and octadecyltrimethylammonium bromides in the presence of lead nitrate and copper(II) nitrate by titration against sodium tetraphenylboron with methyl yellow as indicator

Tetradecyltrimethylammonium bromide					Octadecyltrimethylammonium bromide				
	Determined/g l^{-1}					Determined/g l^{-1}			
			In presence of					In presence of	
Taken/ g l^{-1}	In pure solution	Error, per cent.	$Pb(NO_3)_2$+ $Cu(NO_3)_2$	Error, per cent.	Taken/ g l^{-1}	In pure solution	Error, per cent.	$Pb(NO_3)_2$+ $Cu(NO_3)_2$	Error, per cent.
3·312	3·309*	−0·09	3·343	+0·9	3·880	3·890*	+0·25	3·850	−0·8
0·8230	0·8214†	−0·2	0·8296	+0·8	0·9700	0·9690†	−0·1	0·9753	+0·5
0·1656	0·1676†	+1·2	0·1676	+1·2	0·1940	0·1921†	−0·9	0·1921	−0·9

* Titrated against 0·01 M sodium tetraphenylboron.
† Titrated against 0·005 M sodium tetraphenylboron.

Discussion

It can be seen from Tables I to III that the error is within ±1 per cent. for the determination of CTAB, either alone or in the presence of lead nitrate or copper nitrate, or both, by titrating it against sodium tetraphenylboron with methyl yellow as indicator. At lower concentrations of CTAB (0·0005 to 0·001 M) the end-point is sharp only when the titration is carried out with sodium tetraphenylboron solutions of higher concentration (0·0025 or 0·005 M).

It is shown in Table IV that it is also possible to determine other quaternary ammonium surfactants such as tetradecyltrimethylammonium bromide and octadecyltrimethylammonium bromide in the presence of lead and copper(II) nitrates by titrating them against sodium tetraphenylboron with methyl yellow as indicator. The error in these instances is within ±1 per cent. Uno and Miyajima[8] proposed the formation of a 1:1 complex between methyl orange and the quaternary ammonium salt as the reason for the failure of the indicator to give the red colour at pH 3·0. When such a solution was titrated with potassium cadmium iodide solution, the excess of surfactant was precipitated first and then the surfactant in the complex with consequent liberation of the methyl orange to give the red end-point. It can be assumed that the same mechanism also takes place in the present instance.

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An Improved Test for Cocaine, Methaqualone and Methadone with a Modified Cobalt(II) Thiocyanate Reagent

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The classical cobalt(II) thiocyanate reagent has been rendered more sensitive and selective so as to provide a convenient field test for cocaine, methaqualone and methadone, substances that fail to give clear positive responses in the widely used Marquis procedure.

THE traditional colour test for cocaine consists of the addition of cobalt(II) thiocyanate solution to give a blue flaky precipitate; since Young's first report[1] there have been no important modifications of this test. In searching for a field test for use by non-technical personnel for cocaine, methadone and methaqualone (abused drugs that fail to give distinctive colours in the Marquis test[2]) it was obvious that no single reagent could be specific, but it was hoped that response could be restricted to these three abused drugs and possibly to a limited number of rarely encountered licit preparations.

EXPERIMENTAL

The reagent traditionally used is a 2 per cent. w/v solution of cobalt(II) thiocyanate in water. A blue flaky precipitate is produced on a spot-tile when the reagent is added to cocaine, methadone and pethidine and also procaine, which is a frequently encountered licit local anaesthetic. Only faint blue specks are observed with methaqualone, and this lack of response was attributed to the fact that the reagent does not "wet" the sample.

VARIATION OF THE SOLVENT—

It was observed that the addition of the traditional reagent to the sample followed by one drop of methanol successfully lowered the surface tension to produce an extremely sensitive reagent that "wetted" the sample and so responded to methaqualone as well as to the more common local anaesthetics. This simple alteration enabled the spot-tile to be discarded as the intense turquoise colour produced was produced by a soluble complex and could be displayed on a filter-paper in the same way as in our field tests for cannabis[3] and hallucinogens.[4]

VARIATION OF THE ACID—

An acid is an essential component for rendering the reagent sensitive to the base form of a drug as well as to the salt. Attempts were made to alter the sensitivity of the reagent by altering the acid used. A series of organic acids were investigated and it was found that by replacing hydrochloric acid with oxalic acid the reagent could be rendered less sensitive to procaine.

AVAILABILITY OF WATER—

Aizikov and Udoverko[5] assigned the formula $M(CNS)_2 2B.2H_2O$ to the blue complex formed between metal (M) thiocyanates and organic bases (B), and Babko and Schrechenko[6] assumed that the replacement of halide ions by water in the system cobalt halide - thiocyanate - water, in organic solvents, caused a decrease in colour intensity. Therefore, it appeared that the amount of water that is available for complexing might be a controlling factor in limiting the response of the reagent to the particular controlled drugs. This was investigated in three ways, by determining the optimum methanol-to-water ratio, by incorporating a hygroscopic salt and by incorporating a humectant.

A reduction in the methanol-to-water ratio from 1:1 to 2:3 successfully altered the response time for procaine from less than 5 s to 35 s. No increase in the response time was obtained by adding calcium chloride but the addition of solid metaphosphoric acid produced a marked limitation to the response by procaine. By using 1 per cent. v/v orthophosphoric acid instead of either hydrochloric acid or oxalic acid, the reagent was still capable of reacting to the base form as well as to the salt form of methaqualone, methadone and cocaine.

By adding glycerol to the methanolic oxalic acid reagent it was possible to modify the responses to some local anaesthetics, but the performance of this form of the reagent with methaqualone was unreliable in that atmospheric humidity governed its rate of response. Propan-2-ol was also investigated as a possible humectant but no advantages could be found for its use.

From all these modified reagent solutions, five were chosen that had been found to be the optimum formulation of each modification. These solutions were evaluated by comparing the times required for responses to occur. Table I shows the results obtained for a series of local anaesthetics together with cocaine, methaqualone, methadone and some antihistamines.

TABLE I

RESPONSES OF DRUGS TO MODIFIED COBALT(II) THIOCYANATE REAGENT SOLUTIONS

Sample	Reagent solution A	B	C	D	E
Substances scheduled in the Misuse of Drugs Act, 1971—					
Cocaine	++	++	++	++	++
Methadone	++	++	++	++	++
Pethidine	++	++	++	++	++
Methaqualone	++	−	+	+	++
Benzoylecgonine	++	++	++	−	+
Ecgonine	++	−	−	−	−
Topical anaesthetics—					
Amethocaine	++	++	++	++	++
Bupivacaine	++	++	++	++	++
Butacaine	++	++	++	++	++
Cinchocaine	++	++	++	++	++
Cyclomethycaine	++	++	++	++	++
Lignocaine	++	++	++	++	++
Piperocaine	++	++	++	++	++
Procaine	++	++	−	++	−
Tropacocaine	++	−	−	+	+
Proxymetacaine	++	−	−	−	+
Prilocaine	++	−	−	−	−
Benzocaine	++	−	−	−	−
Antihistamines—					
Diphenhydramine	++	++	++	++	++
Dramamine	++	++	++	−	+
Meclozine	+	−	−	+	++
Tranquilliser—					
Promethazine	++	++	++	++	++

Key: ++, responds in less than 5 s; +, responds between 5 s and 1 minute; and −, responds after 1 minute.

Reagent solutions—

A, 1·6 per cent. w/v solution of cobalt(II) thiocyanate in a 10 per cent. solution of hydrochloric acid (sp. gr. 1·18) in methanol.

B, 2 per cent. w/v solution of cobalt(II) thiocyanate in 2 N hydrochloric acid.

C, 0·8 per cent. w/v solution of cobalt(II) thiocyanate in 1 per cent. orthophosphoric acid (sp. gr. 1·75) solution.

D, 0·5 per cent. w/v solution of cobalt(II) thiocyanate in a 1 + 1 v/v mixture of glycerol with a 2·5 per cent. solution of oxalic acid in methanol.

E, 0·8 per cent. w/v solution of cobalt(II) thiocyanate in a 2 + 3 v/v mixture of methanol with 1 per cent. orthophosphoric acid (sp. gr. 1·75) solution.

METHOD

Place 1 to 2 mg of the suspected substance in the middle of a filter-paper. Add one drop of the modified cobalt(II) thiocyanate reagent and observe any turquoise colour that develops within 5 s of the addition of the reagent. Disregard any colour that develops after 5 s.

Discussion

Comparison between the results obtained with the methanolic hydrochloric acid solution (solution A) and those obtained with the aqueous hydrochloric acid solution (solution B) showed the higher sensitivity of solution A to all the local anaesthetics together with cocaine, methaqualone and methadone. Replacement of hydrochloric acid with phosphoric acid (solution C) successfully excluded procaine from substances having response times of less than 5 s and slightly improved the response to methaqualone in aqueous solution.

No advantage was gained from incorporation of glycerol as a humectant (solution D). At certain glycerol concentrations procaine was excluded from compounds having response times of less than 5 s, but the response to methaqualone at these concentrations was unreliable. The results in Table I were obtained under conditions of humidity and glycerol concentration producing the optimum response of methaqualone. An increase in humidity can limit the response severely.

The final reagent solution contained methanol and orthophosphoric acid (solution E). This reagent gave instantaneous responses to cocaine, methadone, methaqualone and pethidine but also to a number of less frequently encountered topical anaesthetics. Most important, procaine and benzocaine were excluded from compounds having response times of less than 5 s.

Conclusions

In practice, a sequence of field tests* is advised as a screening procedure for misused drugs. The first test should be for hallucinogens,[4] followed by the Marquis test[2] and then, in the event that no reaction occurs with the Marquis reagent within 1 minute, a modified cobalt(II) thiocyanate test should be applied. A negative response to all three tests eliminates the substance from the need for further examination and avoids the unnecessary detention of persons or goods.

For use in screening tests by law enforcement officers in the United Kingdom, solution E would be the most useful modification in that the common local anaesthetics procaine and benzocaine would not be detected. Lignocaine, although widely used, is rarely used in solid form and the probability of encountering, under suspicious circumstances, the other topical anaesthetics that respond to the test is small. Tranquillisers and antihistamines will have been noted by their reaction to the Marquis reagent, and also Mandrax, which is a special case and contains diphenhydramine as well as methaqualone.

Investigating officers in the Armed Services may be also concerned when military personnel are taking drug subsances that have not been prescribed by the Medical Officer, so that the very sensitive hydrochloric acid - methanol reagent (solution A) would be of greater value in such circumstances.

Whether the modified reagent is used as part of a sequence or in a broad-spectrum sorting procedure, its use is intended to limit the number of occasions when professional analysis becomes necessary.

* A provisional patent application has been made.

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An Automated Procedure for the Determination of Sulphur in Plant Tissue

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An automated turbidimetric procedure for the determination of sulphur in plant tissue is described. A double-probe system is used so that the system is washed with a buffer solution on the wash cycle. This ensures a satisfactory return to the base-line. The values obtained are compared with those obtained by the standard gravimetric procedure.

The increasing need for the elimination of pollution in water and the atmosphere has led to a requirement for a method for the determination of sulphur-containing compounds in effluents. Sulphur also occurs in compounds of two essential amino-acids, methionine and cystine, as well as in the vitamin thiamine, and as more basic investigations into the rôle of these compounds in life processes are conducted, greater demands are placed on rapid, precise and accurate methods for determining sulphur. The micro-balance and other sophisticated techniques for handling small amounts of material make the gravimetric procedure (precipitation as barium sulphate) still useful, although completely impractical for routine determinations on a large scale.

In spite of the well known uncertainties inherent in turbidimetric procedures, barium sulphate suspensions have been studied widely and have found useful application in quantitative determinations of sulphur. Canals, Charra and Riety[1] found nephelometric sulphate determinations to be critically dependent on the crystalline form and size distribution of the light-scattering particles. They also found that the degree of light scattering depended on the nature and relative amount of the precipitant and such factors as temperature and standing time before measurement. This would appear to indicate that to carry out a nephelometric or turbidimetric procedure for sulphate with a reasonable amount of precision, considerable care should be taken in maintaining optimum reaction conditions. With an AutoAnalyzer system, however, it is possible to treat every standard as well as sample in exactly the same manner, maintaining reaction conditions rigorously throughout the whole determination.

This paper reports the work carried out in an investigation of the application of the Technicon AutoAnalyzer to the turbidimetric procedure for determining sulphur in plant material. Previous attempts[2,3] to automate this procedure had been relatively unsuccessful because of insufficient washing between samples and a drifting base-line, indicating that absorption of barium sulphate on the colorimeter flow-cell wall was occurring. This necessitated the careful observance of the base-line and insertion of standards between samples at regularly spaced intervals.

Experimental

Apparatus—

Standard Technicon AutoAnalyzer equipment was used. The phototube colorimeter was fitted with a 50-mm tubular flow cell and 420-nm filters.

Reagents—

Unless otherwise specified, all reagents were of analytical-reagent quality.

Barium chloride solution—A 2·5 per cent. solution in 0·006 M hydrochloric acid containing 0·25 per cent. of gelatine was used.

Buffer solution—This consisted of 40 g of EDTA (disodium salt), 7 g of ammonium chloride and 57 cm^3 of concentrated ammonia solution (sp. gr. 0·88), diluted to 1 dm^3 with distilled water.

Standard sulphate solutions—Sulphate solutions containing 10, 20, 40 and 100 p.p.m. were prepared in the same amounts and with types of acids as used in the oxidation of the unknown sample.

The flow was as indicated in Figs. 1 (*a*) and (*b*), with a double-probe system.

Initial studies to determine the optimum conditions for the reactants were undertaken on standard solutions containing sulphate alone by using the flow diagram shown in Fig. 1, *i.e.*, without the double-probe arrangement for introducing the barium chloride solution into the system.

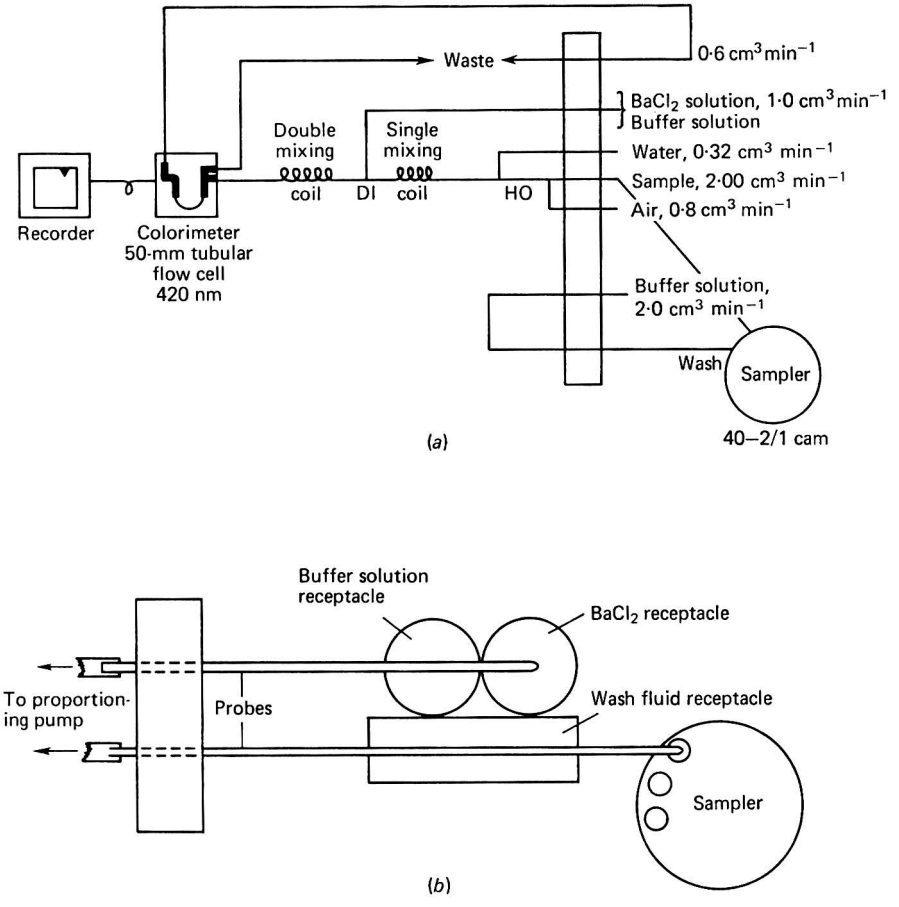

Fig. 1. (*a*), Flow diagram for the turbidimetric determination of sulphate in the range 0 to 100 p.p.m.; and (*b*), top view of probe arm modification to enable sample and $BaCl_2$ solutions to be sampled simultaneously and on the wash cycle for both probes to sample buffer solutions. (The $BaCl_2$ line alternately pumps buffer and $BaCl_2$ solutions. Further, the length is controlled so that it does not overlap with the sample stream)

Results and discussion

When carrying out the initial determinations on standard solutions with only a wash with water between samples, a sharp decrease in the percentage transmission was observed on comparing the peaks obtained from similar standards (Fig. 2). This indicated a shift in the base-line, which was caused by deposits of barium sulphate coating the colorimeter flow-cell wall. Unless this could be rectified the automated turbidimetric procedure would be of little value in determining the sulphate content of samples. Consequently, ways of improving the situation were investigated.

In the Technicon AutoAnalyzer Methodology[3] provision is made for an EDTA - ammonia buffer to be passed through as a wash between samples. For this reason a buffer solution was prepared and used in the proposed system. This buffer solution was pumped into the

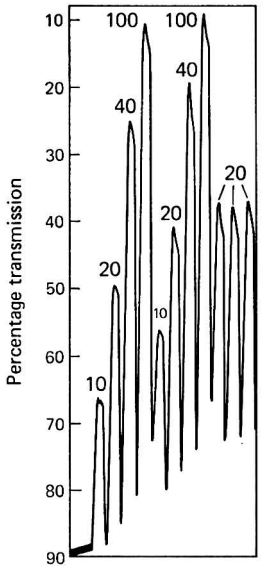

Fig. 2. Recorder chart illustrating the sharp decrease in the percentage transmission caused by the $BaSO_4$ coating on the flow cell wall. Values on peaks are approximate concentrations (p.p.m.) of SO_4^{2-}

sampler reservoir and the determination repeated on a series of standard sulphate solutions. Distinct improvements in carry-over and base-line drift were observed.

In an attempt to improve these conditions larger pump tubes for clearing the flow cell were used. This modification, however, led to an additional problem in that a sharp increase in transmission was observed at the top of the recorded peaks, which became progressively worse with pump tubing of larger internal diameter (Fig. 2). When using a 0·6 cm^3 min^{-1} pump tube for pumping away to waste, smooth and regular peaks were observed and this size was used for all further determinations.

To investigate the influence of the barium chloride concentration on the washing out and the drifting base-line, 0·006 M solutions of hydrochloric acid containing 0·25 per cent. of gelatine and increasing amounts of barium chloride, varying from 0·4 to 10 per cent., were prepared. At low barium chloride concentrations an increase in sensitivity with increasing concentration was observed. Above 2·5 per cent., however, a drift in the base-line occurred again and the reproducibility was also poor; consequently, the concentration was maintained at 2·5 per cent. This was not entirely satisfactory, however, as large differences were observed in the content of the washings at the beginning and end of a determination. At this stage it became evident that a different approach would have to be used to clear the absorbed barium sulphate from the flow cell.

A procedure whereby use is made of a double-probe system, in which the addition of the barium chloride reagent into the flow system is alternated with the EDTA - ammonia buffer solution, was investigated. To obtain satisfactory washing and reproducibility it is important to regulate the flow of barium chloride solution so that it does not overlap with the diluted sample stream. This is conveniently achieved by using coloured solutions to co-ordinate the different flow streams.

To determine the effect of different barium chloride concentrations on determinations with this flow system, two series of standard solutions were prepared. Both comprised 0·5, 1·0, 2·5, 5·0 and 10·0 per cent. barium chloride solutions in 0·006 M hydrochloric acid, but the

second series of solutions also contained 0·25 per cent. of gelatine. An increase in sensitivity with increase in barium chloride concentration for both series of solutions was noted. However, the results obtained with the barium chloride solutions without the added gelatine were of a lower sensitivity and were also less reproducible. At barium chloride concentrations greater than 2·5 per cent. the washing out between samples again became unsatisfactory and for this reason it remains important to maintain the barium chloride concentration at approximately 2·5 per cent.

WET-OXIDATION PROCEDURE FOR THE DETERMINATION OF SULPHATE—

When preparing leaf-tissue samples for analysis, the following wet-digestion procedure was used.

Weigh 0·25 g of the ground sample into a 100-cm^3 Pyrex beaker, add 15 cm^3 of concentrated nitric acid, heat for 20 minutes on a sand-bath, and then add 2 cm^3 of 70 per cent. perchloric acid. Increase the heat and maintain the digestion with gentle boiling until white fumes appear. Evaporate to a volume of approximately 2·5 cm^3.

Remove the beaker from the sand-bath and cool it; then add 20 cm^3 of distilled water and heat the mixture. Filter the solution into a 100-cm^3 calibrated flask and wash the residue with hot 0·1 M hydrochloric acid. This results in a solution containing 20 to 50 p.p.m. of sulphate and 0·15 to 0·24 g-equiv dm^{-3} of hydrogen ion. To determine the effect of the hydrogen ion, and also the type of acid used in the determination, the following solutions were prepared. Firstly, three different series of solutions containing 20 p.p.m. of sulphate were prepared in acids as follows: perchloric acid at concentrations of 0·05, 0·1, 0·27, 0·54 and 1·0 M; hydrochloric acid at 0·05, 0·098, 0·25, 0·5 and 1·0 M; and nitric acid at 0·05, 0·1, 0·27, 0·54 and 1·0 M. Secondly, equal volumes of perchloric and nitric acids were mixed to give mixtures with hydrogen-ion concentrations of 0·05, 0·13, 0·26, 0·52 and 1·30 M.

The results obtained are shown in Fig. 3. The optimum acid concentration appears to be between 0·1 and 0·2 g-equiv dm^{-3}. With perchloric acid a gradual decrease in percentage transmission is observed below 0·2 g-equiv dm^{-3} while at higher concentrations there is a gradual increase in transmission. Hydrochloric acid follows a similar pattern but a lower sensitivity is obtained. With nitric acid optimum values are obtained at approximately 0·12 g-equiv dm^{-3} and a sharp increase in transmission takes place above 0·2 g-equiv dm^{-3}. Nitric and perchloric acids together shift the optimum concentration to the range from 0·15 to 2·0 g-equiv dm^{-3}. This shift is also followed by an increase in percentage transmission, which is not as large as that for nitric acid alone, however. Fig. 4 shows the reproducibility of the procedure.

To minimise errors that may arise because of differences in acid concentration, a standard solution containing the same amount of digestion acid as the samples is carried through the complete digestion procedure.

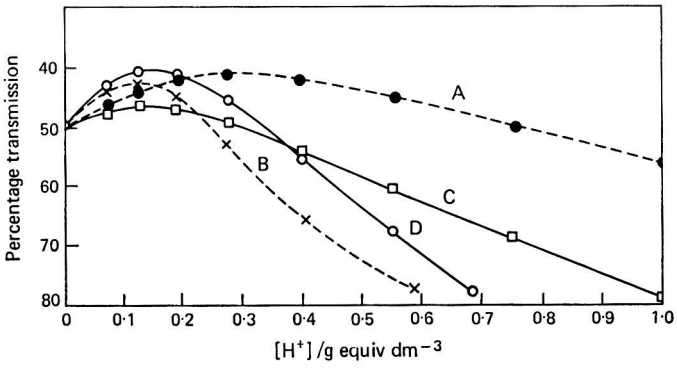

Fig. 3. Effect of type of acid and acid concentration on the turbidimetric sulphate determination on a standard solution containing 20 p.p.m. of sulphate: curve A, perchloric acid; curve B, nitric acid; curve C, hydrochloric acid; and curve D, equal concentrations of perchloric and nitric acids

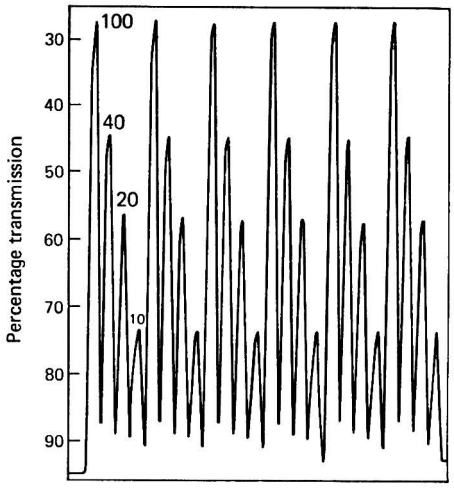

Fig. 4. Recorder tracing showing reproducibility of sulphate procedure. Values on peaks are in p.p.m.

To test the precision and accuracy of this method a series of samples was analysed and the results were compared with those obtained by use of the standard barium sulphate gravimetric procedure. These results are shown in Table I. Very good correlation between the two methods is apparent.

TABLE I

RESULTS OBTAINED FOR THE SULPHUR CONTENT OF LEAF-TISSUE SAMPLES WITH THE AUTOMATED PROCEDURE AND THE STANDARD BARIUM SULPHATE GRAVIMETRIC PROCEDURE

Sample	Sulphur found— by gravimetric procedure, per cent.	Sulphur found— by AutoAnalyzer procedure, per cent.
1	0·25	0·24
2	0·29	0·30
3	0·25	0·25
4	0·28	0·28
5	0·30	0·29
6	0·24	0·24
7	0·35	0·36
8	0·28	0·26
9	0·26	0·27
10	0·28	0·29

CONCLUSION

The automated procedure described here is ideally suited to carrying out large numbers of routine sulphate determinations. The values obtained with this method are, furthermore, in good agreement with the accepted standard procedure.

One of the authors (W.D.B.) thanks the South African Co-operative Citrus Exchange, in whose laboratories part of the work was done. The work was concluded with a grant from the Council for Scientific and Industrial Research.

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A Colorimetric Method for the Determination of 2-Phenylphenol Residues in Citrus Fruits*

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A colorimetric method for the determination of 2-phenylphenol residues in citrus fruit, based on a specific colour reaction given by 2-phenylphenol with sulphuric acid, trace amounts of formaldehyde and iron(III), is described. 2-Phenylphenol is extracted from the fruit with chloroform, transferred into an alkaline medium, and, after acidification of the latter, re-extracted with chloroform. The extract is purified with 86·3 per cent. w/w sulphuric acid and the 2-phenylphenol is then determined colorimetrically. The method permits the determination of small amounts of 2-phenylphenol of the order of 0·05, 0·3 and 0·1 p.p.m. in the pulp, peel and whole fruit, respectively. The recovery of 2-phenylphenol varies between 95 and 106 per cent.

2-Phenylphenol (2-hydroxybiphenyl) is used as a mould inhibitor for the post-harvest treatment of citrus fruits to reduce the incidence of rot in stored fruit. Various methods for determining 2-phenylphenol residues in citrus fruit have been reported and most of them reviewed.[1–8] The recoveries of 2-phenylphenol are not always satisfactory. The errors, of the order of ±15 per cent. and even higher,[1,3–5] seem to be caused essentially by the mode of extraction and by the method of determination used.

2-Phenylphenol is generally cyclically steam distilled from the fruit and trapped in an organic solvent. The extraction of 2-phenylphenol by this procedure is often incomplete even after prolonged distillation. The recovery of 2-phenylphenol depends upon several factors that are difficult to control[1] and varies from 65 to 72 per cent.[4] or from 80 to 100 per cent.[1] More consistent yields are obtained when the trapping solution is replaced after some time with fresh solution for further steam distillation.[1] Direct extraction of 2-phenylphenol from fruit peel by equilibration of the latter with a cyclohexane - isopropyl alcohol mixture permits a recovery of approximately 80 per cent.[4]

Whichever method is applied for determining 2-phenylphenol, ultraviolet spectrophotometry, gas - liquid chromatography or colorimetry, the extracts should previously be purified to eliminate as much as possible of the substances interfering in the determination. However, the purified extracts obtained from non-treated fruit may show a variable apparent 2-phenylphenol content,[1,3–8] because of a highly variable background caused by a natural compound with properties similar to those of 2-phenylphenol, which is difficult to eliminate without loss of 2-phenylphenol.[1,4,6] This compound, which is probably phenolic, gives the same ultraviolet absorption band and the same retention time as 2-phenylphenol, and reacts with some reagents used for colorimetric determination.[1,4–6] This apparent 2-phenylphenol content, which with some reagents may attain 0·8 p.p.m. in the whole fruit,[3] is not negligible when it is necessary to distinguish between treated and non-treated fruit, or to determine extremely small amounts of this compound in the fruit.

The procedure described in this paper permits extraction of 2-phenylphenol with satisfactory accuracy. Its determination is based on the specific colour reaction given by 2-phenylphenol with concentrated sulphuric acid, trace amounts of formaldehyde and iron(III).[9] The reaction is very sensitive and makes possible visual identification and quantitative determination of extremely small amounts of the compound in the fruit.

Method

Apparatus—

All glassware must be scrupulously clean and all joints made of ground glass.

* Contribution from the Volcani Institute of Agricultural Research, Bet-Dagan, Israel, 1970 Series, No. 1777-E.

Blenders—A Vir-Tis "45" homogeniser with a 500-ml stainless-steel container, and a Waring-type blender with a 1000-ml jar, were used.

Shaking machine.

Absorptiometer—A Hilger absorptiometer, Type H810, with 3 × $\frac{5}{8}$-inch tubes, was used.

Distillation apparatus—A round-bottomed 2-litre flask, condenser and connection tube (see Fig. 1, reference 10).

Concentration apparatus—Flat-bottomed 200 and 350-ml flasks with short necks with ground-glass stoppers, a Vigreux reflux column of effective length 15 cm, a condenser and a connection tube.

Test-tubes, 150 × 16 mm.

Graduated tubes—These were 10-ml tubes, graduated at 0·1-ml intervals, with ground-glass stoppers and adaptors for concentration (see Fig. 2, reference 9).

Micropipettes—Curved-tip 0·2-ml pipettes, graduated at 0·001-ml intervals.

Separating funnels, 75, 250, 500 and 1500-ml capacity.

REAGENTS—

All reagents should be of recognised analytical grade.

Standard 2-phenylphenol solution—Prepare as described previously.[9]

Acetic acid reagent—Prepare as described previously.[9]

Determine the formaldehyde content of the glacial acetic acid used and of the acetic acid reagent as follows.

Prepare a standard formaldehyde solution containing 16·6 mg of formaldehyde per litre of water, by mixing 1 ml of 37 per cent. formaldehyde solution with sufficient distilled water to give a volume of 100 ml; then dilute 4·5 ml of this solution to 1 litre with distilled water. Place 0·0, 0·05, 0·10 and 0·15 ml of the standard solution and 0·05, 0·10 and 0·15 ml of the solution to be analysed in separate test-tubes; add 5 ml of chromotropic acid reagent [500 mg of chromotropic acid dissolved in 100 ml of dilute sulphuric acid (4 + 2·8)] to the contents of each test-tube. Place the test-tubes in boiling water for 15 minutes, cool and determine the optical densities at 610 nm, with orange filter No. 61, using the first test-tube as a blank. According to the respective optical densities prepare the standard graph, which is a straight line, and determine the formaldehyde content of the analysed solution. Equal amounts of the acetic acid reagent and of the standard solution should have the same optical densities.

Sulphuric acid reagent—Prepare a stock solution of sulphuric acid containing 0·7 mg ml^{-1} of iron(III) as anhydrous iron(III) sulphate as described previously.[9]

Sulphuric acid, 86·3 per cent. w/w—Dilute 98 per cent. w/w sulphuric acid (4 + 1).

Chloroform (stabilised).

Sodium hydroxide solution, 5 per cent. w/v, aqueous.

Sodium sulphate, anhydrous.

Bone charcoal powder (that does not absorb 2-phenylphenol from chloroform solution)—Check the bone charcoal powder as follows: Add 1 to 2 g of bone charcoal powder to 100 ml of a chloroform solution of 2-phenylphenol of known concentration. Mix and filter through an adequate folded filter-paper (Whatman No. 2 folded filter-paper is suitable). The contents of 2-phenylphenol in the solution before and after treatment with charcoal bone powder should be the same.

Antifoam—Silicone MS Antifoam A (Hopkin & Williams) was used.

GENERAL PROCEDURE

2-Phenylphenol, which is localised mainly in the peel of the fruit, is determined separately in the peel and in the pulp, and calculated, as necessary, for the whole fruit. It is extracted from the peel directly by equilibration of the latter with chloroform; it is steam distilled from the pulp and then extracted from the distillate. The chloroform extract is concentrated and extracted with sodium hydroxide solution; the alkaline phase is then acidified and the 2-phenylphenol re-extracted with chloroform. The extract is treated with 86·3 per cent. w/w sulphuric acid to eliminate remaining substances that may interfere in the determination and the 2-phenylphenol is finally determined colorimetrically.

PREPARATION OF FRUIT EXTRACTS—

Sampling—For an average sample of fruit prepare 100 g of peel and 500 g of pulp.

Weigh, for example, ten fruits and remove their peels carefully to avoid contaminating the pulp with trace amounts of 2-phenylphenol from the peel; during peeling set aside from each fruit an average sample of 10 g of peel, to give a total of 100 g of peel. Weigh the pulps and calculate the weight of all the peels. Take an aliquot from each pulp to make altogether 500 g of pulp.

Preparation of peel sample—Chop the peel on a plate. Place the chopped peel in the 500-ml stainless-steel container of the homogeniser and add exactly 300 ml of chloroform. Locate the shaft with the cutting blades in the container. Weigh the latter with its contents and note the weight. Place the container in an ice-bath and mince the peels for 15 minutes at high speed to an impalpable purée. Do not remove the cutting blades from the container. Adjust, by adding chloroform, the weight of the container with its content to that previously noted to compensate for the loss of chloroform that may occur during mincing. Avoid any change in the volume of the extract during the subsequent operations. Transfer the chloroform extract through a fine sieve into a 500-ml separating funnel. Compress the residue slightly on the sieve to obtain the maximum amount of extract. Decant the chloroform phase into a ground-glass stoppered bottle and add sufficient anhydrous sodium sulphate to clarify the extract and a little bone charcoal powder that does not absorb 2-phenylphenol. Mix and filter the extract through an adequate folded filter-paper (Whatman No. 2). Cover the filtration funnel, to prevent possible evaporation of the chloroform, and collect the filtrate in a graduated cylinder. Note the volume of the filtrate (generally about 75 per cent. of the volume of chloroform used for the extraction), which should be clear. Each 3 ml of filtrate represents 1 g of peel taken for analysis. Transfer the filtrate quantitatively to a 350-ml flat-bottomed flask with a short neck, connect it to the condenser via the Vigreux column and concentrate the extract at atmospheric pressure to a volume of about 5 to 8 ml.

Preparation of pulp sample—Place 500 g of pulp in the 1000-ml jar of the Waring-type blender and blend it for 2 to 3 minutes. Transfer the mashed pulp to a 2-litre distillation flask, rinsing the blender with 500 ml of distilled water and adding the rinsings to the pulp. Add a little antifoam. Connect the distillation flask to the condenser (see Fig. 1, reference 10), which dips into a large glass container of 1250-ml capacity marked at 500 and at 1000 ml. Add a small amount of water to the container to immerse the tip of the condenser. Boil the contents of the flask vigorously, avoiding foaming and charring of the pulp that adheres to the walls of the flask.

After 500 ml of distillate have been collected, stop the distillation, add 500 ml of hot distilled water to the contents of the flask and continue the distillation until a total of 1000 ml of distillate have been collected. Carefully rinse the inner tube and the tip of the condenser with 10 ml of chloroform and collect the rinsings in the distillate. Transfer the distillate to a 1500-ml separating funnel, rinse the container twice with 10 ml of chloroform, add the rinsings to the funnel and shake the funnel for 2 to 3 minutes. Carefully decant off the chloroform phase and extract twice more with 30 ml of chloroform. Collect the chloroform phases in a 100-ml calibrated flask and make the volume up to the mark with chloroform. Add sufficient anhydrous sodium sulphate to clarify the extract and a little bone charcoal powder. Mix and filter the extract as described above. Note the volume of the filtrate; each 1 ml represents 5 g of pulp taken for the analysis. Transfer the filtrate quantitatively to a 200-ml flat-bottomed flask and concentrate as above to about 5 to 8 ml.

Separation of 2-phenylphenol from the bulk of other extractives—The chloroform extract is extracted three times with sodium hydroxide solution in order to transfer the 2-phenylphenol to an alkaline medium, which is then acidified and the 2-phenylphenol re-extracted with chloroform.

Place successively, one beneath the other, three separating funnels, one of 250-ml and two of 75-ml capacity. Transfer the concentrated extract quantitatively to the upper 75-ml separating funnel. Rinse the flat-bottomed flask with 25 ml of 5 per cent. w/v sodium hydroxide solution, add the rinsings to the contents of the separating funnel and shake the funnel for 2 to 3 minutes (gently, to avoid the formation of an emulsion). Carefully run the chloroform phase into the lower 75-ml separating funnel. Rinse the alkaline phase with two portions of 1·5 ml of chloroform and add the rinsings to the chloroform phase. Transfer the alkaline phase to the 250-ml separating funnel. Extract the chloroform phase twice more as follows. Replace the 75-ml separating funnels so that the empty funnel is beneath that containing the chloroform phase. Rinse the flat-bottomed flask and the empty 75-ml

separating funnel with 25 ml of 5 per cent. w/v sodium hydroxide solution. Add the rinsings to the chloroform phase and continue as above, beginning with " . . . shake the funnel for 2 to 3 minutes." After the third extraction discard the chloroform phase. Rinse the combined alkaline phases with three portions of 3 ml of chloroform. Collect the rinsings in a 75-ml separating funnel and extract once with 25 ml of 5 per cent. w/v sodium hydroxide solution, discarding the rinsings. Add the alkaline phase to those collected previously and acidify the combined phases with 5 ml of 86·3 per cent. w/w sulphuric acid. Cool, and extract the acidified solution three times with about 8 ml of chloroform. Collect the chloroform extracts in a 25-ml calibrated flask and make the volume up to the mark with chloroform. (For routine analysis the acidified solution can be extracted once with exactly 25 ml of chloroform.)

Clean-up of interfering substances—Transfer the chloroform extract to a 75-ml separating funnel, add 5 ml of 86·3 per cent. w/w sulphuric acid and shake the mixture mechanically for 5 minutes. Separate the acidic layer carefully and repeat the operation until two consecutive acidic layers remain colourless after shaking and separation. Four extractions are generally sufficient for peel extracts, and five or six for pulp extracts. Wash the purified chloroform extract with 15 to 20 ml of distilled water. Run off the extract, add to it a small amount of anhydrous sodium sulphate, filter and keep the extract in a glass-stoppered flask.

DETERMINATION OF 2-PHENYLPHENOL—

The colour reaction has been described in detail previously.[9] 2-Phenylphenol produces a green colour with concentrated sulphuric acid in the presence of iron(III), and a pink colour in the presence of trace amounts of formaldehyde and iron(III), which is a specific reaction for 2-phenylphenol.

The production of the pink colour results from the quantitative reaction of 2-phenylphenol with formaldehyde, so that for a given amount of 2-phenylphenol the colours produced with increasing amounts of formaldehyde vary, according to the ratio (w/w) of formaldehyde to 2-phenylphenol, and change in the sequence green, greyish pink, pinkish yellow, pink, purple, greyish purple. The colour intensities measured as described below increase rapidly and, after reaching a maximum, which coincides with the production of the pink colour, decrease slowly. The characteristic pink colour is produced and the maximum intensity is attained when the ratio (w/w) of formaldehyde to 2-phenylphenol is between about 0·10:1 and 0·25:1. The intensity of the colours produced with very small amounts of 2-phenylphenol is not affected by an increase in the ratio of formaldehyde to 2-phenylphenol between 0·25:1 and 3·0:1. To ensure instantaneous development of full colour intensity, the chloroform solution containing 2-phenylphenol should first be mixed with the acetic acid reagent in proportions not exceeding 0·1 ml of chloroform per millilitre of the mixture; then the sulphuric acid reagent should be added to the mixture.

PREPARATION OF STANDARD GRAPH—

Prepare a standard graph as described previously.[9]

The Beer - Lambert law is obeyed up to 16 μg of 2-phenylphenol in 5 ml of reagent. The standard graph is reproducible. The intensity of the colour, measured over 12 hours, was found to be stable.

PREPARATION OF THE MIXTURE OF FRUIT EXTRACT WITH ACETIC ACID REAGENT—

One millilitre of the mixture should contain not more than 0·1 ml of chloroform and not more than 80 μg of 2-phenylphenol. To prepare the mixture appropriately, determine the approximate concentration of 2-phenylphenol in the extract. Place 0·01-ml portions of the extract at the bottom of three test-tubes, and add in turn 0·09, 0·14 and 0·19 ml of acetic acid reagent. Mix, then add 5 ml of sulphuric acid reagent to the contents of each tube, as described for the preparation of the standard graph. Measure the optical densities. Three conditions may be observed, as follows.

(*i*) *The colours produced in the three test-tubes are pink, with about the same optical densities*—Examples are extracts from peels containing approximately 20 to 600 p.p.m. of 2-phenylphenol and from pulps containing more than 3 p.p.m. The extract is suitable for the direct preparation of the mixture with acetic acid reagent. Determine from the standard graph the approximate concentration of 2-phenylphenol in the extract. Place in a 10-ml graduated tube the required amount of extract, not exceeding 1 ml and containing not more than 800 μg of 2-phenylphenol, make the volume up to 10 ml with the acetic acid reagent and mix.

If the approximate 2-phenylphenol concentration is very low (less than 160 μg ml^{-1}) it is advisable to concentrate the extract in order to increase the accuracy of the dosage. To achieve this, place exactly 5 ml of the extract in a 10-ml graduated tube, add 1 ml of the acetic acid reagent (to prevent loss of 2-phenylphenol during concentration), close the tube with the concentration adaptor (see Fig. 2, reference 9), and concentrate the solution carefully to a volume of 1 ml under reduced pressure, by maintaining in water at 50 to 60 °C the portion of the tube containing the solution. Rinse the inside of the adaptor and the capillary with a few drops of acetic acid reagent and collect the rinsings in the graduated tube. According to the approximate amount of 2-phenylphenol in the concentrated mixture, up to 400 μg, or from 400 to 800 μg, make the volume up to 5 or 10 ml, respectively, with the acetic acid reagent and mix.

(*ii*) *The colours vary or their optical densities, or both, increase with increased amounts of acetic acid reagent*—The extract is too concentrated and must be diluted. Dilute suitably an aliquot of the extract with chloroform or acetic acid reagent to obtain the results indicated in (*i*) and proceed as in (*i*).

(*iii*) *The colours are not discernible visually*—The extract is too dilute. To determine the approximate concentration of 2-phenylphenol in the extract, the latter should be concentrated. To achieve this place 1 ml of the extract in a 10-ml graduated tube, add 1 ml of the acetic acid reagent and concentrate the mixture to 1 ml as described under (*i*). Place 0·1 to 0·4-ml portions of the concentrated mixture in test-tubes and develop the colours with 5 ml of sulphuric acid reagent. Measure the optical densities. Calculate the approximate concentration of 2-phenylphenol in the extract, and the amount of the extract to be concentrated so as to prepare 2 to 5 ml of the mixture with acetic acid reagent. To concentrate the required amount of the extract, proceed gradually. Place a maximum of 5 ml of the extract in a 10-ml graduated tube, add 1 ml of the acetic acid reagent and concentrate the mixture to 1 ml, as described above. Add a second fraction of 5 ml of the extract through the inlet, rinse with a few drops of chloroform, concentrate the solution to 1 ml and continue until the required amount of the extract has been concentrated to 1 ml. Reduce slightly the volume to eliminate the remainder of the chloroform. Rinse the adaptor with the acetic acid reagent as above and make the mixture up to the required volume with the acetic acid reagent and mix.

DETERMINATION OF 2-PHENYLPHENOL—

Place in turn 0·05, 0·10 and 0·15 ml of the acetic acid reagent mixture (or 0·2, 0·3 and 0·4 ml if the colours are weak) at the bottom of three test-tubes and develop the colours with 5 ml of sulphuric acid reagent. Measure the optical densities. The colours should be clear pink and the optical densities should increase in proportion to the amounts of the mixture taken for the determination. Determine the amount of 2-phenylphenol in 0·1 ml of the mixture. Calculate the 2-phenylphenol content in the peel and in the pulp taken for analysis as follows—

$$\text{2-Phenylphenol content in the peel} = \frac{a \times 10 \times b \times 25 \times 3}{c \times d} \text{ p.p.m.}$$

$$\text{and in the pulp} = \frac{a \times 10 \times b \times 25}{c \times d \times 5} \text{ p.p.m.}$$

where a μg is the amount of 2-phenylphenol in 0·10 ml of mixture, b ml is the volume of prepared mixture, c ml is the volume of extract taken for the preparation of the mixture and d ml is the volume of filtrate taken for the concentration.

Calculate the 2-phenylphenol content in the whole fruit.

DISCUSSION

SENSITIVITY—

The method permits the determination of small amounts of 2-phenylphenol of the order of 0·05, 0·30 and 0·10 p.p.m. in the pulp, the peel and the whole fruit, respectively. The sensitivity can be increased by using larger samples of peel or pulp, or by preparing from the concentrated extract less than 2 ml of the mixture with the acetic acid reagent.

TABLE I

RECOVERY OF 2-PHENYLPHENOL ADDED TO PEELS OF NON-TREATED FRUITS

Sample (100 g of citrus fruit peel)	2-Phenylphenol added/mg	2-Phenylphenol found/mg	Recovery, per cent.
Shamouti oranges	0	0	—
	0·064	0·068	106·2
	0·40	0·404	101·0
	4·00	3·83	95·7
	20·0	19·6	97·8
Valencia oranges	0	0	—
	0·16	0·16	100·0
	1·60	1·57	98·1
	10·0	9·6	96·0
Grapefruit	0	0	—
	0·16	0·166	103·7
	2·40	2·28	95·0
	10·0	10·0	101·0

ACCURACY—

2-Phenylphenol has been determined in the peel (Table I) and pulp (Table IV) of untreated fruit to which known amounts of the compound had been added, and in several samples originating from the same mixture of finely chopped peel of treated fruit (Table II, see also Table III). The results show that the deviations are slight. The recovery of 2-phenylphenol varies between 95·0 and 106·2 per cent. for the peels and between 96·0 and 106·2 per cent. for the pulp of the fruit.

TABLE II

DETERMINATION OF 2-PHENYLPHENOL IN PEELS OF TREATED FRUITS

100-g samples taken from the same mixture of finely chopped peel

Sample No.	1	2	3	4	Average
2-Phenylphenol found/mg	0·219	0·220	0·211	0·214	0·216
Deviation from average value/ mg ..	+0·003	+0·004	−0·005	−0·002	
Deviation from average value, per cent.	+1·4	+1·8	−2·3	−0·9	

VALIDITY—

Extraction of 2-phenylphenol by equilibrating the peel with the solvent—The use of chloroform ensures a satisfactory and rapid extraction of 2-phenylphenol from the peel by equilibrating[11] the minced peels with the solvent. The time of equilibration, which varied from 15 minutes to 48 hours, had no effect on the amount of 2-phenylphenol extracted (Table III).

2-Phenylphenol can also be extracted from the whole fruit by equilibration with the solvent, as described for the peels. The extraction, by equilibration, of 2-phenylphenol from the pulp of the fruit carried out as recommended by Gunther,[11] was not always easy to perform because intractable emulsions formed with the pulp and the chloroform.

Extraction of 2-phenylphenol by distillation—At least 75 to 80 per cent. of 2-phenylphenol added to water or to the pulp of the fruit was recovered in the first fraction of the distillate when its volume reached approximately half that of the mixture prepared for distillation (Table IV); 96 to 106 per cent. of the 2-phenylphenol was recovered in the two or three successive fractions of the distillate (Table IV). The addition of acetic acid to the mixture, or of 2-phenylphenol as its sodium salt, had no effect on the recovery of 2-phenylphenol.

The determination of 2-phenylphenol in the third fraction of the distillate of the pulps (Mode B) was often difficult to perform. The purified extract contained in most instances residues of interfering substances, which were difficult to eliminate by clean-up with 86·3 per cent. w/w sulphuric acid and which were probably formed from the components of the pulp during prolonged boiling.

To detect and determine the 2-phenylphenol it was, in general, necessary to concentrate these extracts greatly and to make the volume of the mixture with the acetic acid reagent up to a volume not exceeding 0·5 ml. In certain instances, on adding the sulphuric reagent, a slightly yellowish colour developed, which was caused by the residues of interfering substances and made it difficult to detect the presence of 2-phenylphenol.

TABLE III

EFFECT OF THE MODE OF EXTRACTION ON THE RECOVERY OF 2-PHENYLPHENOL FROM PEELS OF TREATED FRUITS

100-g samples taken from the same mixture of finely chopped peel

Mode of extraction	2-Phenylphenol found in samples from mixture/mg				Deviation from average value for mixture, per cent.			
	No. 1	No. 2	No. 3	No. 4	No. 1	No. 2	No. 3	No. 4
A	3·79	7·82	0·448	0·240	+4·1	+3·2	+0·9	+2·1
B	3·67	7·62	0·440	—	+0·8	+0·6	−0·9	—
C	3·40	7·36	—	0·230	−6·6	−2·8	—	−2·1
D	3·70	7·51	—	—	+1·6	−0·8	—	—
Average	3·64	7·58	0·444	0·235				

A, Minced for 15 minutes.
B, As in A, and shaken mechanically for 15 minutes.
C, As in A, and stored for 24 hours.
D, As in A, and stored for 48 hours.

Clean-up with 86·3 per cent. w/w sulphuric acid—The chloroform extracts of the acidified alkaline phases may contain residues of substances that interfere in the colorimetric determination of 2-phenylphenol. These products, which yield a yellow coloration with the reagents used for the determination of 2-phenylphenol, might mask to some extent the pink colour and affect the optical density measurements, depending on their concentrations in the acetic acid reagent mixture prepared for the analysis. Concentrated sulphuric acid has been used by several workers for the clean-up of citrus fruit extracts in spectrophotometric and colorimetric determinations of biphenyl.[12] The use of concentrated sulphuric acid may cause a loss

TABLE IV

RECOVERY OF 2-PHENYLPHENOL BY DISTILLATION

Sample	Mode of distillation	2-Phenylphenol added/mg	Found in successive fractions of distillate: mg 1st	mg 2nd	mg 3rd	per cent. of added 1st	per cent. of added 2nd	per cent. of added 3rd	Total found, per cent. of added
300 ml of water	A	0·567	0·456	0·114	—	80·4	20·1	—	100·5
		0·628	0·514	0·114	—	81·8	18·2	—	100·0
		2·85	2·14	0·714	—	75·1	25·0	—	100·1
		4·44	3·77	0·610	—	84·9	13·7	—	98·6
1000 ml of water	B	0·320	0·304	0·020	0	95·0	6·2	—	101·2
		4·00	3·40	0·500	0·157	85·0	12·5	3·9	101·4
500 g of citrus fruit pulp and 500 ml of water—	B								
Valencia oranges		0	0	0	0	0	0	0	0
Shamouti oranges		0	0	0	0	0	0	0	0
Grapefruit		0	0	0	0	0	0	0	0
Valencia oranges		0·080	0·085	0	—	106·2	0	—	106·2
Grapefruit		0·160	0·135	0·024	0·002	84·4	15·0	1·2	100·6
		0·320	0·262	0·070	n.d.	81·9	21·9	—	103·8
Valencia oranges		0·160	0·155		Trace	97·1		—	97·1
		0·320	0·311		n.d.	97·2		—	97·2
Shamouti oranges		0·160	0·166		n.d.	103·7		—	103·7
		0·220	0·221		Trace	100·4		—	100·4
Grapefruit		0·160	0·156		Trace	97·5		—	97·5
Grapefruit		1·60	1·357			96·0			96·0
		4·00	3·986			99·6			99·6

A, Two fractions distilled of 120 to 140 ml each.
B, Three fractions distilled of 500 ml; after the 1st and 2nd fractions, 500 ml of water were added to the sample.
n.d., Not detected.

of biphenyl.[12] Dilute 95·2 per cent. w/w sulphuric acid has been found convenient for the clean-up of the extracts in the colorimetric determination of biphenyl with sulphuric acid, trace amounts of formaldehyde and iron(III) as reagent.[10] No loss of biphenyl occurs, but 2-phenylphenol disappears rapidly during the treatment.[10]

Because of the relatively small amounts of interfering substances remaining in the chloroform extracts of the acidified alkaline phases, the possibilities of clean-up of these extracts with more dilute sulphuric acid and the effect of the sulphuric acid acting on 2-phenylphenol under various conditions were investigated. It was found that it is possible to purify these extracts quickly with more dilute sulphuric acid; and that, as with the loss of biphenyl,[10] the loss of 2-phenylphenol increases with the ratio (v/v) between the sulphuric acid and chloroform solutions and also with the time of shaking, and decreases with increasing dilution of the sulphuric acid (Table V). The 86·3 per cent. w/w sulphuric acid chosen provides a quick clean-up procedure, without the risk of loss of 2-phenylphenol.

Table V

Effect of sulphuric acid on loss of 2-phenylphenol

2-Phenylphenol dissolved in chloroform (800 μg ml^{-1}) treated by shaking with sulphuric acid

Sulphuric acid (90 per cent. w/w) diluted	Sulphuric acid content, per cent. w/w	Ratio (v/v) of sulphuric acid to chloroform solution	Loss of 2-phenylphenol, per cent. after shaking time		
			1 hour	4 hours	7 hours
6 + 1	89·9	1:1	37	50	85
		1:3	18	28	61
5 + 1	88·4	1:1	0	19	31
		1:3	0	11	23
4·5 + 1	87·4	1:1	0	0	10
		1:3	0	0	6
4 + 1	86·3	1:1	0	0	0
		1:3	0	0	0
3 + 1	83·0	1:1	0	0	0
		1:3	0	0	0

With extracts containing sufficient amounts of 2-phenylphenol, and which can be mixed with the acetic acid reagent without previous concentration, the concentration of the interfering substances in the mixture is generally too low to interfere in the determination. The treatment of these extracts with sulphuric acid can be omitted in most instances. On the other hand, extracts that must previously be concentrated, often 100 times or more, should be purified carefully with sulphuric acid. It should be noted that Mestres and co-workers[4,6] used dilute sulphuric acid (2 + 1) to reduce the natural interference in the determination of 2-phenylphenol by gas chromatography, and by colorimetry with 4-aminophenazone as reagent.

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Detection of Chloramine T in Minced Meat and Farinaceous Foods

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The phenothiazine method for the detection of chloramine T in dairy products was modified for application to minced meat and farinaceous foods. A hydrochloric acid extract of the material is treated with Carrez reagent to precipitate the proteins. The acidic solution is extracted with diethyl ether and the ethereal solution is purified with sodium hydrogen carbonate - potassium permanganate solution. Shaking the ethereal solution with phenothiazine and sodium hypochlorite reagents yields a violet reaction product when chloramine T is present. The limit of detection is about 25 p.p.m. of chloramine T.

In a previous paper[1] the detection of chloramine T (the sodium salt of *N*-chlorotoluene-*p*-sulphonamide), added to dairy products as a preservative, was described. Because this disinfectant is also intensively used in plant sterilisation in the food industry, attempts have been made to apply this method to minced meat and farinaceous foods also. The method had to be modified drastically, however, to eliminate the effects caused by strongly interfering (reducing) substances in the meat.

For the reactions between chloramine T and phenothiazine and between toluene-*p*-sulphonamide and phenothiazine - hypochlorite mixture, reference can be made to the previous paper,[1] in which it was also reported that a bacteriostatic effect is obtained only when at least 50 mg of chloramine T per kilogram of material is used.

Experimental

Standard determinations on minced meat—

Determinations were carried out on 10-g samples of minced meat to which known amounts of chloramine T had been added. The material was stirred for 10 minutes after the addition of 60 ml of 0·1 N hydrochloric acid and then immediately filtered through a nylon tea-strainer. The filtrate was collected in a 50-ml centrifuge tube. At this stage various amounts of Carrez reagents I and II were added in a series of determinations. The best results were obtained when 1·5-ml amounts of both reagents were used. It is necessary to stir the mixture for 1 minute between additions. The solutions were then centrifuged for 15 minutes at 3000 r.p.m. and the clear upper layer was immediately decanted into a 100-ml separating funnel and shaken with 30 ml of diethyl ether. Vigorous shaking was avoided to prevent gel formation. The ethereal layer was washed twice with 5-ml volumes of distilled water.

At this stage of the determination a series of experiments was carried out with various amounts of sodium hydrogen carbonate and 0·1 N potassium permanganate solutions to determine the best conditions. It appeared that addition of 4 ml of a 2 per cent. sodium hydrogen carbonate solution and 0·5 ml of 0·1 N potassium permanganate solution yielded the best results, particularly with the lower concentrations of chloramine T. After shaking the mixture occasionally for about 3 minutes the bottom layer was removed and the ethereal layer washed twice with 5-ml volumes of distilled water. Various amounts of phenothiazine and sodium hypochlorite were then added. It appeared that 0·5 ml of each of the reagents (*i.e.*, 0·3 per cent. of phenothiazine in 96 per cent. ethanol and 0·3 per cent. of available chlorine in water) gave the best results. After shaking the solution occasionally a violet product was obtained, the intensity of which increased with higher concentrations of chloramine T. With 250 p.p.m. it takes about 3 minutes for the colour to form, whereas with 25 p.p.m. it takes about 90 minutes. The colour, once it has been formed, remains stable for at least several hours.

STANDARD DETERMINATIONS ON FARINACEOUS FOODS—

Standard determinations on farinaceous foods, such as spaghetti, rice and macaroni products, to which known amounts of chloramine T had been added, by the above method as used for minced meat yielded the same results.

METHOD

REAGENTS—

Carrez I—Dissolve 10·6 g of potassium hexacyanoferrate(II), $K_4Fe(CN)_6.3H_2O$, in 100 ml of distilled water.

Carrez II—Dissolve 22·0 g of zinc acetate, $Zn(CH_3COO)_2.2H_2O$, and 3 g of glacial acetic acid in 100 ml of distilled water.

Hydrochloric acid, 0·1 N—Analytical-reagent grade.

Sodium hydrogen carbonate solution—A 2 per cent. w/v solution in distilled water.

Potassium permanganate solution, 0·1 N—Analytical-reagent grade.

Diethyl ether—Analytical-reagent grade.

Ethanol, 96 per cent.—Analytical-reagent grade.

Phenothiazine reagent—Prepare daily a fresh 0·3 per cent. w/v solution of technical grade phenothiazine (B.D.H. Ltd.) in 96 per cent. ethanol.

Sodium hypochlorite reagent—Prepare daily a fresh solution containing about 0·3 per cent. of available chlorine.

Chloramine T standard solution, 1 mg ml^{-1}—Dissolve 100 mg of chloramine T in 100 ml of 0·1 N hydrochloric acid.

PROCEDURE—

To 10 g of ground material in a 150-ml beaker add 60 ml of 0·1 N hydrochloric acid. Stir the mixture with a stirring rod for 10 minutes and filter it through a nylon tea-strainer into a 50-ml centrifuge tube. Add 1·5 ml of Carrez I reagent. Stir the mixture, add 1·5 ml of Carrez II reagent after 1 minute, then stir for 1 minute. Centrifuge it for 15 minutes at 3000 r.p.m. Decant the clear solution immediately into a 100-ml separating funnel and shake it gently with 30 ml of diethyl ether. Wash the ethereal layer twice with 5 ml of distilled water. Next, add 4 ml of 2 per cent. sodium hydrogen carbonate solution and 0·5 ml of 0·1 N potassium permanganate solution. Shake the mixture occasionally for about 3 minutes. When the lower layer shows no pink coloration add more permanganate solution. Drain off the lower layer and wash the ethereal layer twice with 5 ml of distilled water. Add 0·5 ml of each of the phenothiazine and hypochlorite reagents and shake. The appearance of a violet reaction product in less than 3 minutes indicates a chloramine T content of at least 250 p.p.m. With lower concentrations the time required is longer and, although the reaction is enhanced by occasional shaking, at the lowest concentration of about 25 p.p.m. it may require about 90 minutes for the colour to be produced.

CONCLUSIONS

The method is not specific, as other sulphonamides also give this reaction. However, because of their use as pharmaceuticals, sulphonamides are not expected to be present in foods. The original method had to be modified drastically in order to eliminate the interfering substances in minced meat and farinaceous foods. The presence of too much Carrez reagent caused losses of chloramine T by adsorption. Too little, however, led to gel formation in the ethereal extract. Excess of potassium permanganate also has a disadvantageous effect on the detection of chloramine T.

After centrifugation the clear solution should be decanted immediately, otherwise difficulties may arise because of the diffusion of undesirable substances from the precipitate to the upper layer. For the same reason the material should not remain in the hydrochloric acid solution for longer than 10 minutes. This method is rapid and therefore particularly suitable for series analysis.

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The Determination of Acrylamide in Water by Using Electron-capture Gas Chromatography*

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A method is described for the determination of residual acrylamide in water at levels likely to arise from the use of acrylamide polymers and copolymers in water treatment. After bromination of the sample, the α,β-dibromopropionamide obtained is extracted with diethyl ether and the extract analysed by electron-capture gas chromatography. Replicate samples of acrylamide added to River Thames water at concentrations of 500, 50, 5 and 0·25 μg l^{-1} were analysed with relative standard deviations of ± 5, ± 5, ± 9 and ± 10 per cent., respectively.

A detection limit of 0·1 μg l^{-1} of acrylamide was achieved with River Thames water and 0·25 μg l^{-1} was determined with a relative standard deviation of ± 10 per cent. Yields of α,β-dibromopropionamide vary with water quality, ultraviolet light intensity and other reaction conditions. To overcome effects caused by these variables, when present, an internal standardisation procedure is used. The detection limit in River Thames water remained at 0·1 μg l^{-1} but with this procedure 0·25 μg l^{-1} was determined with a relative standard deviation of ± 20 per cent.

THE water industry is making increasing use of polyelectrolytes in water clarification and sludge treatment. Many of these materials are derived from acrylamide. Polyacrylamide is itself non-toxic but its monomer, acrylamide, has a high chronic toxicity[1] and therefore its concentration in food and drink must be limited to very low levels.[2]

The Ministry of Housing and Local Government set up a committee in 1966 to assess the possible hazards of new chemicals used in water treatment. One of its problems was to consider the use of polyacrylamide coagulant aids and the residual acrylamide monomer in the polymers. The committee therefore determined a permissible level of acrylamide in water. In the absence of a suitable method for the determination of acrylamide in water at levels considered safe, it was necessary to specify the acrylamide content of polymers and to limit the amounts of polymer used so as to ensure that the permissible level of acrylamide monomer in water was not exceeded.

This committee reports from time to time and considers individually each polymer submitted to it. Typical clearance conditions,[3] however, are that if no batch of polymer contains more than 0·05 per cent. of acrylamide, then the average polymer dose should not exceed 0·5 mg per litre of water and the maximum dose should not exceed 1·0 mg l^{-1}. Under these conditions the maximum amount of acrylamide likely to be found in the water would be 0·5 μg l^{-1} and the maximum average content, 0·25 μg l^{-1}. Levels likely to be found in sludge drainage water, and in effluents from other sources, such as gravel or coal washing, in which polyacrylamides are used, could be considerably higher as large doses of products that do not necessarily conform to the specification required for potable water grade polymers may be used for these applications. However, in order to determine acrylamide at the levels likely to be found in potable water, a method of analysis is required which will enable down to at least 0·25 μg of acrylamide per litre of water to be determined.

As no methods capable of determining acrylamide at these very low levels existed, work was undertaken to develop a suitable technique so that the behaviour of acrylamide in water systems could be investigated. A method is described that is capable of determining down to 0·1 μg of acrylamide per litre of water and involves a bromination reaction followed by extraction with an organic solvent and final determination by electron-capture gas chromatography.

* This paper is based on Water Research Association Technical Paper TP.78.

EXPERIMENTAL

α,β-DIBROMOPROPIONAMIDE—

It was considered that bromination of acrylamide in aqueous solution to form α,β-dibromopropionamide would give two distinct advantages for microanalysis. Firstly, the brominated material should have excellent sensitivity towards the electron-capture detector, and secondly, it should be more easily extracted than acrylamide from water with organic solvents. A combination of these features offered the basis for the development of a rapid and relatively simple method for the microdetermination of acrylamide in water. α,β-Dibromopropionamide was synthesised as outlined under Method and its melting-point (132 °C) was in good agreement with that given in the literature.[4] Because the melting-point was the only information available by which the identity of the product of bromination of acrylamide could be confirmed to be α,β-dibromopropionamide, the product was further identified by elemental analysis (C 15·8, H 2·1, Br 69·6, N 5·7 per cent.; calculated for $C_3H_5Br_2NO$: C 15·6, H 2·2, Br 69·3, N 6·1 per cent.), infrared spectroscopy, nuclear magnetic resonance spectroscopy, mass spectrometry and, finally, by independent synthesis via α,β-dibromopropionic acid.

Although this material chromatographed well with the columns and under the conditions used previously for the determination of acrylamide in polyacrylamides,[5] and appeared to be more than 99 per cent. pure under these conditions, the 20 per cent. Carbowax 20M column showed excessive bleed rates at 180 °C for satisfactory operation of the electron-capture detector. A glass column, 1 m long by 3 mm i.d., packed with 60 to 80-mesh acid-washed DMCS-treated Chromosorb W supporting 10 per cent. by weight of FFAP (Carbowax 20M terminated with 2-nitroterephthalic acid) and operated at 180 °C with a nitrogen flow-rate of 25 ml min^{-1}, was found to be more suitable. Under these conditions, α,β-dibromopropionamide had a retention time of 6·5 minutes and approximately 40 pg (40×10^{-12} g) gave a recorder deflection of half full scale at the maximum usable sensitivity of the chromatograph; that is, when the base-line noise was about 0·5 per cent. of full-scale deflection. Straight-line calibration graphs were obtained for up to 300 pg of α,β-dibromopropionamide when $\log_{10}$ peak areas were plotted against $\log_{10}$ amounts of α,β-dibropropionamide injected. Acrylamide showed very poor sensitivity towards the electron-capture detector.

EXTRACTION OF α,β-DIBROMOPROPIONAMIDE FROM WATER—

α,β-Dibromopropionamide proved to be much more readily extracted from water with organic solvents than was acrylamide. Of the solvents tested, diethyl ether was the most suitable, as it combined good extraction efficiency with ease of evaporation down to the final concentration before gas-chromatographic analysis. Extraction of 4 μg l^{-1} solutions of α,β-dibromopropionamide in distilled water with an equal volume of diethyl ether gave 74 per cent. recovery of the amide. The extraction was pH dependent, giving poor recoveries at values below pH 3·0. Recoveries were identical beween pH 3·0 and 10·0, but pH 3·0 was preferred for the extraction because of the rapid decomposition of α,β-dibromopropionamide in alkaline solution. Although the amide was stable at pH 10·0, very rapid decomposition occurred at higher pH, for instance, severe losses could be incurred by adjusting the pH of amide solutions with concentrated sodium hydroxide solution. The momentary rise in pH at the zone of addition of the alkali was sufficient to decompose the α,β-dibromopropionamide in this zone. Successive extractions of 100 ml of a 4 μg l^{-1} aqueous solution at pH 3·0 with 100, 50 and 50-ml volumes of diethyl ether gave 87 per cent. recovery of α,β-dibromopropionamide. It was possible to concentrate solutions of α,β-dibromopropionamide in diethyl ether up to 100 times without loss of the amide by careful evaporation in a stream of dry air at room temperature. The degree of concentration did not give rise to any interferences resulting from impurities in the solvent.

Ethyl acetate was a more efficient extraction solvent than diethyl ether but was more difficult to purify for electron-capture gas chromatography. It could not be concentrated to the degree required for the analysis. Recoveries of 82 per cent. could be obtained by saturating the aqueous layer (100 ml) with sodium sulphate and extracting once with 25 ml of ethyl acetate. Unfortunately, this technique could not be used for concentrations of acrylamide below 1 μg l^{-1} because of the presence in the solvent and sodium sulphate of materials that interfered in the analysis. However, it was a more convenient method of extraction when levels of acrylamide below 1 μg l^{-1} were not of interest.

BROMINATION OF ACRYLAMIDE RESIDUES IN AQUEOUS SOLUTION—

Initially, attempts were made to brominate aqueous solutions of acrylamide at the 30 μg l^{-1} level by using the same reagents and conditions as those used in the preparation of α,β-dibromopropionamide. Yields of α,β-dibromopropionamide were disappointingly low (3 per cent.). When the reaction was performed in daylight, yields improved dramatically, thus showing light to have a major influence on the reaction. The effect of pH was also shown to be a major factor affecting yields of α,β-dibromopropionamide; yields at pH 0·5, 1·0, 2·0 and 5·0 were 49, 62, 41 and 13 per cent., respectively. At pH 1·0, therefore, the yield was at a maximum. Several other factors concerning the brominating agent and its method of addition had minor effects on product yield and the best conditions found consisted in using 4 ml of saturated bromine water for every 100 ml of water sample, the reagent being added quickly. Dropwise addition of the reagent gave poorer yields of α,β-dibromopropionamide. The use of sodium sulphite to stop the bromination reaction gave fewer analytical interferences than sodium thiosulphate.

It was noticed that the yields of α,β-dibromopropionamide varied with the intensity of the daylight for a given reaction time. Irradiation with an ultraviolet lamp was therefore investigated in an attempt to standardise these conditions. It was found that correct use of the ultraviolet irradiation gave yields as good as those obtained in daylight. The yields were consistent over a few days, but if constant reaction conditions were used the yields varied slightly as the lamp aged. Alteration of these conditions restored the yields and a procedure was evolved for optimising the irradiation conditions. It was found that too much or insufficient irradiation gave poor results and it seems that too much decomposes the α,β-dibromopropionamide.

The influence of light on the yields of α,β-dibromopropionamide suggested that formation of this compound occurred via a free-radical reaction mechanism. Experiments in the dark with mercury(II) salts and halogen carriers as catalysts gave poor yields and strengthened this hypothesis. It would appear that although stoicheiometric uptake of bromine is obtained under ionic conditions,[6] the major product of this reaction is not α,β-dibromopropionamide. The initial step in the ionic addition is attack by Br^+ on the molecule. It is possible, therefore, that in an aqueous environment the secondary attack would be by OH^- ions, and not Br^- ions, and would therefore give much reduced yields of α,β-dibromopropionamide but still give stoicheiometric uptake of bromine.

It was found that the maximum yields of α,β-dibromopropionamide attainable fell as the amount of acrylamide in solution decreased. Yields were 50 to 66 per cent. at levels between 1 and 500 μg l^{-1}, but fell sharply below 1 μg l^{-1} to 34 per cent. at 0·25 μg l^{-1}. Thus, recovery factors determined on dissimilar standards can be used to calculate acrylamide levels in samples with reasonable accuracy above 1 μg l^{-1} but not below that level. Below 1 μg l^{-1}, recoveries must be calculated from similar standards. Because some variation in yield with the type of water being analysed was noticed the standards should be analysed in the same water as the sample.

A type of internal standardisation procedure was therefore developed in which a sample was divided into halves, and one half analysed to determine acrylamide. The use of previously determined recovery factors then permitted an approximate determination of the acrylamide in the sample to be made. An equivalent concentration of acrylamide to that calculated was then added to the second sample to give a level approximately double that in the original sample. Analysis of the "spiked" sample enabled a recovery factor to be calculated that gave excellent results, despite its concentration being double that of the sample. The calculation used assumed the recovery to be constant over the concentration range encountered.

ANALYSIS OF RIVER THAMES WATER—

Some analytical interferences arose at the 0·25 μg l^{-1} level when analyses of River Thames water were made. These interferents could not be completely separated from α,β-dibromopropionamide on other gas - liquid chromatographic columns (10 per cent. Uncon 50-HB-2000, 10 per cent. Triton X-305) that give good separations of amides by chromatography. Neither could they be removed by simple column clean-up techniques with alumina, silica gel or Florisil. They could, however, be reduced to acceptable levels by a single extraction

with diethyl ether at pH 1·0 before extraction of the α,β-dibromopropionamide at pH 3·0. Under these conditions, recoveries of α,β-dibromopropionamide were reduced by only 1 or 2 per cent. The final analytical procedure therefore consisted in brominating the sample at pH 1·0, pre-extracting the solution with diethyl ether at pH 1·0, extracting the α,β-dibromopropionamide with diethyl ether at pH 3·0, concentrating the extract and determining it by electron-capture gas chromatography. The concentration factor varied according to the level of acrylamide in the sample and the detector sensitivity, but was usually a factor of 10 in the range 1·0 to 50 μg l^{-1} of acrylamide and 100 in the range 0·1 to 1 μg l^{-1} of acrylamide. Fig. 1 shows gas chromatograms of River Thames water and River Thames water *plus* 0·25 μg l^{-1} of acrylamide determined by this procedure. These extracts were concentrated 100 times before gas-chromatographic analysis.

The method has been used to analyse a large number of water samples containing acrylamide derived from the use of polyacrylamides. No major problems have been encountered, apart from the occurrence in some samples of material retained longer than acrylamide, thereby extending the analysis time. This problem has been overcome by the use of a "back-flushing" device.[7]

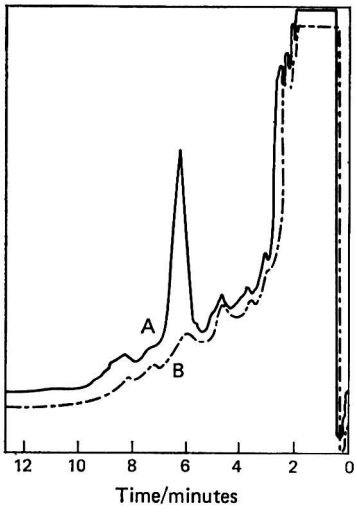

Fig. 1. Gas-chromatographic trace for acrylamide added to a river water: A, river water + 0·25 μg l^{-1} of acrylamide; and B, river water blank

Method

Apparatus—

Gas chromatograph—Pye No. 134 with an electron-capture detector.

Stirrer motor—Citenko, variable speed, with glass stirrer.

Direct-reading pH meter—E.I.L., Model 23A.

Ultraviolet lamp—Hanovia, Model 16, with emissions from 235 to 1140 nm, used on a retort stand without the filter.

Conical flasks—Glass-stoppered, 250-ml capacity.

Centrifuge tubes—Glass-stoppered, 100-ml capacity.

Syringe—Hamilton microlitre, 0 to 10 μl with 2-inch needle.

Glass tube packed with silica gel and molecular sieves—This was connected to an air supply to give a dry air stream flowing at 200 ml min^{-1}.

Gas - liquid chromatographic column—A 1-m long × 3-mm i.d. column, packed with 60 to 80-mesh acid-washed DMCS-treated Chromosorb W, supporting 10 per cent. by weight of FFAP, and operated at 180 °C with a nitrogen flow of 40 ml min^{-1} (10 lb in^{-2} pressure).

REAGENTS—

Bromine water—Shake distilled water, which has been adjusted to pH 1·0 with sulphuric acid, with an excess of bromine for 30 s. Allow it to settle for 1 minute and use the aqueous phase.

Sodium sulphite solution, approximately 0·1 M.

Sodium hydroxide solution, 2 N.

Sulphuric acid solution (1 + 3).

Diethyl ether, boiling-point 35 °C—Re-distilled by using a fractionating column.

Acrylamide—B.D.H. analytical-reagent grade.

ANALYTICAL INTERFERENCES ARISING FROM APPARATUS AND REAGENTS—

When using the gas chromatograph at high sensitivity, solvents absorb impurities from the air and care is needed to keep all solutions well stoppered. It is also necessary to check the solvent for the development of interfering impurities if it has stood for a long period after distillation.

Contact made by solutions with plastics materials, except PTFE, also gives rise to interferences in electron-capture gas chromatography. It is essential that all glassware should have ground-glass or PTFE stoppers. Grease must not be used on joints or stopcocks. A non-ionic detergent such as Stergene must be used for washing glassware as anionic detergents give rise to interferences.

PREPARATION OF α,β-DIBROMOPROPIONAMIDE—

Acrylamide (3·5 g) is dissolved in 10 ml of water in a 300-ml Kjeldahl flask. To this is added 12·5 g of potassium bromide dissolved in 15 ml of water and 10 ml of 6 N sulphuric acid. The flask is fitted with a two-way head with stopcocks and the air is evacuated. The flask is then enclosed in a box to exclude light, but with two "view" holes to enable the reaction to be followed visually. The stopcock inlets are left protruding. Potassium bromate (2·92 g) dissolved in 25 ml of water is then added dropwise with shaking, while preserving the vacuum. Between additions of potassium bromate all the liberated bromine is allowed to react. When half of the bromate has been added a white solid (α,β-dibromopropionamide) will separate. After completing the addition of the bromate the box is stored in a dark cupboard for 30 minutes. The small excess of bromine present is destroyed with sodium sulphite and the vacuum broken. The white solid is filtered off, washed with 10 ml of water and then air dried. This compound should have a melting-point of 132 °C, which should not change with recrystallisation of the compound from benzene.

PREPARATION OF STANDARD SOLUTIONS OF α,β-DIBROMOPROPIONAMIDE FOR GAS CHROMATOGRAPHY—

Weigh accurately 10 mg of α,β-dibromopropionamide into a 100-ml glass-stoppered calibrated flask and dissolve it in, and make up to the calibration mark with, analytical-reagent grade methanol. Dilute an aliquot of this solution 1 + 99 with the same methanol. Dilute aliquots of the further diluted methanolic solution with re-distilled diethyl ether until a range of solutions is obtained containing from 1 to 400 μg l^{-1} of α,β-dibromopropionamide (5 to 2000 pg per 5 μl). These solutions are stable for at least 2 weeks, after which time they deteriorate because of evaporation or decomposition of the amide.

PREPARATION OF ACRYLAMIDE STANDARD—

Weigh accurately 10 mg of acrylamide into a 100-ml glass-stoppered calibrated flask and dissolve it in, and make up to the calibration mark with, fresh distilled water. Dilute an aliquot of this solution successively with distilled water until a range of solutions is obtained giving a set of standards containing 5×10^4, 5×10^3, 5×10^2, 50 and 25 μg l^{-1} of acrylamide; 1-ml volumes of these solutions are used to add acrylamide to water in order to check recoveries.

OPTIMISATION OF ULTRAVIOLET IRRADIATION—

Clamp the ultraviolet lamp in a fixed position 15 cm above the reaction beaker, with the beam shining directly on to the liquid surface. Carry out the bromination reaction with a solution of known acrylamide content for periods of 30 s and 1, 5 and 30 minutes. Extract

the solutions with diethyl ether and determine the concentration of α,β-dibromopropionamide present. Repeat this procedure with the ultraviolet lamp at 25 and 50 cm above the reaction beaker. Calculate from the results the optimum position for the lamp and also the optimum time for irradiation. It may be necessary to repeat the experiments with closer time intervals in order to obtain the maximum yields of α,β-dibromopropionamide. The concentration of acrylamide used in these experiments does not affect the results within the concentration range tested, *i.e.*, less than 1 mg l^{-1}.

BROMINATION AND EXTRACTION PROCEDURES—

Bromination procedure—Measure 100 ml of sample into a squat 250-ml beaker. Adjust the pH of the solution to 1·0 with the sulphuric acid solution. Place the beaker and its contents under the ultraviolet lamp at the pre-determined height with the beam directed on to the surface of the solution. Add 4 ml of the saturated bromine water, stirring vigorously. Allow it to react for the pre-determined time, then destroy the excess of bromine with 4 ml of an approximately 0·1 M solution of sodium sulphite.

Extraction procedure—Pour the aqueous solution resulting from the bromination stage into a 250-ml glass-stoppered separating funnel (the solution being at pH 1). Add 20 ml of re-distilled diethyl ether. Shake the mixture vigorously for 2 minutes and allow the solution to stand for 10 minutes to ensure good phase separation. Discard the ethereal phase. Transfer the aqueous phase to a 250-ml beaker and adjust the pH to 3·0 in the following manner. Stir the sample vigorously, but without splashing, by using a magnetic stirrer. Add 2 N sodium hydroxide solution dropwise from a burette. Follow the pH change by using the pH meter and stop the addition of alkali when pH 3·0 is reached.

Pour the solution into a 250-ml glass-stoppered separating funnel and add 100 ml of diethyl ether. Shake the mixture vigorously for 2 minutes. Allow the phases to separate and run the ethereal phase into a glass-stoppered conical flask. Re-extract the aqueous phase with two further 50-ml volumes of diethyl ether. Combine the ether extracts and measure the volume obtained. If the acrylamide concentration in the sample is above 50 μg l^{-1} this extract can be analysed directly by injecting 5-μl aliquots into the gas chromatograph. For lower concentrations the extract can be concentrated by a factor of up to 100 before the analysis by directing a jet of dry air (at 200 ml min^{-1}) on to the surface of the liquid. Immerse the flask in a bath of water at room temperature during the evaporation to prevent undue cooling. The exact degree of concentration depends on the sample and the sensitivity of the chromatograph but is usually a factor of 10 in the range 1 to 50 μg l^{-1} and 100 in the range 0·1 to 1·0 μg l^{-1} of acrylamide. It has sometimes been possible to concentrate by a factor of only 10 in the range 0·1 to 1 μg l^{-1}.

GAS - LIQUID CHROMATOGRAPHIC ANALYSIS—

Inject 5-μl volumes of the prepared standards into the chromatograph. Calculate the areas of the acrylamide responses by multiplying the peak height by the peak width at half its height. Plot the amount of acrylamide *versus* peak area, by using log - log co-ordinates. A straight line should be obtained over most of its length. The calibration is unlikely, however, to be truly linear.

Inject 5-μl samples of the organic extract into the gas chromatograph and determine the amount of α,β-dibromopropionamide present by measuring the area of the peak obtained. As some day-to-day variation may occur in the response of the detector to α,β-dibromopropionamide it is necessary to re-calibrate the chromatograph each day. The calibration should be checked after each two injections by the injection of a standard. Inject each unknown at least twice and use the average peak area to calculate the amount of α,β-dibromopropionamide.

CALCULATION OF RESULTS—

The concentration of α,β-dibromopropionamide (a) present in the water sample can be calculated as $a = 2xb \times 10^{-3}$ μg per litre of water (assuming a 100-ml water sample and 100 per cent. extraction efficiency), where x pg is the amount of α,β-dibromopropionamide in a 5-μl injection of solvent extract and b ml is the volume of the solvent extract after concentration.

The approximate concentration of acrylamide present in solution before bromination (c)

can then be calculated as $c = a\frac{71}{230} \times \frac{100}{y}$ μg l^{-1}, where $\frac{71}{230}$ is the ratio of the molecular weights of acrylamide and α,β-dibromopropionamide, and y is the approximate percentage yield of α,β-dibromopropionamide in the solvent extract after the complete analysis calculated from the standards. This would be 40 per cent. from 0·1 to 1·0 μg l^{-1}, 50 per cent. from 1·0 to 10 μg l^{-1} and 60 per cent. from 10 to 1000 μg l^{-1}.

In the range 1·0 to 1000 μg l^{-1}, c would probably give a sufficiently accurate figure for most purposes. However, for levels below 1·0 μg l^{-1} the yields fall sharply and are somewhat variable with conditions. It is desirable, therefore, to use acrylamide as an internal standard in order to obtain a more accurate estimate of the yield of α,β-dibromopropionamide. The procedure is as follows.

Perform an analysis and calculate c as above. To another 100-ml aliquot of water sample add sufficient acrylamide to double the amount calculated from the analysis, *i.e.*, add $\frac{c}{10}$ μg per 100 ml of sample. Analyse the "spiked" sample, which will contain about $2c$ μg of acrylamide per litre of water. Calculate a_2 μg, the amount of α,β-dibromopropionamide per litre of water. If the concentration of α,β-dibromopropionamide from the original analysis was a_1 μg l^{-1}, then the amount Z, from c μg l^{-1}, of acrylamide added per litre of water, is given by $Z = a_2 - a_1$.

The percentage yield of α,β-dibromopropionamide under those particular conditions, y_1, is then given by $y_1 = \frac{71\,Z}{230\,c} \times 100$ and the concentration of acrylamide in the sample (q), assuming constant yield over the concentration range q to $q + c$, is $q = \frac{a_1 c}{Z}$ μg l^{-1}.

If peaks that interfere in the acrylamide determination are present in the water before the addition of acrylamide or polymer, the blank from this source must be subtracted, as micrograms of α,β-dibromopropionamide per litre, from the value of a. Blank determinations on the water investigated must therefore always be performed in order to assess this factor.

Results

Samples of acrylamide in water were prepared by adding known volumes of the aqueous standard solutions of acrylamide to 100-ml samples of River Thames water. These solutions were then analysed and the yields calculated by comparing the amount of acrylamide orginally added with the amount of acrylamide detected as the α,β-dibromopropionamide. Ten samples each were analysed at the following levels: 500, 50, 5 and 0·25 μg l^{-1}. The results are shown in Table I.

Table I

Recoveries of acrylamide as α,β-dibromopropionamide

Acrylamide concentration/μg l^{-1}	Calculated α,β-dibromopropionamide concentration/μg l^{-1}	Recovery of acrylamide as α,β-dibromopropionamide, per cent.	Relative standard deviation of results, per cent.
500	1620	66	±5
50	162	64	±5
5	16·2	50	±9
0·25	0·81	34	±10

The blank from the river water was significant only at the lowest level analysed and was determined on ten replicate samples as 0·06 μg l^{-1} of α,β-dibromopropionamide with a relative standard deviation of ±22 per cent.

Four samples made up to contain 0·24 μg l^{-1} of acrylamide, when analysed by using the internal standardisation procedure, gave a mean value of 0·26 μg l^{-1} with a relative standard deviation of ±20 per cent.

The method described by Roos[8] has been used to calculate the limits of detection of the analysis. This figure is 0·1 μg l^{-1} at the 95 per cent. confidence level as calculated from the blank figures and the results derived from the analysis of samples by the internal standardisation procedure.

Discussion

A method has been presented that is capable of determining acrylamide in water at levels down to 0·1 μg l^{-1}. It is the only method available for the determination of acrylamide in water at these very low concentrations. Any method of detecting acrylamide other than electron-capture gas chromatography of the derived α,β-dibromopropionamide would require concentration of the acrylamide by a factor of at least 10^3 and probably 10^4 to detect these low concentrations. Acrylamide itself is extremely difficult to extract from water with organic solvents, and cannot be extracted by any that are easily evaporated. Evaporation of the water itself could lead to hydrolysis of the acrylamide to acrylic acid or possibly its loss by steam distillation. Interfering materials would almost certainly pose major problems in such a procedure. Any possible alternative analytical procedure to the bromination - electron-capture method therefore appears fraught with more difficulties than the method developed. It is intended to use this method of analysis in the investigation of levels of acrylamide in various effluents and of its fate during treatment processes in waterworks.

Despite the non-quantitative yields of α,β-dibromopropionamide, and the variance of these yields with concentration, their reproducibility at any given concentration is good. The method has proved adequate for analyses performed on river waters and effluents at levels ranging from 0·1 μg l^{-1} to 1·0 mg l^{-1}.

The internal standardisation procedure has proved useful and avoids the necessity of brominating a series of closely related acrylamide standard solutions in order to calculate α,β-dibromopropionamide yields. It has still proved necessary to calibrate the chromatograph with α,β-dibromopropionamide standards, for although these calibrations are straight-line graphs on log - log co-ordinates they would not be straight lines on linear co-ordinates. Calculation of results from the one internal standard response was therefore not possible.

Conclusions

A method involving bromination at pH 1·0, pre-extraction at pH 1·0, extraction at pH 3·0 and concentration of the extract prior to analysis by electron-capture gas chromatography has been developed for the determination of acrylamide in water. The limit of detection was 0·1 μg l^{-1} of acrylamide in River Thames water; 500, 50, 5 and 0·25 μg l^{-1} levels were analysed with relative standard deviations of $\pm$5, $\pm$5, $\pm$9 and $\pm$10 per cent., respectively.

Because of the variation in yields of α,β-dibromopropionamide at the bromination stage, a type of internal standardisation procedure has been developed primarily for use at levels of acrylamide below 1 μg l^{-1}; a level of 0·25 μg l^{-1} of acrylamide was analysed with a relative standard deviation of $\pm$20 per cent. by using this procedure.

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An Inexpensive Gas Chromatograph for the Detection and Collection of Labile Organophosphorus Compounds*

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A gas chromatograph suitable for use with labile organophosphorus compounds is described. It incorporates all-glass systems for detection and collection, with an exceptionally short distance from the column to the detector or collector. Easily exchanged thermionic and flame-ionisation detectors are used. The instrument is simple, convenient and cheap to make.

The thermionic detector is sensitive to less than 1 pg of diazinon and responds linearly over a 5000-fold range. Fractions from 50 ng to 50 μg have been collected with 90 to 100 per cent. efficiency.

A gas chromatograph was needed for the detection and collection of the degradation products of organophosphorus pesticides. A thermionic detector for phosphorus compounds, a flame-ionisation detector and a simple trapping device that would collect amounts from about 0·1 to 50 μg with negligible loss were required. As the compounds of interest are usually present in small amounts and are often unstable, an all-glass system with a short path from the column to the detector was necessary. The chromatograph had to be cheap and convenient to use. As no instrument that fulfilled all these conditions was available, the apparatus to be described was constructed. It consists of a column oven, detector and collector linked to a commercial amplifier and temperature control unit.

Description

Fig. 1 is a section through the chromatograph. The oven consists of concentric metal tubes, 1 and 2, which are 5 and 8 inches in diameter. The outer tube is lagged with glass-fibre, 3, covered with aluminised paper, 4. The tubes are screwed to a base, 5, and closed by a lid, 6, both being made of asbestos. (The lifting handles for the lid are not shown.) The U-shaped glass column, 7, of $\frac{1}{8}$ inch o.d., is held by nuts and O-rings in the inlet and exit housings, 8 and 9, attached to the lid. Carrier gas passes through the heating coil, 10, to the inlet housing, and enters the column at the injection septum and nut, 11. The detector, 12, temperature sensor, 13, gas connections, 14, and thermocouple, 15, are all carried by the lid. Heating is by three Nichrome elements, 16, between the two tubes. The turbine, 17, circulates air through large ports at each end of the inner tube as shown by the arrows.

Fig. 2(*a*) shows the column exit housing and the detector. The column, secured by the O-ring assembly, 18, passes through the oven lid and ends within the salt tip, 19, of the thermionic detector. The salt tip is pressed into the brass bushing, 20, which is screwed into the exit housing and sealed by the O-ring, 21. Hydrogen is admitted through the tube, 22, and joins the column effluent immediately below the flame. Air is discharged into the detector body, 12, through the tube, 23. The detector body carries the electrodes, 24 and 25; it is not attached to the lid but is located by the ring, 26.

Details of the salt tip are shown in Fig. 2(*b*). The salt, potassium bromide, is pressed into the brass bush, the bush is machined and holes A and B are drilled in the salt. Hole B can serve as the flame jet or, as shown in this figure, a short piece of stainless-steel tubing, C, is pressed into B to form the jet. The steel-cored tip provides higher sensitivity. To convert the thermionic detector into a flame-ionisation detector, the salt tip is replaced by a stainless-steel tip similarly machined.

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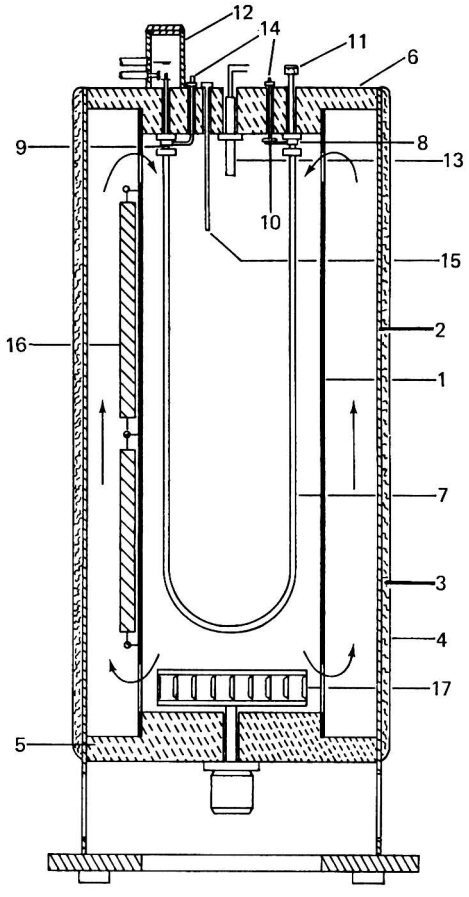

Fig. 1. Section through chromatograph
(not to scale)

When fractions are to be collected a chromatogram is first recorded to determine the retention times of the peaks. The assembly shown in Fig. 3 is then substituted for the detector. The heating block, 27, replaces the detector body and fits closely round the column exit housing. The block is heated by the two soldering iron elements, 28, controlled by an energy regulator. The heater is covered by the asbestos lid, 29, through which passes the glass collection tube, 30, $\frac{1}{4}$ inch o.d. and about 10 inches long, which is packed with glass-wool. The tube is sealed by the O-rings, 21 and 31. For collection, the heater supply is set so that the temperature in the tip of the column is 2 or 3 °C higher than that in the oven (the energy regulator can be calibrated and the block pre-heated). The sample is then injected, and each fraction is collected in a separate tube and eluted with a suitable solvent. If the sample is large a fraction can be collected repeatedly in the same tube without elution between successive collections.

Further details of some of the components are as follows.

Item	
1 and 2	Aluminium sheets, 16 gauge, rolled into tubes 34 inches long and welded
3 and 4	Glass-fibre rigid section, 36 inches in length and of 8 inch bore and $1\frac{1}{2}$-inch wall thickness, covered with aluminised paper (Filter Supply Co. Ltd.)
5 and 6	Sindanyo asbestos, heat resisting (Turner Asbestos Co. Ltd.)
8 and 9	Housing, brass, $\frac{1}{8}$-inch gas inlet tubes brazed in
10	Heating coil, stainless steel, $\frac{1}{16}$ inch o.d., 12 inches long

13	Temperature sensor, resistance thermometer (Pye-Unicam Ltd., Catalogue No. 717087)
14	Gas connections, ⅛-inch copper tube, connected by captive seal couplings (Drallim Industries Ltd.)
16	Heater elements, Nichrome, 1400 W
17	Turbine fan (Airflow Developments Ltd.) driven by motor (Universal Electric Motor Co. Ltd., Model AA1H101K) at 2500 r.p.m.
28	Soldering iron heating elements ("Antex," Southern Watch & Clock Supplies Ltd.)
29	As 5 and 6

Results and discussion

Column heating—

The absence of separate heaters for the detector and injector might be expected to aggravate the difficulty of heating a tall, cylindrical oven satisfactorily. The temperature in the thermocouple pocket was found to be constant within ±0·5 °C from the top of the turbine to about 3 cm below the oven lid, but decreased above this level. The exit limb of the column is heated by the detector flame, however, and measurements of its temperature while carrier gas was flowing showed that the cool region extended for only about 5 cm within the packed part of the column. At 170 °C, the minimum temperature in this section was 7 °C below that of the column oven. In practice the sensitivity of the instrument has shown that the slight cooling causes no trouble, although the arrangement would probably be unsatisfactory with a detector that did not generate its own heat. Micheletti and Bryan[1] have described a chromatograph fitted with a flame-ionisation detector within the column oven. Their design avoids the risk of condensation but makes the detector less accessible.

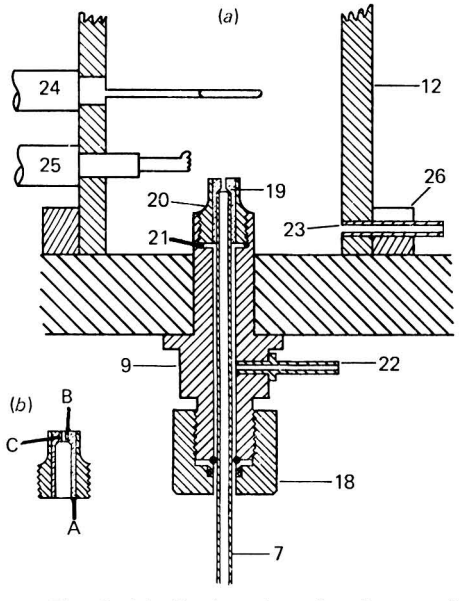

Fig. 2. (*a*), Section through column exit housing and detector; and (*b*), details of salt tip (not to scale)

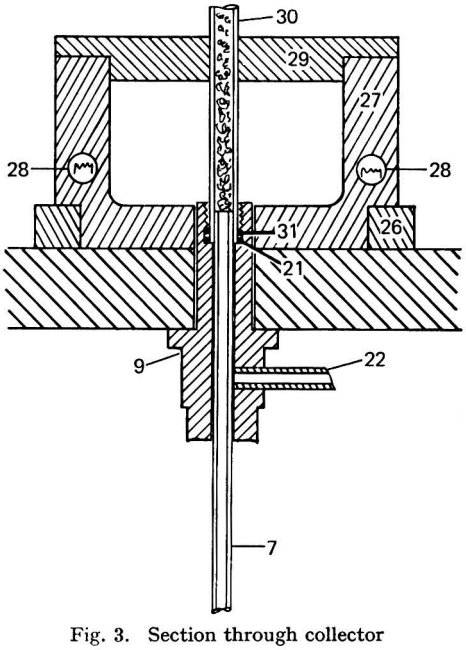

Fig. 3. Section through collector (not to scale)

Detection—

A particular feature of the detector is the very short path (about 2 mm) from the column exit to the flame with a dead volume between the two of about 5 μl. Several commercial gas chromatographs have the detector vertically above the column with a steel capillary connecting the two, and Oster[2] has described a flame-ionisation detector with a short distance between the column and the flame tip. In the present design, the column is probably as close to the flame as is possible, and this is likely to be an important factor in achieving high sensitivity, particularly to labile compounds.

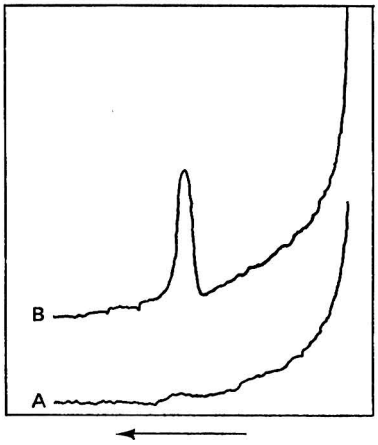

Fig. 4. Chromatogram of 1 pg of diazinon (actual size): A, acetone (0·5 μl); and B, 0·002 p.p.m. of diazinon in acetone (0·5 μl). Column 2 per cent. XE-60 on Aeropak 30; and temperature 143 °C. Retention time of diazinon 3·0 minutes. The peak represents a signal of $0{\cdot}07 \times 10^{-12}$ A (7 per cent. full-scale deflection on a 1-mV recorder)

The sensitivity of the thermionic detector was examined by injecting solutions of diazinon, diazoxon, chlorfenvinphos and pyrimithate. Fig. 4 shows chromatograms of 1 pg of diazinon and of its solvent blank, chromatographed on a 3-foot column containing 2 per cent. of XE-60 on Aeropak 30. Similar responses were given by 5 pg of diazoxon, 7 pg of chlorfenvinphos and 10 pg of pyrimithate. These results were obtained with a steel-cored salt tip and were recorded at an amplifier sensitivity of 10^{-12} A full-scale deflection with a 1-mV recorder. With a plain salt tip, noise was excessive at sensitivities higher than about 10^{-10} A. In some recent commercial thermionic detectors, the salt is pressed into a cup surrounding a steel capillary tube and the increased sensitivity of this design compared with a simple salt pellet has been pointed out.[3,4] The sensitivity provided by the thermionic detector was ample, but higher sensitivities have been reported by Mees and Spaans,[4] who examined the effects upon sensitivity of the design and position of the electrodes, the hydrogen flow, the polarisation potential and the type of salt. They obtained the highest sensitivity with the salt pressed into a steel cup and an axial rod as the collector electrode. They also found that a potential difference of only 4 V was best and that careful purification of the salt was essential for very sensitive detection. The sensitivity of the present detector might be increased by paying attention to these features, but their influence has not been examined. As the chromatograph was not intended for precise quantitative work, the linearity of the detector response was not particularly important. A brief test, however, showed a linear response to diazinon from 1 pg to 5 ng. The response to larger amounts was not determined.

COLLECTION—

Table I shows the recoveries of diazinon, diazoxon, trimethyl phosphate and triethyl phosphate at various levels. It can be seen that 90 to 100 per cent. of each compound was recovered and that at least 96 per cent. of this was in the intended fraction. Recoveries were determined by chromatography of the collected fractions.

Leathard and Shurlock[5] have reviewed the different types of collection system that have been used. The arrangement described here, which is simpler than most, worked well for its intended purpose but has not been tried for compounds more volatile than trimethyl phosphate. It would be difficult to use it with some commercial chromatographs, for which a device recently described by Cronin[6] for trapping sub-microgram amounts from PLOT

columns might be more suitable. The main disadvantages of the present method are that the peaks are not recorded and that for amounts much greater than 1 μg, rather large volumes of eluting agent (up to 15 ml) are required. The first disadvantage causes little inconvenience in practice and the second is slight provided that eluting agents are carefully purified. The advantages are the absence of metal connecting tubes, the very short distance beween the end of the column and the condensation region, and the general simplicity. A further slight advantage is that none of the sample is wasted by splitting the effluent.

TABLE I

RECOVERY OF COLLECTED FRACTIONS

Compound	Weight injected	Recovery in intended fraction, per cent.	Recovery before or after intended fraction, per cent.	Total recovery, per cent.
Diazinon	50 ng	92	3	95
	1 μg	90, 87, 99, 90, 90	6, 4, 0, 1, 5	96, 91, 99, 91, 95
	50 μg (5 × 10 μg)	90	3	93
Diazoxon	2 μg	90, 95	4, 7	94, 102
Trimethyl phosphate ..	250 ng	90	0	90
Triethyl phosphate ..	250 ng	87	2·5	90

CONVENIENCE AND COST—

The chromatograph has been in constant use for about 16 months, and has required no attention apart from occasional cleaning of the detector body and electrodes. The location of the main components on the lid makes them easily accessible. A column can be changed in less than 4 minutes, and if a spare lid is kept fitted with a column the complete unit can be changed in less than 1 minute. The detector can be replaced by the collector in about 2 minutes. The salt tips are quickly installed and are very robust. One that was fitted originally shows no signs of deterioration and it seems that a tip should last for a few years.

The total cost of the materials (including the fan and motor) and time spent in construction was about £75. A Pye-Unicam amplifier and temperature control unit can conveniently be used, bringing the total cost to about £350, excluding the recorder. Full details of the construction of the instrument will be provided on request.

The authors are grateful to Mr. F. Randall for most of the work of construction, and to Mr. D. E. Mundy for his evaluation of various aspects of the performance.

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The Gas-chromatographic Determination of Residues of Barban and its Major Metabolites in Crops

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A method is described for the determination of residues of barban [4-chlorobut-2-ynyl *N*-(3-chlorophenyl)carbamate] and its major water-soluble metabolites in harvest grain (wheat and barley) and sugar beet. Hydrolysis of barban and its metabolites yields 3-chloroaniline. Bromine water quantitatively converts 3-chloroaniline into its tribromo derivative, which can be detected by electron-capture gas chromatography. The limit of detection is 0·01 p.p.m. of barban. Mean recoveries of barban added to sugar beet, wheat and barley samples are 84·0, 68·8 and 78·0 per cent., respectively. The possibility of interference from other herbicides is discussed.

The method is more rapid than other published procedures and allows smaller samples to be extracted without loss of sensitivity.

Barban [4-chlorobut-2-ynyl *N*-(3-chlorophenyl)carbamate] is the active ingredient of the wild-oat herbicide marketed under the trade name Carbyne. The commercial product contains 12·5 per cent. of active ingredient and the recommended rate of application in the field is 0·35 to 0·70 kg of active ingredient in 110 to 225 l ha^{-1} (5 to 10 oz in 10 to 20 gal $acre^{-1}$).

Barban residues in numerous crop species have been determined previously by the method of Riden and Hopkins.[1] This method required a chromatographic clean-up of the extract and a hydrolysis procedure followed by steam distillation of the hydrolysis product, 3-chloroaniline, into an acid. This acidic product was diazotised and coupled with *N*-(1-naphthyl)ethylenediamine dihydrochloride to form a dye for spectrophotometric determination. Extracts of older plants often required further chromatographic clean-up to eliminate interference from the dye formed by *o*-aminoacetophenone (the product of the alkaline hydrolysis of naturally occurring tryptophan).[2] A subsequent method[3] enabled unextracted residues of water-soluble metabolites containing the 3-chloroaniline moiety to be determined in a similar manner. In these methods, samples of 100 to 120 g were necessary to attain a sensitivity of 0·01 p.p.m. of barban. A gas-chromatographic method has recently been described[4] for the individual determination of numerous substituted phenylurea and phenylcarbamate herbicides (including barban) in river water. In this method, thin-layer chromatographic separation, hydrolysis and dinitrophenylation of the liberated aniline moieties were used to enhance their electron-capture response.

The development of a gas-chromatographic method that requires little clean-up was prompted by the need for residue investigations in crops following recent trials with a 25 per cent. formulation of barban. The method has been used to determine residues of barban and its water-soluble metabolites in grain (wheat and barley) and sugar beet. Extraction and hydrolysis to 3-chloroaniline are followed by bromination to 3-chloro-2,4,6-tribromoaniline for electron-capture detection. The sensitivity is 0·01 p.p.m. of barban in 25-g samples.

Bromination to enhance electron-capture response has been reported[5–7] for several pesticides with the use of solvents containing iodine and bromine. 3-Methylaniline (obtained by hydrolysis of residues of the herbicide phenmedipham) has been converted into 3-methyl-2,4,6,-tribromoaniline, for gas-chromatographic determination, by treatment with an aqueous solution of potassium bromide, 2,4,6-trimethylaniline and potassium bromate.[8] For the gas-chromatographic method described here, bromination of 3-chloroaniline with bromine water - hydrobromic acid has been found to be quantitative, rapid and convenient. Aniline moieties found in other phenylurea and phenylcarbamate herbicides can also be detected but, with two exceptions, do not interfere (see under Interference). The bromination products of *o*-aminoacetophenone also appear on the metabolite chromatogram but do not interfere.

Experimental

Reagents—

Solvents—Distil all organic solvents and water in glass apparatus. Anhydrous diethyl ether is unsuitable because some batches produce peaks on the chromatogram having long retention times relative to those of analytical interest.

Light petroleum, boiling range below 40 °C.

Hydrochloric acid—Dilute the concentrated acid to 20 per cent. v/v with water.

Bromine water (1 + 100 *v/v*).

Hydrobromic acid—Dilute 167 ml of 48 to 50 per cent. w/w acid to 1 litre with water, to give an approximately 1 N solution.

Sodium sulphite ($Na_2SO_3.7H_2O$), *AnalaR, saturated aqueous solution.*

Silicone MS—Antifoam A (Hopkin and Williams), 10 per cent. v/v solution in benzene

Standard solutions—

3-Chloroaniline—Re-distil and reject the first and last 10 per cent. by volume. Prepare 1000 μg ml^{-1} (1000 p.p.m.) and thence 5 μg ml^{-1} (5 p.p.m.) solutions in approximately 1 N hydrobromic acid.

Barban—Prepare 1000 and 10 μg ml^{-1} solutions of barban (Fisons Agrochemical Division, purity >99 per cent.) in benzene.

Dieldrin, 2 μg ml^{-1} solution in diethyl ether.

Apparatus—

An Aerograph, Series 1200, gas chromatograph equipped with a concentric tube, tritium-foil electron-capture detector operated at 90 V, d.c. mode, was used, with a column 1·5 m × 3 mm o.d. packed with 2·5 per cent. of silicone E301 *plus* 0·25 per cent. of Epikote 1001 on Celite, 100 to 120 mesh. The operating conditions were: column temperature, 160 °C; injector temperature, 170 °C; detector base temperature, 270 °C; nitrogen flow-rate, 60 ml min^{-1}; range, 1; attenuation, 64.

Under these conditions, 3-chloro-2,4,6-tribromoaniline has a retention of 0·53 relative to dieldrin, which is used as an internal standard to allow for injection variations.

Formation of derivatives—

In a preliminary study to determine the optimum reaction conditions, 1·5-μg amounts of 3-chloroaniline were added to 10-ml volumes of hydrobromic acid for bromination under the various conditions. The normality of the hydrobromic acid (in the range 0·5 to 4·0 N), the volume of bromine water added (in the range 0·25 to 2·0 ml) and the reaction time (in the range 1 to 30 minutes) had such a small influence on the yield of 3-chloro-2,4,6-tribromoaniline that the conditions described in the bromination section were chosen entirely on practical considerations. In this way, calibrations were obtained by bromination of 3-chloroaniline directly from standard solutions or after hydrolysis of standard solutions of barban. Synthetic 3-chloro-2,4,6-tribromoaniline standards were subjected to the same bromination conditions. In all instances the calibration points fell on the same straight line for the range 0·25 to 2·0 μg of 3-chloroaniline (or equivalent) taken.

A sample of 3-chloro-2,4,6-tribromoaniline was prepared by the addition of excess of bromine water to 3-chloroaniline in approximately 1 N hydrobromic acid, filtration of the precipitate and recrystallisation from ethanol. The melting-point of the product was 124 to 125 °C, in agreement with the literature value.[9]

Preparation of samples—

Grain—Grind batches of about 50 g of threshed grain for 1 minute in a Moulinex grinder. Shake 25 g of ground grain with 100 ml of diethyl ether - light petroleum (1 + 3 v/v) for 30 minutes in a 250-ml conical flask. Filter the mixture into a 250-ml separating funnel through a No. 1 filter-paper and rinse the flask with a further 50 ml of solvent; combine the filtrates.

Sugar beet—Remove lumps of soil from the beet and remove the top and tap root. Chop the beet into small pieces and mix them thoroughly before sub-sampling. Macerate a 25-g sub-sample twice in 60 ml of ethyl acetate - hexane (1 + 3 v/v). Filter on a Buchner funnel. Decant the filtrate and 25 ml of solvent used to rinse the flask into a 250-ml separating funnel.

Retain the solid fractions from both types of sample and air-dry them, until free from organic solvent, for determination of the metabolites. Clean up the extracts in the following manner for determination of barban residues.

Determination of barban—

Shake the extract with 20 ml of 20 per cent. hydrochloric acid solution and discard the aqueous layer. Run the organic phase into a 250-ml round-bottomed flask, add antibumping granules and evaporate the solution to dryness on a rotary evaporator at water-bath temperatures of 45 and 50 °C for grain and beet extracts, respectively.

Rinse the inside of the flask with 1 ml of benzene, then add 10 ml of methanol, swirl the mixture and add 50 ml of 2·5 N sodium hydroxide solution. Finally, add 2 drops of antifoam solution with a Pasteur pipette and reflux the mixture for 1 hour under a condenser with a glass-wool plug. Cool and rinse the condenser with 20 ml of water. Add 60 ml of benzene to the flask, swirl the contents and transfer them back into the 250-ml separating funnel. Shake the mixture and discard the aqueous layer. Extract the benzene with 10 ml and then 5 ml of hydrobromic acid, and combine the acidic extracts in a 50-ml conical flask for bromination.

Determination of water-soluble metabolites—

Place the air-dried solid into a 500-ml round-bottomed flask (the whole of the sample for beet or three fifths of the total weight for grain). Add, with swirling, 50 ml of methanol, 250 ml of 2·5 N sodium hydroxide solution and 5 ml of antifoam solution. Reflux the mixture for 2 hours under a plugged condenser.

Note—Grain solids foam even with antifoam present, which is the reason for the use of the smaller sample; set the simmerstat carefully.

Cool and rinse the condenser with 20 ml of water. Proceed as follows, according to the type of crop.

Grain—Add 100 ml of water and stir the mixture. Pour it into a 250-ml centrifuge tube and spin it at 1700 r.p.m. for 15 minutes. Decant the liquid phase, which is viscous, into a 500-ml separating funnel. Add 100 ml of benzene to the centrifuge bottle, swirl the liquid gently and decant it into the funnel.

Sugar beet—Filter the mixture through a glass-wool plug into a 500-ml separating funnel containing 100 ml of benzene. Rinse the flask and glass-wool with 20 ml of water and 20 ml of benzene.

For both types of sample, shake the separating funnel, discard the aqueous layer and extract the benzene with hydrobromic acid as under Determination of barban.

Bromination and gas - liquid chromatography—

Add 1 ml of bromine water to the hydrobromic acid extract. Swirl the mixture, stopper the vessel and allow it to stand for 15 minutes. Add about 8 drops of sodium sulphite solution to destroy excess of bromine and swirl the mixture. Add 3 ml of 10 N sodium hydroxide solution. Cool the mixture for 5 minutes, add 5 ml of dieldrin solution, shake, then inject 1 μl of the organic layer into the gas chromatograph.

Calibration and calculation—

Transfer aliquots of standard 3-chloroaniline solution equivalent to 0·25, 0·5, 1·0, 1·5 and 2·0 μg of 3-chloroaniline into 50-ml conical flasks with a pipette. Make the volume up to 15 ml with hydrobromic acid. Brominate the solution and inject the product into the gas chromatograph as described above. Plot the peak height ratio of 3-chloro-2,4,6-tribromoaniline to dieldrin against the amount (in micrograms) of 3-chloroaniline added. The calibration graph is linear over the range of 3-chloroaniline standards used and is reproducible for one set of brominated standards for several days if evaporation of diethyl ether is prevented.

Use this calibration graph to determine the concentration of 3-chloroaniline in the unknown samples (p μg in 5 ml). If necessary, dilute the unknown sample solution with more dieldrin solution to bring the peak height to within this concentration range.

The residue, r, as parts per million of barban, is given by the equation—

$$r = \frac{2{\cdot}03 \times p \times d}{w}$$

where w is the weight of sample taken (in grams), d is the dilution factor of the diethyl ether solution in units of 5 ml, and 2·03 is the molecular weight conversion factor for 3-chloroaniline to barban. For water-soluble metabolites in grain, an additional factor ($\times 5/3$) is necessary to allow for the sub-sampling of the solid prior to digestion.

Results

Blanks and recoveries—

Mean apparent barban residues found at harvest in untreated grain samples were <0·01 p.p.m. For metabolites, the mean blank residues were 0·03 p.p.m. in barley and 0·02 p.p.m. in wheat. Blank determinations on untreated sugar beet samples gave mean residues of <0·01 p.p.m. for both barban and the metabolites.

Recoveries for barban on the three harvest crops are summarised in Table I. Metabolite recoveries were simulated by adding 3-chloroaniline to extracted solids (as pure metabolites have not been isolated). No such recovery experiments were reported in the earlier work.[3]

Table I

Recovery results

Crop	Chemical added	Level added (as p.p m. of barban)	Number of determinations	Mean recovery, per cent.	Coefficient of variation, per cent.
Sugar beet	Barban	0·02 to 0·10	5	84·1	9·0
	3-Chloroaniline	0·05 to 0·10	5	77·6	10·7
Wheat	Barban	0·02 to 1·00	6	68·8	7·1
	3-Chloroaniline	0·05 to 0·17	5	56·0	8·4
Barley	Barban	0·02 to 1·00	5	78·0	7·4
	3-Chloroaniline	0·05 to 0·10	4	48 7	7·0

The recoveries are corrected for blank determinations.

Interference—

o-Aminoacetophenone—This compound gives two gas - liquid chromatographic peaks with retentions of 0·38 and 0·54 relative to 3-chloro-2,4,6-tribromoaniline. Similar peaks occur in chromatograms of the metabolite determination. This observation supports the claim[1] that *o*-aminoacetophenone is the interfering agent in the colorimetric determination.

Other pesticides—Consideration of commonly used pesticides in terms of their structures and probable chemical fate in the method described indicated that substituted phenylureas and phenylcarbamates were the most likely sources of interference. Hydrolysis and bromination of nineteen herbicides in these two groups, or bromination of their aniline moieties,

Table II

Residues of barban and its metabolites in wheat grain

Formulation, per cent. of active ingredient	Dosage rate/kg ha^{-1}	Residue (as p.p.m. of barban)	
		Barban	Metabolites
12·5	0·25	0·01	0·01
	0·35	0·01	0·01
	0 49	<0·01	0 02
	0·49	<0·01	0 01
	0·70	<0·01	0 01
	0·70	<0 01	0 03
	0·70	<0 01	0 03
25·0	0·25	<0 01	*
	0·35	<0·01	0·01
	0·49	0·01	0·01
	0·49	0·01	0·01
	0·70	0·01	0·01
	0·70	0 02	0·03
	0·70	0·01	0·03

* Sample not analysed.

TABLE III

RESIDUES OF BARBAN AND ITS METABOLITES IN BARLEY GRAIN

Formulation, per cent. of active ingredient	Dosage rate/kg ha^{-1}	Residue (as p.p.m. of barban)	
		Barban	Metabolites
12·5	0·25	0·01	0·04
	0·25	<0·01	0·03
	0·25	<0·01	0·05
	0·35	<0·01	0·06
	0·49	<0·01	0·01
	0·70	<0·01	0·01
	0·70	<0·01	<0·01
25·0	0·25	<0·01	—
	0·25	<0·01	—
	0·35	0·01	0·01
	0·49	0·01	0·01
	0·70	<0·01	0·03
	0·70	<0·01	0·03

were carried out by the procedure described here. All the anilines examined could be detected after bromination but only those which yielded 3-chloro-2,4,6-tribromoaniline as the final product interfered. These interfering compounds were chlorpropham [isopropyl *N*-(3-chlorophenyl)carbamate] and chlorbromuron [*N*-(4-bromo-3-chlorophenyl)-*N'*-methoxy-*N'*-methylurea], but they are unlikely to be used with barban in practice. However, residues of 0·4 and 2·0 p.p.m., respectively, can be distinguished from 0·1 p.p.m. of barban in crop extracts by the thin-layer chromatographic procedure summarised below.

Thin-layer chromatographic procedure—Apply the unhydrolysed extract in diethyl ether as a streak on to one half of a 20 cm × 20 cm × 0·5 mm silica layer (Merck HF_{254}, activated for 2 hours at 105°C). Develop the spot in an unlined Shandon Chromatank to the top of the layer by using heptane (equilibrated with methanol) *plus* diethyl ether (65 + 35 v/v) as solvent. R_F values for chlorpropham, barban and chlorbromuron are 0·57, 0·46 and 0·21, respectively. Use standard spots (visible under 254-nm ultraviolet light) to select the area containing barban. Scrape this area into a round-bottomed flask and proceed with the hydrolysis. Recoveries of barban for all three crops averaged 55 per cent. at the 0·1 p.p.m. level.

ANALYSIS OF FIELD-TREATED CROPS—

The method has been used to determine residues of barban and its metabolites in harvest samples of three crops, following the application of either of two different formulations. The results for wheat, barley and sugar beet are shown in Tables II, III and IV, respectively.

TABLE IV

RESIDUES OF BARBAN AND ITS METABOLITES IN SUGAR BEET

Formulation, per cent. of active ingredient	Dosage rate/kg ha^{-1}	Residue (as p.p.m. of barban)	
		Barban	Metabolites
12·5	0·70	<0·01	<0·01
	0·70	<0·01	—
	1·05	<0·01	<0·01
	1·05	<0·01	—
25·0	0·49	<0·01	<0·01
	0·49	<0·01	—
	0·70	<0·01	<0·01
	0·70	<0·01	—

DISCUSSION

Earlier studies of the decline of residues of barban in a range of crops after treatment showed that they quickly fell to below 1 p.p.m. and at harvest time were generally not detectable.[1] Similarly, residues of the water-soluble metabolites that are hydrolysed to 3-chloroaniline had been shown[3] to approach zero in 4 to 6 weeks after treatment.

The results of this investigation with the new gas - liquid chromatographic method are in agreement with these earlier findings and also with unpublished results obtained in this laboratory with a similar spectrophotometric procedure. Barban residues were not greater than 0·02 p.p.m. in any of the crops that had been treated at application rates of 0·25 to 1·05 kg ha^{-1}. Metabolite levels ranged from less than 0·01 p.p.m. to 0·06 p.p.m. No significant difference was found in residue levels resulting from the two formulations.

Conclusion

The method described is sensitive and rapid. With four heating devices available for the hydrolysis step, eight samples per day were analysed to determine barban and its metabolites. The method is not subject to interference from pesticides that are likely to be used with barban in the three crops investigated.

Helpful discussions with Mr. M. Crofts are acknowledged.

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A Dual-purpose Basal Medium for the Microbiological Assay of Nicotinic Acid (Niacin) and Pantothenic Acid with *Lactobacillus plantarum*

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A dual-purpose medium for the microbiological assay of nicotinic acid (niacin) and pantothenic acid with *Lactobacillus plantarum* has been devised.

The advantage of the use of the dual-purpose medium described in this paper for the assay of nicotinic and pantothenic acids is that it is simple to prepare and gives steeper standard graphs for the assay of nicotinic acid than the basal media that have been proposed previously.[1] Moreover, the same medium can be used for the assay of pantothenic acid, which avoids the disadvantage of having to prepare a special peptone solution free from pantothenic acid with a life of only 2 to 3 weeks, and was found to be as sensitive as the basal medium previously described.[2]

Experimental

Maintenance of organism—

Lactobacillus plantarum is maintained as an agar stab culture.[1] Cultures should be renewed weekly.

Basal medium—

The modified basal medium has the composition given in Table I.

Table I

Basal medium for assay of nicotinic and pantothenic acids

Ingredient	Amount	Ingredient	Amount
Vitamin-free Casamino Acids (Difco)*	5·0 g	Sodium chloride	5·0 g
DL-Tryptophan	0·1 g	Ammonium sulphate	3·0 g
L-Cystine	0·1 g	Thiamine hydrochloride	1000 μg
Peptone - liver - yeast solution*	100 ml	Nicotinic acid†	2000 μg
Glucose	20·0 g	Calcium *d*-pantothenate‡	1000 μg
Sodium acetate (anhydrous)	20·0 g	Riboflavin	500 μg
Dipotassium hydrogen orthophosphate	2·5 g	Pyridoxine hydrochloride	1000 μg
Potassium dihydrogen orthophosphate	2·5 g	*p*-Aminobenzoic acid	100 μg
Adenine	10·0 mg	Biotin	4·0 μg
Guanine	10·0 mg	L-Tyrosine	0·1 g
Uracil	10·0 mg	L-Phenylalanine	0·2 g
Xanthine	10·0 mg	Inorganic salt solution*	5·0 ml
		Water	to 500 ml

* See text.
† Omitted for the assay of nicotinic acid.
‡ Omitted for the assay of pantothenic acid.

Vitamin-free Casamino Acids—It is advisable to treat this material once with activated charcoal to remove any traces of nicotinic acid and pantothenic acid. Dissolve 10 g of the Casamino Acids in 100 ml of water and adjust the pH to 3·0 with concentrated hydrochloric acid with BDH Universal Indicator as external indicator. Add 5 g of good-quality activated charcoal and stir the mixture mechanically for 30 minutes, filter it and re-adjust the volume to 100 ml. Preserve the solution under sulphur-free toluene at a temperature not exceeding 4 °C.

Peptone - liver - yeast solution—Dissolve 25 g of Difco peptone, 10 g of proteolysed liver (Paines and Byrne) and 10 g of Difco yeast extract in 400 ml of 1 N sodium hydroxide solution,

heat the solution in an autoclave for 15 minutes at 15 p.s.i., allow it to cool, adjust the pH to 0·8 to 1·0 electrometrically with concentrated hydrochloric acid and make the volume up to 500 ml with water. Add 45 g of high-quality activated fuller's earth (Lloyd's reagent) and stir the mixture mechanically for 30 minutes, filter it and repeat these operations twice, re-adjusting the pH to 0·8 to 1·0 if necessary. Add 18 g of dipotassium hydrogen orthophosphate, adjust the pH to 6·6 to 6·8 with sodium hydroxide solution, steam the solution for 30 minutes, then cool and filter it. Adjust the pH to 3·0 with concentrated hydrochloric acid, add 25 g of good-quality activated charcoal, stir the mixture mechanically for 30 minutes and filter it. Re-adjust the pH to 6·6 to 6·8 with sodium hydroxide solution and steam the mixture for 30 minutes. Preserve the solution obtained under sulphur-free toluene at a temperature not exceeding 4 °C. The solution will maintain its activity for 3 months. Any precipitate that forms during storage should be filtered off before the solution is used.

Inorganic salt solution—Dissolve 10 g of magnesium sulphate ($MgSO_4.7H_2O$), 0·5 g of manganese(II) sulphate ($MnSO_4.4H_2O$) and 0·1 g of iron(III) chloride in 250 ml of water and add 5 drops of concentrated hydrochloric acid. This solution will maintain its activity indefinitely at room temperature.

After mixing, adjust the pH of the basal medium to 6·8 with 30 per cent. sodium hydroxide solution, with bromothymol blue as external indicator, or electrometrically, and make the volume up to 500 ml with water.

ASSAY PROCEDURE—

A new standard graph must be used for each assay. The amounts of nicotinic acid and pantothenic acid required to establish the standard graphs are 0·05 to 0·25 μg and 0·02 to 0·16 μg, respectively.

To establish the standard graph for pantothenic acid, 108 mg of calcium *d*-pantothenate should be dissolved in 100 ml of water. The resulting solution contains the equivalent of 1000 $\mu g\ ml^{-1}$ of pantothenic acid.

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Voltammetric Reduction of Cations in Mixed Solvents*

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By using a mercury-pool indicator and a molybdenum-wire reference electrode, the reduction of various ions, including Cd^{2+}, Tl^{+}, Eu^{3+}, CrO_4^{2-}, was studied on fast voltage-scanning instruments. The shifts in the reduction potentials were studied for different mixtures of water with methanol, propanol, acetone, methyl cyanide, dioxan and formamide. For all the ions under test except CrO_4^{2-}, in every solvent except water - formamide, the shift is towards negative potentials. In water - formamide the shift is towards more positive potentials. The relevant factors determining the shift in potential, such as solvation, ion-pair formation, increased resistance of the solution, adsorption, structure of the solvent and composition of the supporting electrolyte, were considered. The contribution of factors other than solvation was found to be minimal. For the ions considered it was found that the changes in the activity coefficients of the metal ions for various aqueous mixtures of organic solvents play an important part in shifting the reduction potential. The theoretical shifts were calculated and compared with the experimental observations. The trend in both cases was found to be in the same direction.

FOLLOWING the addition of an organic solvent to an aqueous solution, the half-wave potential of the electroactive ion shifts either to a more positive or a more negative potential. Several reasons have been given to explain these shifts,[1–3] chief among them being solvation of the metal ion associated with the change in dielectric constant.

The factors that lead to a positive shift of peak potentials are: a decrease in dielectric constant, changes in ion - dipole interactions, reduced solvating properties of the solvent with reduced donor properties, greater liquid-junction potentials of the reference electrode and a reduction in the basic nature of the organic solvent compared with water, leading to reduced solvation in the solvent. Conversely, the following factors lead to negative shifts: ion-pair formation by the electroactive ion as well as by the supporting electrolyte, adsorption of the solvent on the electrode surface, complex formation with solvents, specific interaction with the solvents, increased resistance of the solution, a decrease in the electrode reaction rate, an increase in the viscosity of the solution, greater solvation of the supporting electrolyte, particularly the anion, and the more acidic nature of the solvent compared with water.

In any given situation one or more of the reasons stated above may apply, and it becomes very difficult to predict the shift in a particular solvent for a particular ion. It is therefore necessary to study the reduction of several cations in a number of solvents in order to reach some general conclusions.

Solvents can be classified in several different ways, *viz.*, according to their chemical character, differentiating and levelling properties, affinity towards H^+ or OH^- ions, or co-ordinating tendency.

The solvents used in this work were formamide, methyl cyanide, methanol, acetone, propanol and dioxan; all are miscible with water. As Table I shows, they present a wide cross-section of different solvent characteristics. The range of dielectric constants varies from 2·23 for dioxan to 109·50 for formamide.

Choice of the electrode system when using mixed and non-aqueous solvent media presents difficulties. The conventional dropping-mercury electrode is not very reliable as the solvent tends to creep up the capillary, making the drop-time irregular. Pool-type mercury electrodes were therefore used. A cup-type electrode was tried first but the results obtained were not very reliable. A J-type mercury-pool electrode was then used and was

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found to give satisfactory results. Similarly, the usual calomel electrode could not be used with reliability in this work because of the high junction potential generated across its boundary. In our laboratory a molybdenum wire has been found to be an excellent reference electrode,[4-8] and proved to be satisfactory in the present work.

TABLE I

CHARACTERISTICS OF WATER-MISCIBLE SOLVENTS

Solvent	Nature	Dielectric constant at 25 °C	Dipole moment	Viscosity at 25 °C/cP	Density at 25 °C/ g cm^{-3}
Formamide	Basic	109·50	3·37	3·302	1·1292
Methyl cyanide ..	Polar electron donor	37·45 (20 °C)	3·37	0·325 (30 °C)	0·7768
Methanol	Amphiprotic	32·66	1·66	0·445	0·7867
Acetone	Amphiprotic	20·25	2·72	0·316	0·7846
Propanol	Amphiprotic	19·70	1·66	2·004	0·7995
Dioxan	Non-polar electron donor	2·23	0·45	1·087	1·0269
Water	Amphiprotic	78·54	1·84	0·8937	0·9973

EXPERIMENTAL

APPARATUS—

Cup-type mercury-pool electrode—This electrode was made by bending Pyrex tubing (0·5 cm i.d.) into a J form, the length of the two arms being 7·5 and 1·0 cm. A cup was sealed on to the top of the short arm. A short platinum wire with its ends in the cup and tube served to make electrical contacts. The cup was filled to the top with treble-distilled mercury, which acts as a cathode.

J-type mercury-pool electrode—This electrode was made by bending a Pyrex tube of uniform diameter into a J form with no cup at the top of the shorter arm. The J-tube was filled with treble-distilled mercury so that the level of mercury in the short arm was about 1 mm below the tip. Electrical contact was made by platinum wire passed through the longer arm. The mercury meniscus in the shorter arm served as the cathode.

Polarograph—Most of the work reported in this paper was carried out on a Model A1660 differential cathode-ray polarograph, manufactured by Southern Analytical Instruments.

Beckman instrument—A three-electrode system involving a J-type mercury pool (area 0·1215 cm^2), platinum-wire auxiliary and molybdenum-wire reference electrodes was used in the work carried out on an Electroscan-30. The Electroscan-30, manufactured by Beckman Instruments Inc., is a versatile instrument that can be used in all the electro-analytical techniques requiring either constant potential or constant current.

Beaker-type cell—A 50-ml beaker, fitted with a rubber bung containing five holes, served for a polarographic cell and was used mainly in the work with the Electroscan-30.

Semi-micro cell—The semi-micro cell used with the cathode-ray polarograph was similar to the J-type mercury-pool electrode described above. The i.d. of the J-form capillary was 0·156 cm. The wider tube, fused to the short arm of the J-tube, had an i.d. of 0·6 cm and a height of 6·0 cm, and served as the cell to hold 0·5 to 1 ml of the solution. The molybdenum-wire anode was inserted into the wider tube through a rubber bung.

REAGENTS AND SOLUTIONS—

AnalaR grade methanol, acetone and dioxan, supplied by B.D.H., were used. Pure propanol was supplied by Fluka A.G. (Switzerland) and pure formamide and methyl cyanide were supplied by Aldrich Chemical Co. Inc., Milwaukee, Wisconsin, U.S.A.

All other reagents used in the work were either of AnalaR grade, from B.D.H., or G.R. grade, from E. Merck.

NITROGEN PURIFICATION—

Nitrogen, for the de-aeration of the polarographic solutions, was purified by passing the gas through a train of acidified ammonium metavanadate solutions equilibrated with zinc amalgam,[9] and then through a solution having a composition similar to that of the medium of the test solution.

TEMPERATURE—

The cells were kept in a toluene-regulated and thyratron relay operated thermostat. All the work was carried out at 25 ± 0·1 °C.

RESULTS AND DISCUSSION

The reduction of Tl^+ and Eu^{3+} was studied in 0·02 M potassium nitrate solution as supporting electrolyte in all the organic solvent - water mixtures. The proportion of the organic solvent varied from 0 to 95 per cent. by volume. In addition, the reduction of Cd^{2+} in 0·1 and 0·05 M potassium chloride, Pb^{2+} in 0·1 M potassium chloride, Sb^{3+} in 1 M hydrochloric acid and CrO_4^{2-} in 0·5 M sodium hydroxide, as well as Tl^+ in 0·07 M sodium sulphate and Eu^{3+} in 0·1 M ammonium chloride, was studied in aqueous mixtures containing up to 90 per cent. of methanol, acetone and formamide. For each cation, the linearity of the current *versus* concentration graph was confirmed in a given solvent mixture, a typical curve for Tl^+ being given in Fig. 1 (*a*).

For a fixed concentration, 0·5 mM of Tl^+, the changes in peak current and peak potentials with increasing methanol concentration are shown in Fig. 1 (*b*).

Comparative shifts of peak current and peak potential with different solvent compositions for the ions studied are shown in Fig. 2. Over-all observations regarding the peak currents and potentials are summarised in Tables II and III. Some of the more important findings are discussed below.

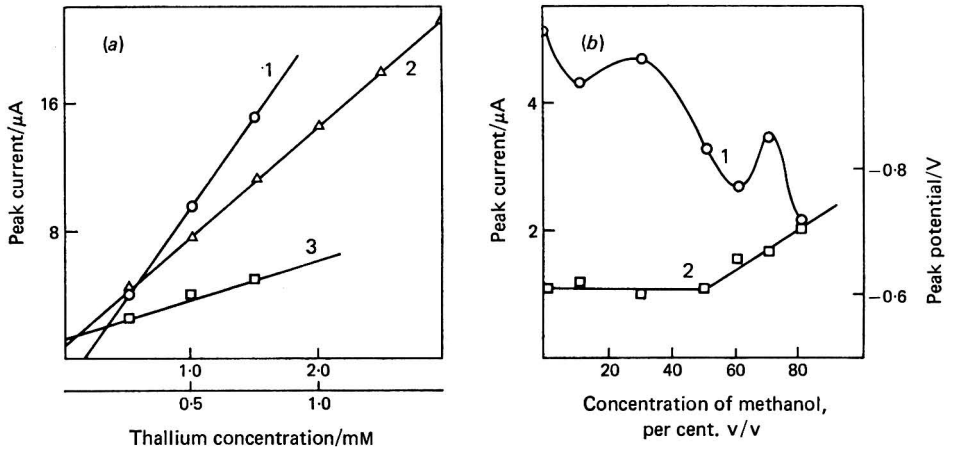

Fig. 1. Thallium ion reduction in water - methanol. Semi-micro cell, mercury-pool cathode and molybdenum-wire anode connected to differential cathode-ray polarograph. (*a*), Graphs of peak current against Tl^+ concentration at methanol concentrations of 30 per cent. (1), 50 per cent. (2) and 80 per cent. (3). Lines 1 and 2 were determined in the higher range of Tl^+ concentration, and line 3 in the lower range. Supporting electrolyte, 0·0704 M sodium sulphate solution. (*b*), Graphs of peak current (1) and peak potential (2) against concentration of methanol. Tl^+ concentration, 0·5 mM; supporting electrolyte, 0·1408 M sodium sulphate solution

PEAK CURRENTS (i_p)—

Peak currents in general decreased with increasing organic solvent concentration, except for the reduction of Eu^{3+} in acetone and formamide and the reduction of Tl^+ in formamide, dioxan and methyl cyanide. On addition of the solvent, in some instances the current became greater than in water and then decreased afterwards. In many instances, however, the current gave maxima and minima as shown in Fig. 1 (*b*), the former occurring between 20 and 30 per cent. and the latter between 70 and 90 per cent. of organic solvent (Fig. 2). The probable explanation is that the solvent molecules enter the primary hydration sheath of the cation, changing the radius of the solvated entity.[10] The minima may be explained either by Walden's rule, when for solvents with low dielectric constants the minimum equivalent conductivity occurs at concentrations of about 70 to 90 per cent. of solvent, or, as has been

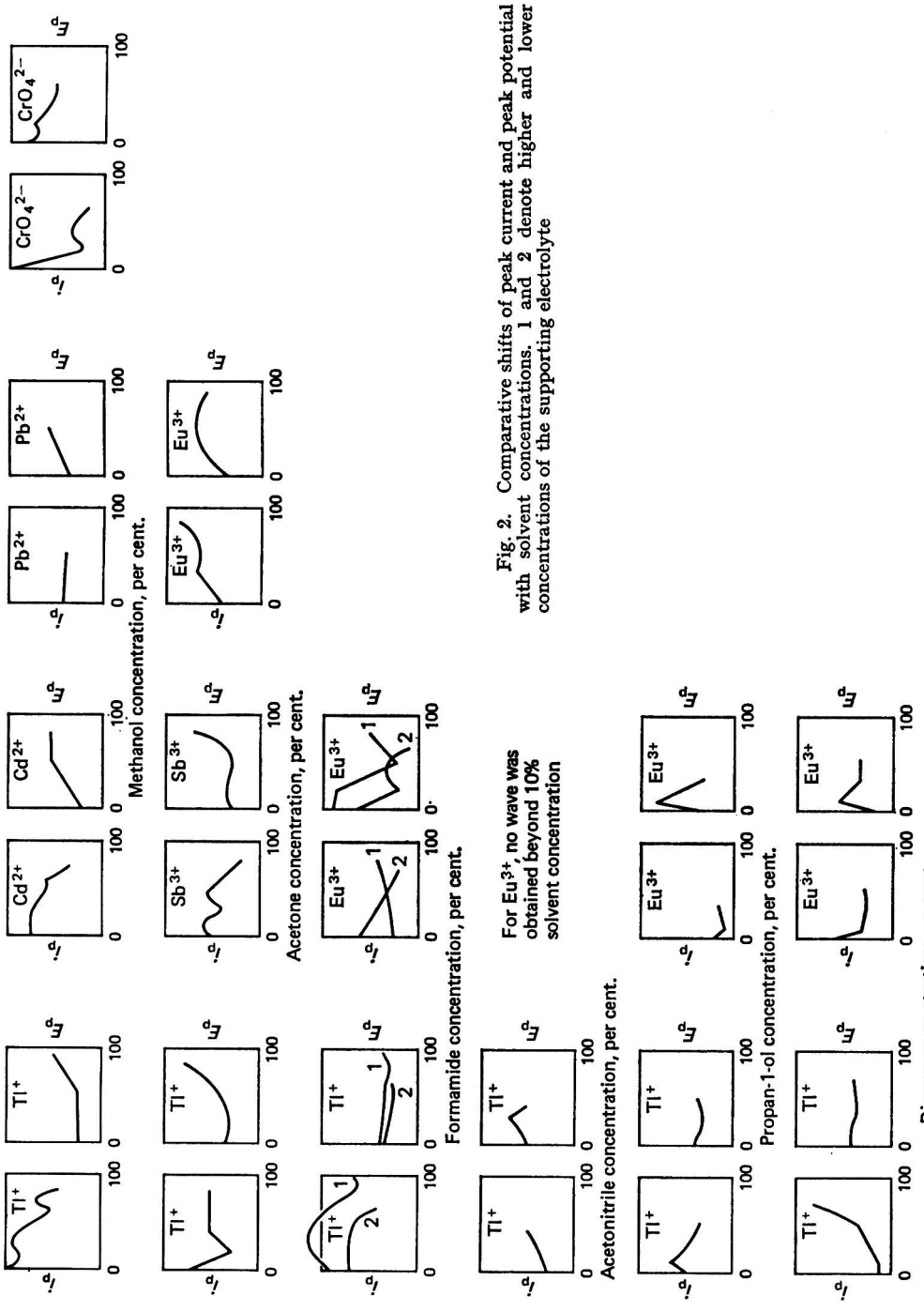

Fig. 2. Comparative shifts of peak current and peak potential with solvent concentrations. 1 and 2 denote higher and lower concentrations of the supporting electrolyte

found with methanol, the fact that the basicity of the methanol - water system passes through a minimum at about 70 per cent., reflecting in the minimum of the peak current.[11]

The extent of the current shift does not bear any relation to the nature or charge of the ion. For example, in acetone - water the current for Eu^{3+} increases by 0·21 μA whereas that for Sb^{3+} decreases by 20·4 μA. Neither is it related to the dielectric constant, because in 50 per cent. methanol - water and 40 per cent. acetone - water mixtures, which have similar dielectric constants, the current for 1 mM of Tl^{+} is greater by 2·0 μA in the latter solvent. By using the differential cathode-ray polargraph, i_p is usually found to be proportional to $n^{3/2}$, but this does not apply with those solvents for which the trend is towards i_p being proportional to n.

TABLE II

CHANGES IN E_p AND i_p

Organic solvent	Metal ion concentration	Change in solvent volume, per cent.	Extent of shift: i_p/μA	Extent of shift: E_p/mV	Remarks
Methanol	1·0 mM Cd^{2+}	0 to 70	−3·30	−160	Peak changes to S-shape at higher solvent concentrations.
	0·5 mM Tl^{+}	0 to 80	−3·00	−100	i_p decreases, minima at 10 and 60 per cent., maxima at 30 and 70 per cent. E_p constant up to 50 per cent., negative shift afterwards.
	1·0 mM CrO_4^{2-}	0 to 60	−3·40	+150	i_p sharply decreases up to 20 per cent., then remains constant up to 30 per cent., rises at 40 per cent. and then decreases.
	1·0 mM Pb^{2+}	0 to 50	−0·25	−110	
Acetone	0·6 mM Cd^{2+}	0 to 80	−3·40	−140	
	1·0 mM Tl^{+}	0 to 40	−1·20	Nil	+20 mV shift at 20 per cent. and no shift at 40 per cent.
	0·2179 mM Eu^{3+}	0 to 80	+0·21	−140	i_p increases.
	0·5 mM Sb^{3+}	0 to 80	−20·4	−180	i_p maxima at 10 and 40 per cent., minimum at 30 per cent.
	1·0 mM Pb^{2+}	0 to 40	−11·1	−340	
Formamide	0·1 mM Cd^{2+}	0 to 80	+0·09	−10	
	0·5 mM Sb^{3+}	0 to 80	−25·56	+20	
	0·5 mM Tl^{+}	0 to 95	−1·44	+30	
	0·1666 mM Eu^{3+}	0 to 80	+0·469	+0·255	E_p shows positive shift, maximum of 380 mV at 50 per cent.

Apparatus: differential cathode-ray polarograph; semi-micro cell; mercury-pool cathode; molybdenum-wire anode.

PEAK POTENTIALS (E_p)—

The E_p shift bears no relation to the size of the ionic charge, but is dependent on its sign. For example, in methanol a negative shift is obtained for all cations and a positive shift only for CrO_4^{2-}. The shift is also independent of the dielectric constant. For Tl^{+} in 50 per cent. methanol - water and 40 per cent. acetone - water mixtures the peak potentials differ by 50 mV.

For the majority of ions the shift is towards more negative potentials in all solvents except formamide and propanol. The maximum negative shift obtained is for Pb^{2+}, 340 mV in 50 per cent. acetone. Maxima and minima are obtained in the graph of E_p *versus* the percentage of organic solvent present, but not to the same extent as in the case of the graphs of i_p *versus* the percentage of organic solvent present in the solvent mixture.

GENERAL CONSIDERATIONS

SUPPORTING ELECTROLYTE—

The supporting electrolyte assumes an unusual importance in mixed-solvent work. The peak potential of Tl^{+} in 70 per cent. formamide and a concentration of 0·05 M of sodium sulphate in the solution is positive compared with that in a 0·1 M solution of the salt. Moreover, in the former concentration the peak currents are double those in the latter (Fig. 2).

Similarly, for Eu^{3+} the concentration of the supporting electrolyte becomes important, unlike that in aqueous solution, probably because the solvation of the ions of the supporting electrolyte also affects the results. For 0·5 mM Tl^+ in 50 per cent. methanol with a 2 per cent. sodium sulphate solution as supporting medium, almost no change is obtained in E_p, whereas with 0·02 M potassium nitrate solution, E_p shifts by 180 mV towards more positive potentials in the same solvent mixture. The peak current is decreased by 0·78 μA in the former electrolyte and 2·1 μA in the latter. It is, therefore, necessary to specify accurately the concentration of the supporting electrolyte in this work.

TABLE III

CHANGES IN E_p AND i_p

Organic solvent	Metal ion concentration	Change in solvent volume, per cent.	Extent of shift		Remarks
			$i_p/\mu A$	E_p/mV	
Dioxan	0·2 mM Tl^+	0 to 50	+6·6	+50	
	0·2 mM Eu^{3+}	0 to 50	−1·5	−80	
Propanol	0·2 mM Tl^+	0 to 50	−1·0	+20	
	0·2 mM Eu^{3+}	10 to 30	+1·4	+210	Well defined peak at 30 per cent.
Methyl cyanide	0·2 mM Tl^+	10 to 50	+4·3	Practically no change	
	0·2 mM Eu^{3+}	10 to 50			Wave at 10 per cent. only. No wave at 30 and 50 per cent.

Apparatus: Electroscan-30; J-type mercury-pool cathode; molybdenum-wire anode; platinum-wire auxiliary electrode.

The results show that the shift is not dependent on the nature of the solvent or on ion-pair formation, which takes place at the macroscopic dielectric constant of about 15.[12] For the solvents used in the present work, even at a 90 or 95 per cent. level, the dielectric constant hardly goes down below 20. Moreover, the negative shift occurs even when only a small amount of solvent is added to the water and the dielectric constant is considerably higher.

The donor number theory[13] also cannot account for the negative shift in most of the solvents, because according to this theory, water is nearer to acetone than methyl cyanide, but compared with water the potential shifts are on the negative side for all cations in both solvents. Again, the negative shift cannot be explained as being due to adsorption, although this becomes important in purely non-aqueous solvents. Considerable changes are observed even in 10 to 30 per cent. of organic solvent, when adsorption is not significant.

The structure of the solvent seems to have very little bearing on the results. For example, the E_p shift is greater for methanol than for propanol. The current for 0·2 mM Tl^+ is 1·5 times greater in 50 per cent. propanol than in methanol. The Tl^+ ion is generally considered not to be solvated and has been used to compare results in non-aqueous media.[14] Our results show otherwise. Except for propanol and dioxan, considerable changes in peak potentials and currents occur in all solvents (Table IV).

The increased resistance of the solution is also not responsible for the negative shift. The resistance of the solution was measured and the shift due to the increased resistance calculated. The actual shift obtained was found to be 50 to 100 times greater than that obtained due to the increased resistance alone.

TABLE IV

REDUCTION OF Tl^+ IN AQUEOUS SOLVENTS

Solvent	Dielectric constant	$i_p/\mu A$	E_p/V
Water	78·54	7·2	−0·65
50 per cent. methyl cyanide	58·25	11·5	−0·66
50 per cent. methanol	56·28	5·1	−0·47
50 per cent. acetone	48·2	9·0	−0·54
50 per cent. propanol	42·50	8·8	−0·58
50 per cent. dioxan	34·26	8·4	−0·56

Tl^+ concentration 0·2 mM; supporting electrolyte 0·02 M potassium nitrate; apparatus: Electroscan-30; J-type mercury-pool cathode (0·1215 cm); platinum-wire auxiliary electrode; molybdenum-wire anode; voltage scan rate 100 mV s^{-1}.

SOLVATION—

The extent of solvation of ions is considered to be related to the dielectric constant, *i.e.*, the smaller the dielectric constant, the less the solvation contributes to the positive shift of the peak potential. The dielectric constant is, however, a poor measure of solvation as it fails to take into account specific interactions of the solvent, including complexation and hydrogen and other types of bonding. It does not automatically follow, therefore, that with decreasing dielectric constant E_p will shift towards more positive potentials. Whenever the potential is shifted to more positive values, however, it can undoubtedly be considered that the ion involved is less solvated. This occurs in the instances of Tl^+ reduction in propanol, Eu^{3+} reduction in formamide and CrO_4^{2-} reduction in methanol.

A negative shift is not a certain indication that the ion is more solvated. This will become clear from the following discussion.

EFFECT OF ACTIVITY COEFFICIENTS—

The effect of activity coefficients has not been considered thoroughly, although the effect of dielectric constant on ions and ionic dissociation is mentioned in the literature.[1] As will become clear, this effect is substantial.

For a reversible reduction of a simple ion, $(E_{\frac{1}{4}})_s$, the following equation applies—

$$(E_{\frac{1}{4}})_s = E_s^\circ - \frac{RT}{nF} \ln \left(-\frac{f_a\, k_s}{f_s\, k_a} \right) \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where E_s° is the standard potential of the metal ion - metal amalgam couple, f_a and f_s are the activity coefficients of the metal atoms in the amalgam and the solution, respectively, k_a is the ratio of the anodic diffusion current to the concentration of metal in the amalgam, k_s is the ratio of the cathodic diffusion current to the concentration of metal ion in solution, T is the absolute temperature, R is the gas constant, F is Faraday's constant and n is the number of electrons involved in the reduction of an anion.

According to the Ilkovic equation—

$$-\frac{k_s}{k_a} = \left(\frac{D_s}{D_a}\right)^{\frac{1}{2}} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

where D_s and D_a are the diffusion coefficients of the metal ion in the solution and metal in the very dilute amalgam formed at the dropping-mercury electrode, respectively.

Substituting equation (2) in equation (1)—

$$(E_{\frac{1}{4}})_s = E_s^\circ - \frac{RT}{nF} \ln \left(\frac{f_a\, D_s^{\frac{1}{2}}}{f_s\, D_a^{\frac{1}{2}}} \right) \quad .. \quad .. \quad .. \quad .. \quad (3)$$

According to Kolthoff,[15] $\left(\frac{D_s}{D_a}\right)^{\frac{1}{2}}$ normally has the value unity, and as no metal ions are present in the mercury-pool cathode initially, the value of f_a may also be taken as unity. Therefore, $f_a \left(\frac{D_s}{D_a}\right)^{\frac{1}{2}} = 1$.

Equation (3) then becomes, at 25 °C—

$$(E_{\frac{1}{4}})_s = E_s^\circ + \frac{0{\cdot}059\ 15}{n} \log f_s \quad .. \quad .. \quad .. \quad .. \quad (4)$$

The Debye-Hückel equation is—

$$-\log f_s = \frac{1{\cdot}82 \times 10^6 \times Z_i^2\, I^{\frac{1}{2}}}{(\epsilon T)^{3/2}\, [1 + 50\, (\epsilon T)^{-\frac{1}{2}}\, a^\circ I^{\frac{1}{2}}]} \quad .. \quad .. \quad .. \quad (5)$$

where f_s is the activity coefficient of a metal ion in solution, ϵ is the dielectric constant of the solvent, Z_i is the ionic charge, I is the ionic strength and a° is the ion size parameter.

Substituting the value of $\log f_s$ thus obtained in equation (4), we obtain—

$$(E_{\frac{1}{4}})_s = E_s^\circ - \frac{0{\cdot}059\,15}{n} \times \frac{1{\cdot}82 \times 10^6 \times Z_i^2\, I^{\frac{1}{2}}}{(\epsilon T)^{3/2}\, [1 + 50\, (\epsilon T)^{-\frac{1}{2}}\, a^\circ I^{\frac{1}{2}}]} \quad .. \quad .. \quad (6)$$

The term 50 $(\epsilon T)^{-\frac{1}{2}}$ $a^{\circ}I^{\frac{1}{2}}$ is much less than unity and can be neglected. Equation (6) then becomes—

$$(E_{\frac{1}{2}})_s = E_s^{\circ} - \frac{0{\cdot}059\,15}{n} \times \frac{1{\cdot}82 \times 10^6 \times Z_i^2\, I^{\frac{1}{2}}}{(\epsilon T)^{3/2}} \quad .. \quad .. \quad (7)$$

Equation (7) is used to calculate $(E_{\frac{1}{2}})_s$ values, inserting the values of I, ϵ and T.

The changes in peak potential in various mixtures of the organic solvent and water have been calculated for different ions (Table V). It can be seen that when the dielectric constant decreases as more organic solvent is added, the shift always tends to take place in the negative direction. The extent of the calculated shift is also considerable, *e.g.*, for 1 mM of Cd^{2+} in a mixture of 0·1 M potassium chloride in 90 per cent. dioxan the potential is 256·8 mV, while in 100 per cent. dioxan it becomes 1105 mV for an ionic strength of 0·104.

The observed and calculated shifts for Tl^+ are near to each other in 10 per cent. dioxan and they differ in methanol by 8·8 mV with 10 per cent. of solvent, 42·5 mV with 60 per cent. of solvent and 74·9 mV in 80 per cent. of solvent, the observed shift being greater than that calculated. Similar results are obtained for Tl^+ in propanol. In 60 and 90 per cent. formamide the potential shift for Tl^+ occurs in the positive direction, the observed shift being much greater than that calculated. The dielectric constant of formamide in various aqueous mixtures is always greater than that of pure water. A positive shift is obtained in formamide for all the other metal cations tested, although it is not included in Table V.

TABLE V

EFFECT OF ACTIVITY COEFFICIENTS ON $E_{\frac{1}{2}}$ AND E_p

Volume of organic solvent, per cent.	Dielectric constant	Metal ion concentration and supporting electrolyte	Changes in $E_{\frac{1}{2}}$ or E_p compared with that in water: $E_{\frac{1}{2}}$/mV (calculated)	E_p/mV (observed)
60 per cent. formamide ..	97·0	0·4 mM Tl^+, 0·1 M Na_2SO_4	+4·48	+40·0
90 ,, ,, ,, ..	106·5	0·5 mM Tl^+, 0·05 M Na_2SO_4	+5·1	+40·0
10 per cent. methanol ..	75·5	0·5 mM Tl^+, 0·1408 M Na_2SO_4	−1·20	−10·0
60 ,, ,, ,, ..	56·5	0·5 mM Tl^+, 0·1408 M Na_2SO_4	−12·50	−55·0
80 ,, ,, ,, ..	44·0	0·5 mM Tl^+, 0·1408 M Na_2SO_4	−25·10	−100·0
30 per cent. propanol ..	61·0	0·2 mM Tl^+, 0·02 M KNO_3	−1·98	−30·0
50 ,, ,, ,, ..	47·0	0·2 mM Tl^+, 0·02 M KNO_3	−4·97	−20·0
50 per cent. dioxan ..	34·0	0·2 mM Tl^+, 0·02 M KNO_3	−10·75	−80·0
20 per cent. methanol ..	72·0	1 mM Cd^{2+}, 0·1 M KCl	−0·676	−60·0
50 ,, ,, ,, ..	59·0	1 mM Cd^{2+}, 0·1 M KCl	−2·60	−160·0
60 ,, ,, ,, ..	56·5	1 mM Cd^{2+}, 0·1 M KCl	−3·10	−160·0
80 per cent. acetone ..	32·5	0·6 mM Cd^{2+}, 0·05 M KCl	−9·49	−140·0

The observed change for Cd^{2+} peak potentials in all the solvents, except formamide, is also more negative than that calculated.

The results show that in all instances the direction for the observed and calculated shifts is the same, although the magnitude of the shift is different.

The calculations referred to above have been made on the basis of the macroscopic dielectric constant. However, in the vicinity of the ion, the molecular solvent dipoles are influenced predominantly by the field of the ion, so that the effective dielectric constant near the ion is less than that in the bulk of the solution.[15] The use of the macroscopic dielectric constant is the most serious error in Born's theory. It is, therefore, logical to assume that if the potential shifts were calculated on the basis of microscopic dielectric constants, instead of the macroscopic ones, the magnitude of the observed and calculated shifts would be comparable. Unfortunately, because the values of microscopic dielectric constants were not available and cannot readily be measured, these calculations could not be made.

The contribution from solvation should not be underestimated. Nevertheless, for low percentages of solvent, when significant solvation does not occur, the changes in activity coefficient due to the dielectric constant, macroscopic or microscopic, assume great importance. To summarise, the dielectric constant gives rise to effects that shift the potentials in opposite directions; in the positive direction, owing to decreasing solvation with decreasing dielectric constant, and in the negative direction, owing to changes in the activity coefficients caused by the decrease in the dielectric constant.

This work has revealed that the contribution due to the changes in the activity coefficients of the metal ion towards the changes in peak potentials is as important as the contribution due to solvation. At low solvent concentrations it is even more important. By taking into account the changes in the activity coefficients it is now possible to predict the direction in which the peak potential will shift, but not the exact magnitude of the shift.

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The Coulometric Determination of Sulphur Dioxide by Using Differential Electrolytic Potentiometry for End-point Location

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The application of differential electrolytic potentiometry to end-point detection in the coulometric titration of sulphur dioxide is described; the results obtained with simple, semi-automatic apparatus on samples of sulphur dioxide of the order of 10^{-6} mol are discussed. The method is rapid, sensitive, precise and simple.

COULOMETRY is now established as one of the most important and reliable methods available for the determination of sulphur compounds, especially at low concentrations. The commonest procedure involves the electrogeneration of bromine from an acidic bromide medium and the location of the end-point by any one of a number of electroanalytical techniques; such techniques have recently been reviewed by Bishop.[1]

During the course of kinetic studies it became necessary to determine sulphur dioxide in aqueous samples rapidly and as a matter of routine, in the concentration range 10^{-4} to 10^{-5} M, to an accuracy of better than 1 per cent. It was necessary that the procedure should be semi-automated and rapid for the routine work although speed was also essential because of the volatility and moderately fast aerial oxidation of sulphur dioxide.

In coulometric titrations of this type, potentiometry has provided the most trouble-free method for end-point detection, being less dependent on temperature, stirring speed, electrode condition and related parameters than amperometry and other alternative methods. Of the various potentiometric methods available, differential electrolytic potentiometry (DEP) offers the greatest advantages in terms of rapidity of response and sensitivity, as well as requiring comparatively simple apparatus.

DEP has previously been used for end-point detection in the microcoulometric titration of hydrazine,[2] whereas bipotentiometry, with its much larger and less stable indicator currents, has been applied in the coulometric determination of bromine number.[3]

APPARATUS—

Titration cell—The cell consisted of a glass tube of length 6 cm and internal diameter 2 cm, sealed and flat at the base and having three side-arms of internal diameter 1·5 cm that radiated at 45° to the stem from a point about 3 cm from the base. The capacity of the cell was approximately 10 cm^3 and the sample was introduced through the open top by pipette. The three side-arms accommodated: (1), the agar-gel bridge to the remote cathodic auxiliary electrode and the generating anode; (2), the DEP electrodes; and (3), the emptying and rinsing tube. The cell is illustrated in Fig. 1.

Electrodes—The coulometric generating and auxiliary electrodes consisted of 2-cm long 22 s.w.g. platinum wires. The auxiliary electrode was immersed in dilute hydrochloric acid in a beaker and connected to the cell by a short length of glass tubing filled with 10 per cent. agar in saturated potassium chloride solution.

The DEP electrodes were made from two 30 s.w.g. platinum wires sealed into the end of a single soda-glass tube and insulated inside with glass-fibre sleeving. A 1-cm length of each wire protruded from the glass and was bent into a circle.

Coulometric circuit—A constant-current supply was constructed from two Fenlow A2 chopper-stabilised operational amplifiers.[4] When the circuit had warmed up the stability of the output current easily exceeded the required $\pm 0{\cdot}1$ per cent. This supply was switched in four nominal ranges of 1, 5, 10 and 25 mA; only the lowest range was used in these experiments. The generating current was started by a switch that simultaneously triggered the timer (S.E. Ltd., Model SM200). The current was measured by monitoring the potential drop across a standard 100-Ω resistor.

DEP circuit—The two DEP electrodes, incorporated in the same probe, were in series with a high-value resistance box (10^6 to 10^{12} Ω) and a high-voltage d.c. supply (0 to 1000 V in steps of 100 V).[5] The high values of voltage and resistance ensured a virtually constant indicating current between the DEP electrodes. The potential across the electrodes was measured with a E.I.L. 33B electrometer connected to a Honeywell-Brown chart recorder (Model 153 X 17). The recorder was fitted with a simple two-relay circuit, activated by reed switches, enabling a fall in potential to switch off the timer at a pre-selected voltage. A diagram of the circuit is included in Fig. 1.

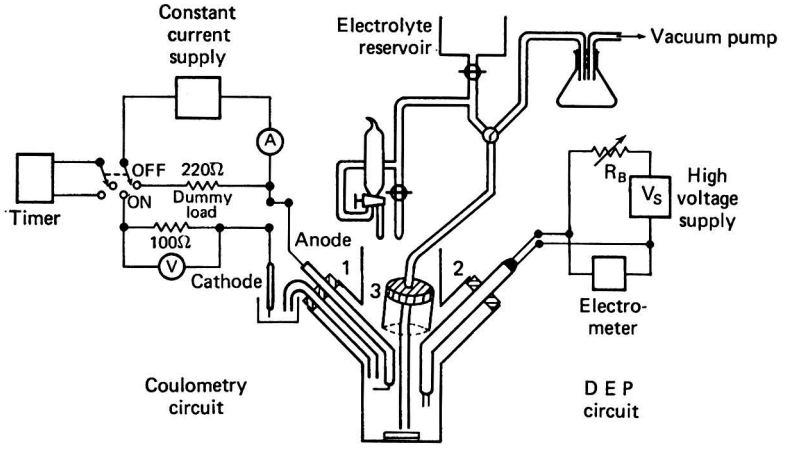

Fig. 1. Diagram of the apparatus, showing the cell assembly

SOLUTIONS—

The base electrolyte, of concentration 0·5 M with respect to potassium bromide and to hydrochloric acid, and also the iodine and standard sodium thiosulphate solutions, were prepared from AnalaR chemicals. The sulphur dioxide solutions were prepared by bubbling sulphur dioxide from cylinders into distilled water.

PROCEDURE—

Before each analysis the titration cell was rinsed twice with base electrolyte from an overhead reservoir, the cell being emptied each time by means of the vacuum system (operated by a water-pump that was attached to a tube in one of the side-arms of the cell). The cell was then charged with 5 ml of electrolyte from an automatic pipette mounted over the cell and the appropriate sample volume of sulphur dioxide solution was run into the cell by pipette. The solution was stirred with a small magnetic paddle. Having primed the relay circuit, the coulometric circuit was switched on, thus starting the timer (the DEP circuit was left on continuously). At the end-point, the drop in potential across the indicator electrodes switched off the timer and the digital time display was recorded.

RESULTS

In these experiments, at low sulphur dioxide concentrations, a low generating current must be used. Thus, for a 5-ml sample a current of 1 mA generates bromine at a rate equivalent to approximately 10^{-6} mol l^{-1} s^{-1} of sulphur dioxide. Because of instrumental electrical and mechanical lag at such low generation rates, there is a short time interval between the equivalence point being reached and the end-point being recorded. This interval can be minimised by adjustment of the voltage and resistance in the DEP circuit and the pre-set cut-off potential; however, the voltage and resistance adjustments also affect the stability of the indicator signal, and thus conditions must be found under which the time interval is both short and reproducible, and also the potential jump at the end-point is large.

The time lag was determined by analysing volumes of sulphur dioxide solution with

a ratio between successive volumes of 2:1; comparison of the various generation times allowed the time lag to be calculated.

A set of typical results is shown in Table I.

TABLE I

APPRAISAL OF TITRATION PARAMETERS

Experiment	High-voltage supply to DEP circuit (V_B)/V	Resistance at resistance box (R_B)/Ω	Indicator			
			Current density/A cm^{-2}	Time lag/s	Stability	Potential jump/mV
A	200	10^9	2×10^{-6}	9·0	Fair	900
B	100	10^{10}	10^{-7}	6·4	Poor	600
C	200	10^{10}	2×10^{-7}	7·2	Fair	700
D	1000	10^{11}	10^{-7}	5·6	Good	900

Generating current, 0·95 mA; sample concentration, 0·5 to 3 $\times$ 10^{-4} M.

These tests indicated that the conditions of experiment D were the most suitable. The magnitude of the potential jump at the end-point also varies with the age of the DEP electrodes. The electrodes used in these experiments had been in constant use for over 2 months; however, new electrodes produce jumps up to 200 mV smaller. With the conditions of experiment D and new electrodes, the recorder trace of the DEP signal during the course of a typical coulometric titration of a sulphur dioxide sample, close in size to the extreme lower limit set by the standards of precision and reproducibility required, was as shown in Fig. 2. The cut-off potential was usually chosen to be 200 mV below the maximum plateau; alteration of this value affects the magnitude of the time lag, but 200 mV was found to be the most convenient setting.

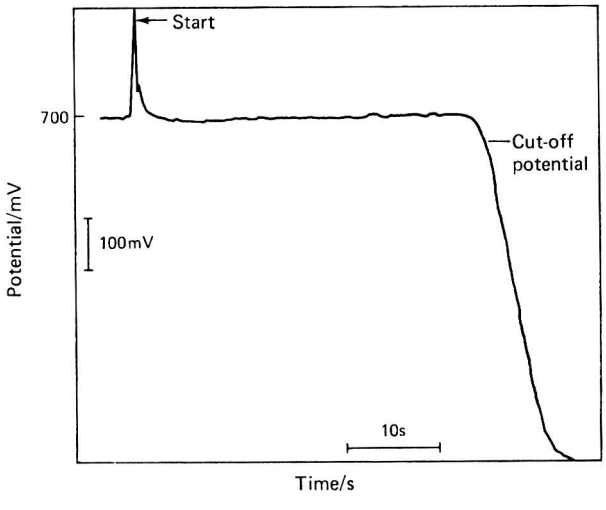

Fig. 2. A recorder trace of the DEP signal obtained during the coulometric titration of 1·72 $\times$ 10^{-7} mol of sulphur dioxide

A further important parameter is the bromide concentration. It was found by experiment that this concentration should be greater than 0·2 M in the final solution to prevent loss of efficiency in the anode reaction.

As the electrogeneration of bromine is virtually 100 per cent. efficient at this acidity and bromide concentration, and as the reaction between sulphur dioxide and bromine is fast even at these concentrations, the amount of sulphur dioxide in a sample can be calculated directly from Faraday's law. Nevertheless, analyses of standardised sulphur dioxide solutions were performed to check the system.

A strong solution of sulphur dioxide (about 0·04 M) was standardised by the iodine-thiosulphate method. A 2-ml volume of this standardised solution was then diluted to 200 ml with oxygen-free water, and samples of this diluted solution were analysed coulometrically to determine sulphur dioxide. A series of readings for each sample size was taken, and the readings were plotted against time elapsed since dilution on a graph and extrapolated back to zero time to correct for the aerial oxidation of the sulphur dioxide. The results of several experiments are tabulated in Table II; the current differed slightly in the various experiments, but was always approximately 0·95 mA. Before each series of readings the DEP electrodes were pre-conditioned by carrying out two or three runs on any dilute sulphur dioxide solution.

TABLE II

ANALYSES OF STANDARD SULPHUR DIOXIDE SOLUTIONS

Experiment	Number of readings	Sulphur dioxide taken/mol	Sulphur dioxide found/mol	Generating time/s	Relative error, per cent.
1	7	$1·989 \times 10^{-6}$	$1·991 \times 10^{-6}$	409·1	+0·1
2	9	$1·704 \times 10^{-6}$	$1·709 \times 10^{-6}$	351·6	+0·3
3	3	$7·96 \times 10^{-7}$	$8·01 \times 10^{-7}$	176·7	+0·6
4	5	$6·82 \times 10^{-7}$	$6·79 \times 10^{-7}$	148·7	−0·4
5	5	$3·98 \times 10^{-7}$	$4·01 \times 10^{-7}$	95·5	+0·7
6	6	$3·41 \times 10^{-7}$	$3·39 \times 10^{-7}$	79·8	−0·6

As expected, the analyses are more accurate at higher concentrations of sulphur dioxide, but the method remains useful down to sample sizes of 2×10^{-7} mol, or concentrations down to 4×10^{-5} M.

The correction for aerial oxidation was never more than 0·3 per cent., that is, 1 s in 400 s. Even in alkaline media the rate of loss is low, as is shown in Table III, and the standardised solutions are acidic with a consequently lower decay rate.

TABLE III

AERIAL OXIDATION OF SULPHUR DIOXIDE SOLUTION AT 26·5 °C

pH	Concentration of sulphur dioxide/mol l^{-1}	$-\frac{d[SO_2]}{dt}$/mol l^{-1} s^{-1}
8·18	$2·6 \times 10^{-4}$	$1·5 \times 10^{-9}$
8·17	$1·0 \times 10^{-4}$	$1·6 \times 10^{-9}$
7·42	$1·0 \times 10^{-4}$	$1·1 \times 10^{-9}$

An analysis requires no more than 30 s *plus* the generation time shown in Table II.

CONCLUSION

DEP provides a sensitive, rapid and simple method for end-point detection in the coulometric titration of dilute sulphur dioxide samples, producing a potential change of up to 900 mV at the end-point, which is independent of sulphur dioxide concentration.

A simple apparatus making use of this technique has now been in satisfactory routine operation for more than 6 months.

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Fractionation and Identification of Commercial Hydrocolloid Stabilising Agents

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A rapid, simple procedure is described for the separation and identification of the individual components of a mixture of common stabilisers, including agar, alginate, gum arabic, carrageenan, carboxymethylcellulose, methylcellulose, pectate, pectinate, gum tragacanth, guar gum and locust bean gum. Separation of the anionic stabilisers from the neutral stabilisers is effected initially by precipitation with cetylpyridinium chloride. Complete fractionation of the two major groups is then performed by using selective precipitation techniques. Reliable confirmatory tests are reported for all the stabilisers.

THE extensive use of polysaccharides as stabilising agents in the food industry has created the need for techniques for the identification and determination of these compounds when they occur in mixtures and in food products.

Several chemical and some physical methods are already available for the identification of stabilisers; however, many of them are unsatisfactory. For example, the procedure of Jacobs and Jaffe,[1] which classifies stabilisers according to their reaction with Millon's reagent, requires the operator to assess the physical appearance of a precipitate and thus assign the stabiliser in question to one of four groups. As all the stabilisers tested form a precipitate with Millon's reagent, the identification of the components of a mixture is impossible. The method of Cannon,[2] adopted by the Association of Official Agricultural Chemists,[3] is based on four group tests involving zinc chloride - iodine, tincture of iodine, ruthenium red and concentrated sulphuric acid. The colours produced by these reagents are observed microscopically and the stabiliser is assigned to a particular group on this basis. Here also, the components of mixtures cannot be fully resolved. Other schemes of precipitation reactions developed by several workers[4–7] are similarly unsuitable for mixtures. The analytical procedures available for stabiliser identification have been reviewed by Glicksman[8] and by Mantell.[9] The latter author concluded that no convenient method exists for the determination of the constituents of a mixture.

It is well established that polyanions form complexes with cationic detergents such as cetylpyridinium chloride (CPC) and that the concentration of neutral salt required to break such complexes depends on the nature of the polyanion.[10] By using this information, together with the results obtained by Deuel and co-workers[11,12] on the minimum amount of certain electrolytes required to coagulate a variety of polyanions, a successful fractionation scheme has been formulated.

EXPERIMENTAL

STABILISERS—

The following stabilisers were used: (1) agar-agar granular (Fisons Scientific Apparatus Ltd.); (2) Manucol SS/LH alginate (Alginate Industries Ltd.); (3) gum arabic powder (British Drug Houses Ltd.); (4) Kraysta M carrageenan (Kraft Food Industrial Division); (4) Gelcarin HMR carrageenan (Algin Corporation of America); (5) sodium carboxymethylcellulose (Hercules Incorporated); (6) guar gum MM (Meers Corporation); (7) locust bean gum PP2 200 (Meers Corporation); (8) methylcellulose (Hopkin and Williams); (9) pectate, as sodium polygalacturonic acid (Eastman Organic Chemicals); (10) pectinate as apple pectin, 250 grade (British Drug Houses Ltd.); and (11) gum tragacanth powder (one sample from Griffin and George Ltd. and the other from an unknown source).

SEPARATION AND IDENTIFICATION PROCEDURE—

A 0·5 per cent. solution of the stabiliser mixture is prepared by sprinkling the stabiliser on to the surface of vigorously agitated water. In this way, clumping and partial hydration of the stabiliser are avoided. The solution is then heated to 90 to 95 °C and stirred vigorously, cooled to room temperature, centrifuged and the clear liquid decanted. Clarification on cooling indicates the presence of non-ionic cellulose ethers, *e.g.*, methylcellulose, hydroxypropylcellulose (HPC), methylethylcellulose (MEC) or ethylhydroxyethylcellulose (E-EC). If the solution gels, agar is indicated. The solution must be diluted and re-heated until gelation does not occur on cooling. The formation of hydrated beads on the side of the vessel is characteristic of sodium carboxymethylcellulose (SCMC).

To a portion of the solution 2 volumes of 4 M sodium chloride solution are added followed by 1 volume of 1 per cent. CPC. Any precipitate that forms, which would indicate carrageenan, should be centrifuged off and the confirmatory test for carrageenan applied. An equal volume of 1 per cent. CPC is added to the bulk of the solution, as indicated in Fig. 1; any precipitate formed is allowed to flocculate and is then centrifuged off. If no precipitate forms, anionic stabilisers are absent. The precipitate is dissolved by stirring and heating it in 4 M sodium chloride solution. Only a carrageenan - CPC complex, the presence of which will have been indicated in the preliminary test above, will remain insoluble under these conditions.

The clear liquid from the precipitation with 1 per cent. CPC, containing neutral stabilisers, is divided into two portions. To the first portion 3 volumes of ethanol are added. At this stage, locust bean gum and guar gum will precipitate and the appropriate confirmatory tests should be applied. The second portion is boiled almost to dryness and centrifuged while hot. A precipitate formed on boiling indicates methylcellulose (or HPC, MEC or EHEC), and the confirmatory tests for cellulose ethers are applied.

The solution of CPC - polyanion complexes in 4 M sodium chloride solution prepared above is now treated with 3 volumes of ethanol and the resultant precipitate is washed with 80 per cent. ethanol, finally being dissolved in water to give an approximately 0·5 per cent. solution. To this solution an equal volume of 2 M hydrochloric acid is added and the solution centrifuged. The precipitate, which may contain SCMC, alginate or pectate, is dissolved in 0·2 M sodium hydroxide solution to give a 0·5 per cent. solution at pH 7. Addition of 20 per cent. v/v of trichloroacetic acid precipitates alginate and pectate, and confirmatory tests for these samples can be applied to separate portions after re-dissolving the precipitate in 0·2 M sodium hydroxide solution at a pH of just 7 (*i.e.*, the solution must not be made alkaline). The clear liquid from the precipitation step with trichloroacetic acid is neutralised with dilute sodium hydroxide solution (again, the solution is not made alkaline) and is then treated with 3 volumes of ethanol. At this stage the development of a precipitate indicates SCMC.

The clear liquid retained after precipitation with 2 M hydrochloric acid is now neutralised with dilute sodium hydroxide solution (but not made alkaline) and is then treated with 3 volumes of ethanol. The precipitate is re-dissolved in water (to give a 0·5 per cent. solution) and divided into three portions. To one portion 4 per cent. v/v of 1 M copper(II) sulphate solution is added and the solution is centrifuged. The clear liquid is decanted and a confirmatory test for gum arabic applied to it. The formation of a precipitate indicates the presence of pectinate. Then 4 per cent. v/v of 0·6 M iron(III) chloride solution is added to the second portion and the solution is centrifuged. The clear liquid is decanted and a confirmatory test for agar applied. A precipitate indicates the presence of pectinate and tragacanth or tragacanth alone. One volume of iron(III) chloride solution is added to the precipitate. If, under these conditions, the precipitate re-dissolves, tragacanth is indicated and pectinate is absent. Partial dissolution of the precipitate indicates a mixture of both compounds. The final portion of solution is used for the confirmation of pectinate; however, gum karaya will also give a positive result.

CONFIRMATORY TESTS—

Agar—To the clear liquid following treatment with iron(III) chloride solution 1 drop of 9 per cent. tannic acid solution is added. A green - black precipitate confirms agar.

Alginate[13]—To the re-dissolved precipitate formed with trichloroacetic acid an equal

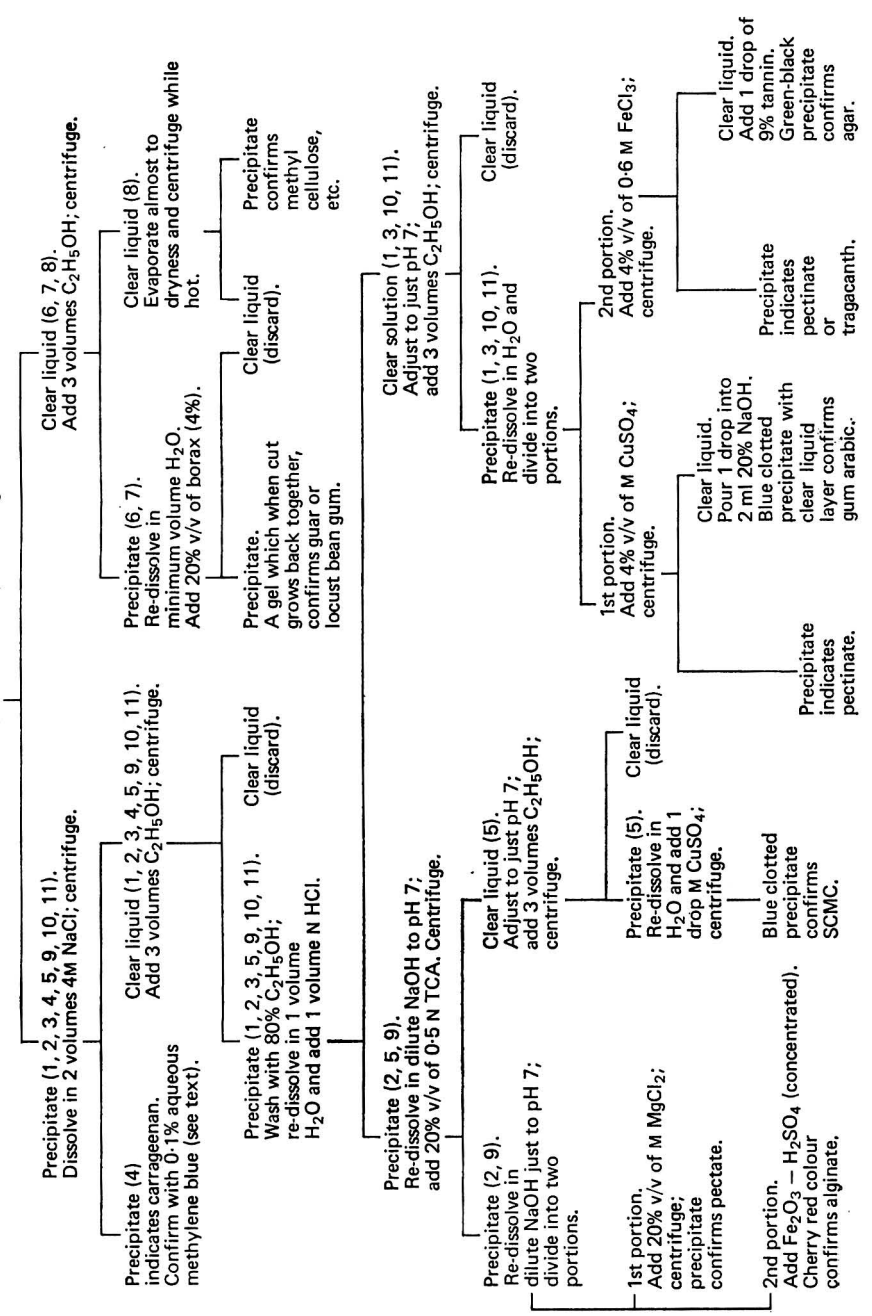

Fig. 1. Stabiliser fractionation scheme

volume of the iron(III) oxide - concentrated sulphuric acid mixture is added. The development of a cherry-red colour confirms alginate.

Gum arabic[9]—One drop of the clear liquid obtained after treatment with copper(II) sulphate solution is poured into 2 ml of 5 M sodium hydroxide solution. Formation of a blue, clotted precipitate with a clear solution above it confirms gum arabic.

Carrageenan[14]—The precipitate insoluble in 4 M sodium chloride solution is washed twice with water and dissolved in 70 per cent. propanol by application of heat. It is then re-precipitated with 2 volumes of ethanol *plus* 1 volume of 4 M sodium chloride solution and the precipitate is washed twice with 80 per cent. ethanol. The precipitate is finally re-dissolved in the minimum amount of water and 1 drop of 0·1 per cent. aqueous methylene blue is added. A blue - black fibrous precipitate confirms carrageenan.

SCMC—To the clear, neutral liquid resulting from the addition of trichloroacetic acid 1 drop of 1 M copper(II) sulphate solution is added; a blue, clotted precipitate confirms SCMC.

Guar and locust bean gum—The precipitate obtained on addition of ethanol is re-dissolved in water to give a 0·5 per cent. solution and 20 per cent. v/v of 4 per cent. borax solution is added. Formation of a gel which, when cut, grows back together, confirms locust bean or guar gum, or both. These gums cannot be distinguished by chemical tests.

Methylcellulose—The precipitate obtained after separation of the above two gums is re-dissolved in water and the solution is boiled. Formation of a precipitate, which re-dissolves on cooling and which cannot be formed on addition of 2 volumes of ethanol and 1 volume of 4 M sodium chloride solution, confirms methylcellulose (or HPC, MEC or EHEC).

Pectate[11]—To the re-dissolved precipitate obtained on addition of trichloroacetic acid in neutral solution a 20 per cent. volume of 1 M magnesium chloride solution is added. Formation of a precipitate confirms pectate.

Pectinate—A solution of phenolphthalein and sodium hydroxide (0·1 M; 2 drops) is prepared and divided into two portions. The test solution is added to one portion and both solutions are boiled. The pink colour fades in the presence of pectinate, karaya and tragacanth.

Discussion

It has been stated by several workers[1–7] that tragacanth in a 0·5 per cent. solution is precipitated by 5 per cent. iron(III) chloride solution. However, this observation is at variance with the findings of Deuel and Solms,[11] who claim that precipitation does not occur. In this investigation, two samples of the stabilisers were examined and both were coagulated by 1 drop of 0·6 M iron(III) chloride solution. However, addition of excess of iron(III) chloride solution resulted in the precipitate re-dissolving. Further, neither sample gave a yellow, stringy precipitate on boiling with 10 per cent. potassium hydroxide solution.[6,7] Contrary to the findings of Deuel and Solms, neither sample of tragacanth was coagulated by copper(II) sulphate solution. We therefore consider this test to be specific for pectinate.

It has previously been reported[11] that, in agreement with our findings, 0·1 per cent. agar solution is coagulated by 9 per cent. tannic acid. Other workers[6,7] have been unable to observe this reaction, and in our experience the precipitate was difficult to detect and the test was not very reproducible.

In the presence of iron(III) chloride solution tannin gave a green - black solution; when agar was present a green - black precipitate was formed. This reaction did not occur with the other stabilisers in this group, *viz.*, gum arabic, pectinate and tragacanth.

The fractionation procedure described is relatively rapid, and reliable identification of a mixture of eleven stabilisers can be effected in about 5 hours. Because mixtures commonly encountered industrially are less complex, the time required for analysis will be correspondingly shorter in practice. The procedure can be used on stabiliser mixtures that have been isolated from food products by methods previously reported in the literature.[15,16] The method does not rely on the experience of the operator in assessing macroscopically or microscopically the physical form of the precipitate in order to identify a constituent. Finally, no specialised or expensive apparatus is required and the method is therefore suitable for use within small analytical laboratories.

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Book Reviews

CHEMICAL ANALYSIS. AN INTENSIVE INTRODUCTION TO MODERN ANALYSIS. By W. E. HARRIS and B. KRATOCHVIL. Pp. x + 222. New York: Barnes and Noble Inc.; London: Chapman and Hall Ltd. 1971. Price £2·25.

This book outlines the course used at the University of Alberta; even there it is supplemented by a modern analytical textbook. Part I gives a general introduction to laboratory tools and operations (8 to 10 hours' work); Part II describes non-instrumental experiments (45 to 60 hours) and Part III gives instrumental experiments (50 to 65 hours). The course is accompanied by 75 hours of lectures on the theory and practice of analytical chemistry.

This book is not suitable for direct use in schools because it does not cover the G.C.E. "A"-level syllabus thoroughly, either as a whole or in part. It is probably not of use as the single textbook for any first-year university course because that would, among other considerations, depend on exactly the same range and make of instruments being available. SI enthusiasts will note that ergs appear, weight is used (with apology) when mass is meant, and that ml are used to express volumes to five significant figures; others will find that manganese dioxide and sodium bicarbonate are mentioned. The definition of a "titer" on page 41 needs scrutiny; Example 1.1 and Table 1.2 need the units of amounts to be included, and the value given for a Faraday is wrong.

But, having said the above, the book does have a use—a copy should be in the library because it may inspire a teacher at school or university to improve a laboratory course, and, as each section finishes with some searching calculations, it is a useful source of questions (and answers). Isn't there a cheaper way of facilitating the interchange of ideas and details of courses?

J. S. CLARKE

SPECTROCHEMICAL METHODS OF ANALYSIS. QUANTITATIVE ANALYSIS OF ATOMS AND MOLECULES. Edited by J. D. WINEFORDNER. Pp. xiv + 530. New York, Sydney, Toronto and London: Wiley-Interscience. 1971. Price £10·50.

This book comprises Volume 9 of a series in "Advances in Analytical Chemistry" and deals with flame spectrometry and molecular spectrometry. Chapters are contributed by various workers in these fields.

With a collection of authors it is always useful and interesting to see what was the editor's intention in gathering together these sections, and how truly the aim of the book has or has not been realised. The editor states that the book was intended to stress the quantitative aspects of the analysis for elements and molecules and was designed especially for the applied spectroscopist. The editor made his field broader by hoping to make the book educational, in that the basic principles as well as the instrumentation and applications of each method would be reviewed. "The book is intended for analytical chemists as well as scientists using spectrochemical methods of analysis." (Does this mean that analytical chemists are not scientists?)

The theoretical principles are fairly well presented, indeed there is a chapter at the beginning of each of the two sections, one of which deals with excitation and de-excitation processes in flames and the other with excitation and emission in solution, which covers the theoretical background. The first does mention some aspects of quantitative work and devotes four of the 120 pages to "Some Conclusions for the Analytical Application of Flame Spectroscopy." The other chapter does not deal with either. Although the book is not intended as a laboratory handbook, in view of the editorial promise one could have hoped for a more "applied" approach. There is some overlap in the presentation from chapter to chapter, which is to be expected with various authors contributing to the book; this overlap serves to connect the various areas covered by the different authors. Most chapters have a fair amount of information but there are few instances when the book will be of immediate use to the practising analytical chemist. One chapter, the appendix, deals with signal-to-noise ratio in spectrochemical analysis. This is timely and could perhaps serve as a basis for future work in this area.

For those who wish to be able to obtain any working information about spectrochemical methods of analysis, this book is not suitable, perhaps not intended. For those who want to know the basic principles of some of the methods available in a series of review articles, the book is adequate.

L. S. BARK

A Colorimetric Method for the Determination of 2-Phenylphenol Residues in Citrus Fruits

A colorimetric method for the determination of 2-phenylphenol residues in citrus fruit, based on a specific colour reaction given by 2-phenylphenol with sulphuric acid, trace amounts of formaldehyde and iron(III), is described. 2-Phenylphenol is extracted from the fruit with chloroform, transferred into an alkaline medium, and, after acidification of the latter, re-extracted with chloroform. The extract is purified with 86·3 per cent. w/w sulphuric acid and the 2-phenylphenol is then determined colorimetrically. The method permits the determination of small amounts of 2-phenylphenol of the order of 0·05, 0·3 and 0·1 p.p.m. in the pulp, peel and whole fruit, respectively. The recovery of 2-phenylphenol varies between 95 and 106 per cent.

ANNA RAJZMAN

The Volcani Institute of Agricultural Research, Division of Fruit and Vegetable Storage, Rehovot, Israel.

Analyst, 1972, **97**, 271–278.

Detection of Chloramine T in Minced Meat and Farinaceous Foods

The phenothiazine method for the detection of chloramine T in dairy products was modified for application to minced meat and farinaceous foods. A hydrochloric acid extract of the material is treated with Carrez reagent to precipitate the proteins. The acidic solution is extracted with diethyl ether and the ethereal solution is purified with sodium hydrogen carbonate - potassium permanganate solution. Shaking the ethereal solution with phenothiazine and sodium hypochlorite reagents yields a violet reaction product when chloramine T is present. The limit of detection is about 25 p.p.m. of chloramine T.

W. F. van GILS and GERDA G. HIDSKES

Provinciale Keuringsdienst van Waren, Assen, The Netherlands.

Analyst, 1972, **97**, 279–280.

The Determination of Acrylamide in Water by Using Electron-capture Gas Chromatography

A method is described for the determination of residual acrylamide in water at levels likely to arise from the use of acrylamide polymers and copolymers in water treatment. After bromination of the sample, the α,β-dibromopropionamide obtained is extracted with diethyl ether and the extract analysed by electron-capture gas chromatography. Replicate samples of acrylamide added to River Thames water at concentrations of 500, 50, 5 and 0·25 $\mu g\ l^{-1}$ were analysed with relative standard deviations of ± 5, ± 5, ± 9 and ± 10 per cent., respectively.

A detection limit of 0·1 $\mu g\ l^{-1}$ of acrylamide was achieved with River Thames water and 0·25 $\mu g\ l^{-1}$ was determined with a relative standard deviation of ± 10 per cent. Yields of α,β-dibromopropionamide vary with water quality, ultraviolet light intensity and other reaction conditions. To overcome effects caused by these variables, when present, an internal standardisation procedure is used. The detection limit in River Thames water remained at 0·1 $\mu g\ l^{-1}$ but with this procedure 0·25 $\mu g\ l^{-1}$ was determined with a relative standard deviation of ± 20 per cent.

B. T. CROLL and G. M. SIMKINS

The Water Research Association, Medmenham, Marlow, Bucks., SL7 2HD.

Analyst, 1972, **97**, 281–288.

An Inexpensive Gas Chromatograph for the Detection and Collection of Labile Organophosphorus Compounds

A gas chromatograph suitable for use with labile organophosphorus compounds is described. It incorporates all-glass systems for detection and collection, with an exceptionally short distance from the column to the detector or collector. Easily exchanged thermionic and flame-ionisation detectors are used. The instrument is simple, convenient and cheap to make.

The thermionic detector is sensitive to less than 1 pg of diazinon and responds linearly over a 5000-fold range. Fractions from 50 ng to 50 μg have been collected with 90 to 100 per cent. efficiency.

A. F. MACHIN and C. R. MORRIS

Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, New Haw, Weybridge, Surrey.

Analyst, 1972, **97**, 289–293.

The Gas-chromatographic Determination of Residues of Barban and its Major Metabolites in Crops

A method is described for the determination of residues of barban [4-chlorobut-2-ynyl *N*-(3-chlorophenyl)carbamate] and its major water-soluble metabolites in harvest grain (wheat and barley) and sugar beet. Hydrolysis of barban and its metabolites yields 3-chloroaniline. Bromine water quantitatively converts 3-chloroaniline into its tribromo derivative, which can be detected by electron-capture gas chromatography. The limit of detection is 0·01 p.p.m. of barban. Mean recoveries of barban added to sugar beet, wheat and barley samples are 84·0, 68·8 and 78·0 per cent., respectively. The possibility of interference from other herbicides is discussed.

The method is more rapid than other published procedures and allows smaller samples to be extracted without loss of sensitivity.

R. J. HARRIS and R. J. WHITEOAK

Fisons Agrochemical Division, Chesterford Park Research Station, Nr. Saffron Walden, Essex.

Analyst, 1972, **97**, 294–299.

A Dual-purpose Basal Medium for the Microbiological Assay of Nicotinic Acid (Niacin) and Pantothenic Acid with *Lactobacillus plantarum*

A dual-purpose medium for the microbiological assay of nicotinic acid (niacin) and pantothenic acid with *Lactobacillus plantarum* has been devised.

E. C. BARTON-WRIGHT

Galloway and Barton-Wright, Haldane Place, London, SW18 4NA.

Analyst, 1972, **97**, 300–301.

Voltammetric Reduction of Cations in Mixed Solvents

By using a mercury-pool indicator and a molybdenum-wire reference electrode, the reduction of various ions, including Cd^{2+}, Tl^{+}, Eu^{3+}, CrO_4^{2-}, was studied on fast voltage-scanning instruments. The shifts in the reduction potentials were studied for different mixtures of water with methanol, propanol, acetone, methyl cyanide, dioxan and formamide. For all the ions under test except CrO_4^{2-}, in every solvent except water - formamide, the shift is towards negative potentials. In water - formamide the shift is towards more positive potentials. The relevant factors determining the shift in potential, such as solvation, ion-pair formation, increased resistance of the solution, adsorption, structure of the solvent and composition of the supporting electrolyte, were considered. The contribution of factors other than solvation was found to be minimal. For the ions considered it was found that the changes in the activity coefficients of the metal ions for various aqueous mixtures of organic solvents play an important part in shifting the reduction potential. The theoretical shifts were calculated and compared with the experimental observations. The trend in both cases was found to be in the same direction.

V. T. ATHAVALE, S. V. BURANGEY and R. G. DHANESHWAR

Analytical Division, Bhabha Atomic Research Centre, Modular Laboratories, Trombay, Bombay-85 (AS), India.

Analyst, 1972, **97**, 302–310.

The Coulometric Determination of Sulphur Dioxide by Using Differential Electrolytic Potentiometry for End-point Location

The application of differential electrolytic potentiometry to end-point detection in the coulometric titration of sulphur dioxide is described; the results obtained with simple, semi-automatic apparatus on samples of sulphur dioxide of the order of 10^{-6} mol are discussed. The method is rapid, sensitive, precise and simple.

P. L. BAILEY and E. BISHOP

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

Analyst, 1972, **97**, 311–314.

Fractionation and Identification of Commercial Hydrocolloid Stabilising Agents

A rapid, simple procedure is described for the separation and identification of the individual components of a mixture of common stabilisers including agar, alginate, gum arabic, carrageenan, carboxymethylcellulose, methylcellulose, pectate, pectinate, gum tragacanth, guar gum and locust bean gum. Separation of the anionic stabilisers from the neutral stabilisers is effected initially by precipitation with cetylpyridinium chloride. Complete fractionation of the two major groups is then performed by using selective precipitation techniques. Reliable confirmatory tests are reported for all the stabilisers.

R. G. MORLEY, G. O. PHILLIPS, D. M. POWER

Department of Chemistry and Applied Chemistry, University of Salford, Salford, Lancashire, M5 4WT.

and R. E. MORGAN

The Medical Center, University of Alabama, Birmingham, Alabama, U.S.A.

Analyst, 1972, **97**, 315–319.

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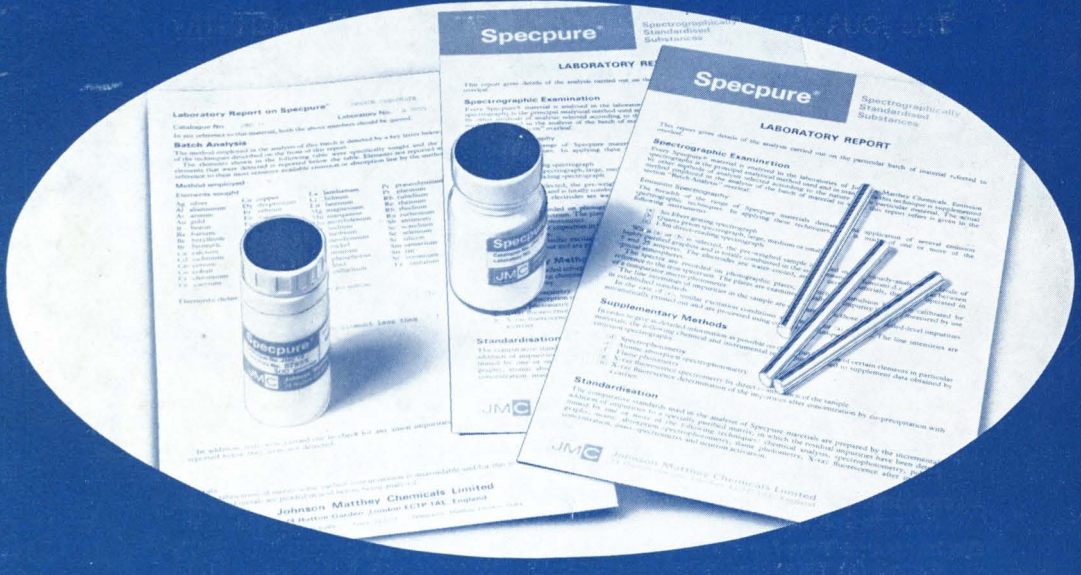

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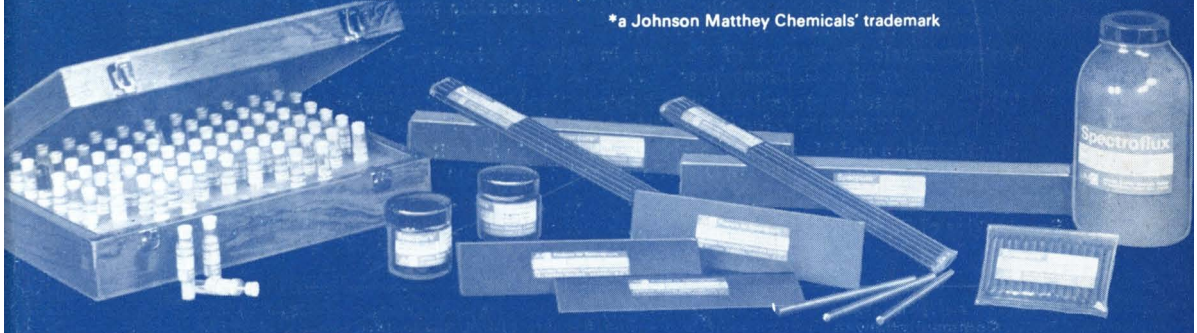

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