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Summaries of Papers in this Issue

Spectrophotometric Determination of Dissolved Titanium in Sea Water after Sodium Diethyldithiocarbamate Pre-concentration

A simple and rapid method for the determination of dissolved titanium in sea water is described. Using sodium diethyldithiocarbamate as precipitant, titanium, iron(III), zinc and part of the magnesium and calcium ions that are naturally present in sea water are coprecipitated. After collecting the precipitate by filtering through a 0.45-μm Metricel membrane filter, the qualitative recovery of titanium and the coprecipitation of titanium with other ions were studied by X-ray fluorescence spectroscopy. The precipitate was easily dissolved in dilute hydrochloric acid and then a sensitive 4,4′-diantipyrylmethane - thiocyanate extraction method was applied, followed by spectrophotometric determination of titanium. The concentration of dissolved titanium in Tong Shiau sea water was found to be 0.11 μg l^{-1} with a relative standard deviation of 5%.

Keywords: *Titanium determination*; *sea water*; *spectrophotometry*; *sodium diethyldithiocarbamate*

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Analyst, 1981, **106**, 385–388.

Determination of Residues of Furalaxyl and Metalaxyl in Nutrient Solution, Peat Compost and Soil Samples by Gas Chromatography

Rapid and sensitive techniques for the determination of residues of the fungicides furalaxyl and metalaxyl in agricultural samples are described. Air-dried soils and peat composts are extracted with acetone in a Soxhlet apparatus; the extract is applied directly to the gas chromatograph and detected using a nitrogen-selective detector. Plants are macerated with acetone and, after filtration and dilution with water, partitioned with chloroform; nutrient solutions are similarly extracted with chloroform. The extracts are subjected to gas chromatography after removal of chloroform and dissolution in acetone. Recoveries are generally better than 80% with detection limits of 0.5 mg kg^{-1} for peat, 0.1 mg kg^{-1} for soils and plants and 0.02 mg kg^{-1} for nutrient solutions.

Keywords: *Furalaxyl determination*; *metalaxyl determination*; *fungicides*; *agricultural samples*; *gas chromatography*

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Analyst, 1981, **106**, 389–393.

High-performance Liquid Chromatographic Determination of Four Biogenic Amines in Chocolate

Some biogenic amines occur in a wide variety of foods including cheese, fish, bakery products, milk products and chocolate. This study was undertaken to analyse and quantify four of the biogenic amines thought to occur in chocolate. Tyramine, tryptamine, 2-phenylethylamine and serotonin (5-hydroxytryptamine) were chosen as the amines of interest. Two high-performance liquid chromatographic (HPLC) systems were used for the final analysis of amine extracts. Both systems employed dual detection, with the first using ultraviolet absorbance at 254 nm and the formation of a post-column *o*-phthaldehyde derivative. The second method used ultraviolet absorbance at 254 nm and the natural fluorescence of the four amines. Thin-layer chromatography (TLC) was performed on all of the extracts to provide further confirmation. All four amines were detected and quantified at varying levels in extracts of several kinds of chocolate and chocolate liquors.

Keywords: *High-performance liquid chromatography*; *biogenic amines*; *chocolate*; *food*

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Analyst, 1981, **106**, 394–402.

Standard Atmosphere Generator: a Dynamic System for the Controlled Dilution of Organic Vapours in Air

An apparatus is described for the production of standard atmospheres of compounds at the concentrations normally encountered in air pollution studies. It is a glass-blown, flow system with continuous syringe injection of the compounds into a vaporiser, prior to their dilution in the main flow line. Statistical analyses of performance tests on a range of compounds show that stable atmospheres are produced within 5 min of start-up. The instrument produces the predicted concentrations of non-polar compounds with a high degree of accuracy, and tests on atmospheres of a relatively involatile compound, 1-methylnaphthalene, reveal no diminution in performance even with concentrations approaching that of its saturated vapour.

Keywords: *Standard atmosphere*; *syringe pump*; *organic vapour*; *air sampling*

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Analyst, 1981, **106**, 403–411.

Comparison of Some Porous Polymers as Adsorbents for Collection of Odour Samples and the Application of the Technique to an Environmental Malodour

A range of Chromosorbs and Tenax-GC have been compared with regard to their efficiency as adsorbents for volatile odorous compounds using a very simple model system. When desorption was accomplished by solvent elution using acetone, Chromosorb 103 provided the best recoveries of those investigated (between 90 and 95%). In situations where thermal lability of trapped compounds is not a problem, use of Tenax-GC and thermal desorption at about 250 °C would be recommended on the basis of this survey, during which this approach consistently provided recoveries greater than 96% from small sample volumes. The latter procedure was applied to the analysis of an industrial malodour from an animal rendering factory, and using gas chromatography - mass spectrometry over 35 compounds (92% of the total odour sample) were positively identified. In particular, a range of alkylthiophenes may be characteristic of this particular odour.

Keywords: *Odour analysis*; *porous polymer adsorbents*; *Chromosorbs*; *Tenax-GC*; *animal rendering odour*

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Analyst, 1981, **106**, 412–418.

Simultaneous Determination of Trace Metals in Sea Water Using Dithiocarbamate Pre-concentration and Inductively Coupled Plasma Emission Spectrometry

A method based on dithiocarbamate pre-concentration and inductively coupled plasma emission spectrometry is described for the simultaneous determination of cadmium, copper, iron, molybdenum, nickel, vanadium and zinc in sea water. The metals are extracted from 500 g of sea water with ammonium tetramethylenedithiocarbamate - diethylammonium diethyldithiocarbamate in chloroform and back-extracted into nitric acid; the sea water concentration factor is 250 or 500. Advantages of the method include high precision, simplicity of calibration and a detection capability in the nanograms per litre range. The method has been applied to Japan Sea, Pacific Ocean and Atlantic Ocean samples.

Keywords: *Trace metals*; *sea water*; *dithiocarbamate pre-concentration*; *inductively coupled plasma emission spectrometry*

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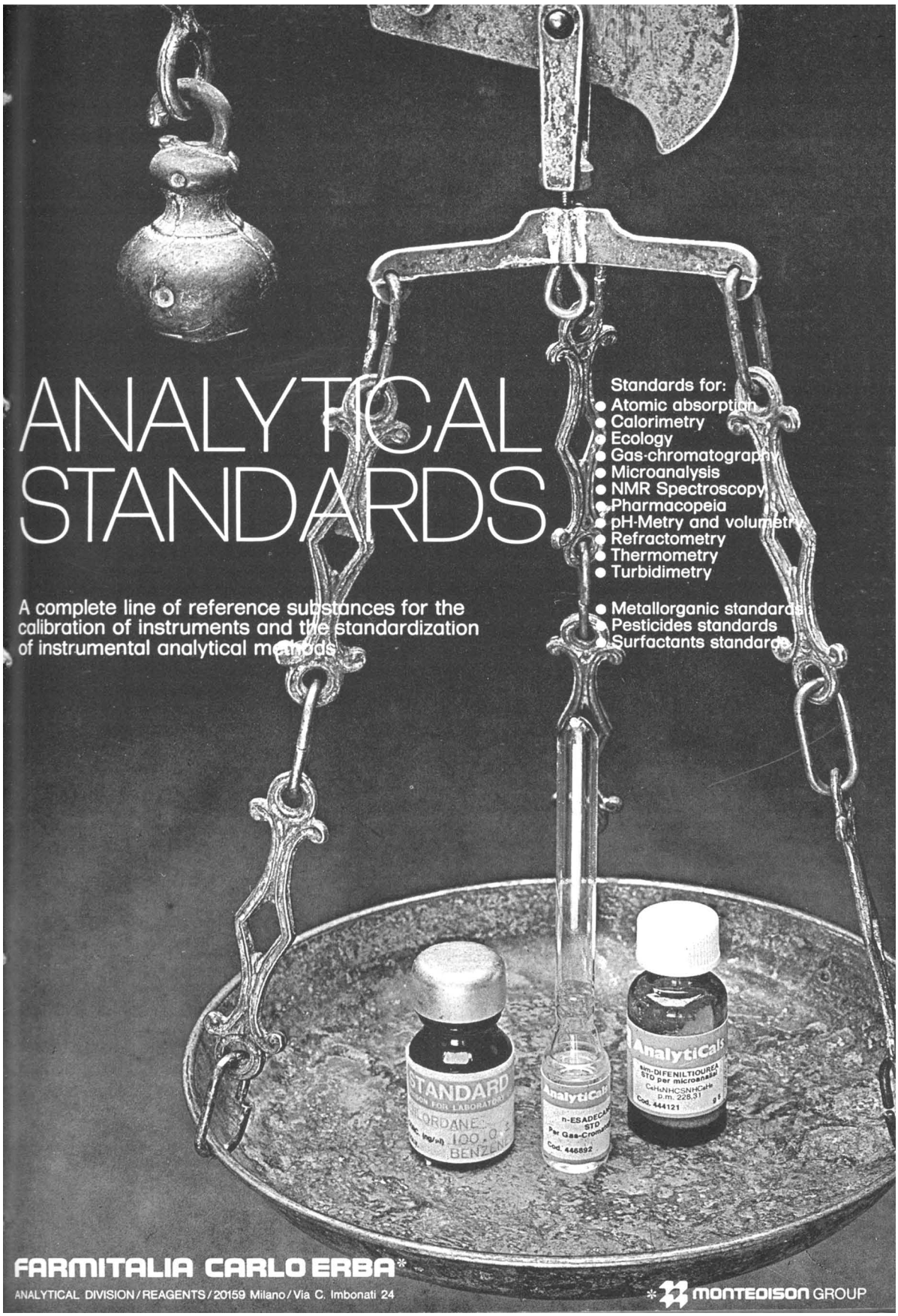
Analyst, 1981, **106**, 419–428.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Kinetic Parameters and Current Efficiencies for Manganese(III) Generation from Manganese(II)

Electrogeneration of manganese(II) at platinum, gold and glassy carbon was investigated by rotating disc electrode voltammetry. Anodic charge-transfer kinetic parameters were determined and used to compute generation current efficiencies. The maximum value obtained was 99.91% at a generating current density of 8.95 mA cm^{-2}, in sulphuric acid. The most appropriate electrode material is gold.

Keywords: *Manganese(III)*; *rotating disc electrode*; *charge-transfer kinetic parameters*

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Analyst, 1981, **106**, 429–432.

Generation of Chlorine at Glassy Carbon. Study of Kinetic Parameters and Current Efficiencies by Rotating Disc Electrode Voltammetry

A glassy carbon electrode is used for the anodic generation of chlorine. The reaction mechanism determined allows the use of approximate current - potential relationships for calculating charge-transfer kinetic parameters. The values found are interpreted by the condition of the electrode surface and adsorbed chlorine. Generating current efficiencies were calculated using the determined kinetic parameters. A maximum of 99.99% was found for a current density of 58.4 mA cm^{-2}. Electrode surface attack was detected at potentials above 1.7 V *vs.* S.C.E.

Keywords: *Glassy carbon*; *chlorine generation*; *rotating disc electrode*; *charge-transfer kinetic parameters*

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Analyst, 1981, **106**, 433–438.

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Spectrophotometric Determination of Dissolved Titanium in Sea Water after Sodium Diethyldithiocarbamate Pre-concentration

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A simple and rapid method for the determination of dissolved titanium in sea water is described. Using sodium diethyldithiocarbamate as precipitant, titanium, iron(III), zinc and part of the magnesium and calcium ions that are naturally present in sea water are coprecipitated. After collecting the precipitate by filtering through a 0.45-μm Metricel membrane filter, the qualitative recovery of titanium and the coprecipitation of titanium with other ions were studied by X-ray fluorescence spectroscopy. The precipitate was easily dissolved in dilute hydrochloric acid and then a sensitive 4,4′-diantipyrylmethane - thiocyanate extraction method was applied, followed by spectrophotometric determination of titanium. The concentration of dissolved titanium in Tong Shiau sea water was found to be 0.11 μg l^{-1} with a relative standard deviation of 5%.

Keywords: *Titanium determination*; *sea water*; *spectrophotometry*; *sodium diethyldithiocarbamate*

The concentration of titanium in sea water was first reported by Griel and Robinson,[1] who used thymol as a chromophoric reagent to determine titanium spectrophotometrically, but they admitted that a more sensitive method was required. Subsequently a few other papers[2–5] appeared, but in most of them attention was concentrated on the determination of the titanium content of sediments and particulate matter in sea water using emission spectrography. However, accurate data[6] for the dissolved titanium content of sea water were still not available. As titanium, like other heavy metal ions, is often concentrated in the particulate phase[5] and the total concentration of titanium in sea water is only a few parts per 10^9,[1,5,7] and also it is easily hydrolysed and has a short residence time, the determination of very low concentrations of dissolved titanium is a challenging problem.

Hydrous titanium dioxide is well known as a good adsorbent[8] for extracting uranium from sea water. In order to obtain information on the mass balance of titanium in the process of extraction of uranium from sea water, the determination of titanium in sea water is necessary.

Sodium diethyldithiocarbamate is commonly used in spectrophotometric analysis,[9] and recently it and other dithiocarbamates have been used to form precipitates with transition metal ions from matrices.[10–13] In this work, titanium was first coprecipitated with zinc and iron(III) ions by using sodium diethyldithiocarbamate as the precipitant and also coprecipitated with magnesium hydroxide, then a membrane filter made of cellulose triacetate (pore size 0.45 μm) was used to retain the precipitates for analysis. Owing to the high void volume of membrane filters, large numbers of sea water samples could be handled in a short time by using high suction flow-rates.

The chloroform extraction of ion pairs of titanium thiocyanates and 4,4′-diantipyrylmethane[14] was one of the most sensitive methods for the spectrophotometric determination of titanium. The ion pair $[(HDAM^+)_2][Ti(SCN)_6^{2-}]$[15] was formed in aqueous solution, and the distribution coefficient of the complex was so high that the pre-concentration of titanium by extraction with a small volume of an organic extractant was easily achieved and only a single extraction was required.

Experimental

Samples

Sea water was collected 10 m below the surface, 1.5 km off the shore of Tong-Shiau, Taiwan, and first filtered through a plastic coarse-pore filter to remove muds and grains of sand, then through a 0.45-μm cellulose triacetate membrane filter. Subsequently the water was acidified to pH 2 with hydrochloric acid and stored in acid-leached polyethylene containers until taken for analysis.

Apparatus

A Varian Techtron 634 ultraviolet spectrophotometer was used to determine titanium. A Gelman magnetic filter funnel of polysulphone construction with an alkylbenzenesulphonate (ABS) plastic support screen, magnetic coupling and an effective filtration area of 9.62 cm^2, and a 0.45-μm GA-6 Metricel cellulose acetate membrane filter were used to retain particulates for analysis. X-ray fluorescence spectroscopy was carried out using a Tracor North, Model NS-880, instrument equipped with a lithium-drifted silicon energy-dispersive detector.

Chemicals

All chemicals were of analytical-reagent grade.

Water. Water was distilled in quartz after de-ionisation with a Millipore reverse-osmosis membrane system. Titanium could not be detected spectrographically in this water following Hughes *et al.*'s procedure[16] for evaluating ultra-pure water quality.

Hydrochloric acid, concentrated. Obtained from Merck, purified by isoosmotic distillation and diluted as necessary.

Ammonia solution, sp. gr. 0.88. Obtained from Merck and purified by isoosmotic distillation.

Sodium diethyldithiocarbamate solution, 4% *m/V.* This was purified by extraction with 4-methylpentan-2-one.

Standard titanium solution, 1 μg ml^{-1}. Prepared by dissolving ammonium titanium oxalate in 1% hydrochloric acid at 50 °C.

Ammonium titanyl oxalate [*ammonium dioxalatooxotitanate(IV)*]. This is commercially available, but can be synthesised by a combination of Zátka and Hoffmann's method[17] and Van de Velde *et al.*'s method.[18]

Diantipyrylmethane. Tokyo Kasei Co.

Tin(II) chloride. Merck.

Potassium thiocyanate. Merck. Dissolve in water to give a 30% *m/V* solution.

Procedure

To 1–6-l volumes of acidified sea water in polyethylene containers, add 0, 0.5 and 1.0 ml of standard titanium solution (in recovery tests only), then to each add 1 ml of 4% sodium diethyldithiocarbamate per litre of sea water, ammonia solution (sp. gr. 0.88) to adjust the pH to 9.0–9.5, mix for 5 min and filter through a 0.45-μm Metricel membrane filter using a water aspirator. After completing the filtration separation, dissolve the precipitate in 10 ml of 2 N hydrochloric acid and heat gently. To the clear solution, add 5 ml of 30% *m/V* potassium thiocyanate solution, 3 ml of 5% *m/V* diantipyrylmethane solution, 2 ml of concentrated hydrochloric acid and 2 ml of 10% *m/V* tin(II) chloride solution. Extract the titanium complex with 5 ml of chloroform for 2 min and measure the absorbance at 420 nm using a reagent blank as reference. Perform the reagent blank test using 4 l of de-ionised, distilled water and the same procedure as for sea water samples.

Results and Discussion

The presence of trace amounts of titanium in sea water was confirmed by the absorption spectrum shown in Fig. 1. The molar absorptivity of $[(HDAM^+)_2][Ti(SCN)_6^{2-}]$ at 420 nm in chloroform is 6.0×10^4 l mol^{-1} cm^{-1}.

A qualitative recovery test was performed by adding 2 μg of titanium to 1 l of sea water, precipitating with sodium diethyldithiocarbamate and collecting the filtrate on a membrane filter, followed by counting the membrane directly using an energy-dispersive X-ray fluorescence spectrometer. The titanium was coprecipitated with zinc and iron(III) ions in sea

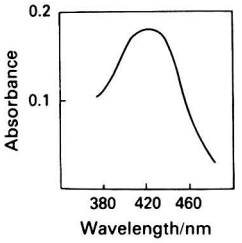

Fig. 1. Absorption spectrum of $[(HDAM^+)_2][Ti(SCN)_6^{2-}]$ in chloroform using a 6-l sea-water sample.

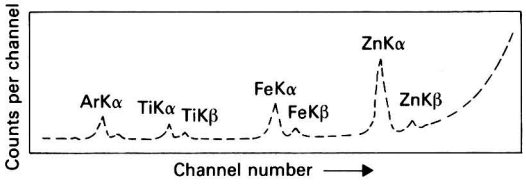

Fig. 2. Coprecipitation X-ray fluorescence spectrum of 2 μg of titanium spiked in 1 l of sea water.

water, as shown by the X-ray fluorescence spectrum (Fig. 2). Unfortunately, owing to its low sensitivity, coprecipitation followed by X-ray fluorescence spectroscopy cannot be applied to the determination of titanium in sea water; further studies on improving the sensitivity by varying the experimental conditions are currently being carried out in our laboratory.

Standard titanium solution (1 ml) was added to 5 l of sea water in order to carry out a quantitative recovery test by using precipitation pre-concentration followed by spectrophotometric measurement. In four assays carried out during the precipitation and separation steps, the recovery of titanium was 95 ± 5%.

The titanium concentration of the Tong Shiau sea water is reported in Table I. Results for the seven aliquots that were spiked with titanium are also included.

The effect of pH on the recovery of titanium from sea water spiked at the level of 1 μg l^{-1} is shown in Fig. 3. As pH values higher than 9.8 cause too much precipitation of magnesium hydroxide and interference in the subsequent filtration process, the pH value in the procedure was fixed between 9.0 and 9.5. A 1-ml volume of 4% *m*/*V* sodium diethyldithiocarbamate precipitant per litre of sea water was found to be sufficient for a satisfactory recovery of titanium.

The only four ions in sea water that, according to Marczenko,[19] could be considered to be potential interferents in diantipyrylmethane - thiocyanate extraction and direct spectrophotometric determination of titanium were Mo(VI), Cu(II), Co(II) and Fe(III). Therefore, 20 μg of Cu(II), 80 μg of Mo(VI), 5 μg of Co(II) and 30 μg of Fe(III) were added to 4 l of sea water (these levels are about double those found in natural sea water) and the proposed procedure was carried out. No interference from these four ions was observed. Zinc ions form a colourless complex with diantipyrylmethane, so they do not interfere in the determination of titanium. Iron(III) ions were reduced with tin(II) chloride, so that they would no longer interfere.

In order to prevent adsorption losses, the collected sea water should be acidified to pH 2[20] and all experimental procedures be completed within 24 h.

Contamination control was always checked by carrying out a blank test. The blank value was about 10–20 ng of titanium. Extra care was taken to prevent contamination from laboratory vessels, reagents and filters. The cleaning procedures for plastic containers suggested by

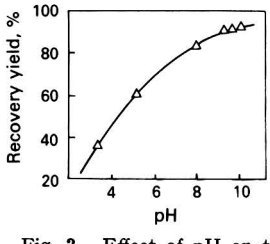

Fig. 3. Effect of pH on the recovery of titanium from sea water spiked at the 1 μg l^{-1} level.

TABLE I

CONCENTRATION OF TITANIUM FOUND IN SEA WATER SAMPLES AND TOTAL TITANIUM FOUND IN THE SAME SAMPLES SPIKED WITH ADDITIONAL TITANIUM

Volume of sea water/l	Amount of titanium added/μg	Total titanium found/μg	Titanium concentration in sea water/μg l^{-1}
1	0	— *	—
1	0.5	0.55	—
1	1.0	1.00	—
2	0	—	—
2	0.5	0.65	
2	1.0	1.20	0.10
4	0	0.44	0.11
4	0	0.44	0.11
4	0	0.40	0.10
4	1.0	1.40	0.10
5	0	0.55	0.11
5	0	0.50	0.10
5	0	0.55	0.11
5	1.0	1.55	0.11
6	0	0.65	0.11
6	0	0.64	0.11
6	0	0.66	0.11
6	1.0	1.68	0.11

* —, Below detection limit.

Moody[21] were followed. Samples were handled inside the laminar flow hood of a clean air bench to prevent contamination from dust.

Christensen[22] mentioned that the reported concentrations of some trace metals in sea water had generally been decreasing over the years. The results of this work are in accordance with such a trend.

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Determination of Residues of Furalaxyl and Metalaxyl in Nutrient Solution, Peat Compost and Soil Samples by Gas Chromatography

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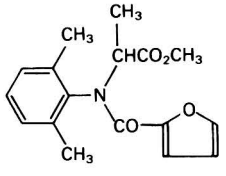
Rapid and sensitive techniques for the determination of residues of the fungicides furalaxyl and metalaxyl in agricultural samples are described. Air-dried soils and peat composts are extracted with acetone in a Soxhlet apparatus; the extract is applied directly to the gas chromatograph and detected using a nitrogen-selective detector. Plants are macerated with acetone and, after filtration and dilution with water, partitioned with chloroform; nutrient solutions are similarly extracted with chloroform. The extracts are subjected to gas chromatography after removal of chloroform and dissolution in acetone. Recoveries are generally better than 80% with detection limits of 0.5 mg kg^{-1} for peat, 0.1 mg kg^{-1} for soils and plants and 0.02 mg kg^{-1} for nutrient solutions.

Keywords: *Furalaxyl determination*; *metalaxyl determination*; *fungicides*; *agricultural samples*; *gas chromatography*

Furalaxyl* [methyl *N*-(2,6-dimethylphenyl)-*N*-(2-furoyl)alaninate] (**I**) and metalaxyl* [methyl *N*-(2,6-dimethylphenyl)-*N*-(2-methoxyacetyl)alaninate] (**II**) are new fungicides with residual and systemic activity against fungi of the order Peronosporales, which attack a wide range of commercial crops. Over-application of chemical can lead to crop damage.[1] Maier-Bode and Riedman[2] made a study of the nitrogen-selective detector and its application to the detection of nitrogen-containing pesticides.



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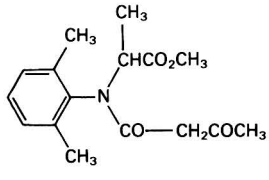

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The procedures described here are sensitive and rapid and can be applied to a wide range of agricultural materials. Soils and peat composts are dried and extracted with acetone in a Soxhlet apparatus, and the extract is analysed, without clean-up, by gas chromatography using a nitrogen-selective detector. Plant samples are chopped and stored deep-frozen; they are macerated with acetone and, after filtration and dilution of the extract with water, the fungicides are extracted into chloroform. The solvent is removed and the residue dissolved in acetone for gas chromatography. Nutrient solutions are extracted with chloroform, which is then treated as previously described for plant samples.

Experimental

Reagents

Analytical-reagent grade materials should be used whenever possible. Blank determinations should be carried out frequently and interferents in solvents removed by redistillation.

* Proposed common name.

The pesticides used were the 25% formulated products, pure specimens being prepared by Soxhlet extraction with acetone and recrystallisation from ethanol.

Acetone.

Chloroform.

Sodium sulphate solution, 5% m/V.

Fungicide stock solutions. Dissolve 20 mg of fungicide in acetone and dilute to 200 ml with acetone.

Fungicide working standard solution. Dilute 10 ml of stock solution to 100 ml with acetone. This solution contains 10 μg ml^{-1} of fungicide.

Apparatus

A Pye, Series 104, gas chromatograph equipped with a rubidium chloride thermionic detector and a glass column (0.9 × 4 mm i.d.) packed with a 5% high-vacuum silicone grease on 80–100-mesh Gas-Chrom Q was used [stationary phase as supplied by ICI (grade M494) or BDH Chemicals].

The carrier gas was nitrogen at a flow-rate of 40 ml min^{-1}. The flow-rate of hydrogen was 40 ml min^{-1} and that of air was approximately 250 ml min^{-1}. The column temperature was 190 °C for metalaxyl and 210 °C for furalaxyl. An almost linear response of peak height and amount of fungicide applied was obtained with 30 and 20 ng of furalaxyl and metalaxyl, respectively, giving full-scale deflection.

Extraction apparatus. This apparatus consisted of an electrically heated series of mantles, Soxhlet extractors with thimbles (28 × 120 mm) and 150-ml flat-bottomed flasks.

Separating funnels. Capacity 100 and 250 ml.

Homogeniser. "ATO-MIX MSE" emulsifier with a 1-l stainless-steel container, obtainable from Scientific Supplies Ltd., London.

Procedure for Soils and Compost

To 25 g of soil or 10 g of peat compost, dried at room temperature and ground to pass a 2-mm mesh sieve, add 2 and 4 ml of water, respectively, mix and allow to stand for 1 h. Transfer the mixture into an extraction thimble, measure approximately 100 ml of acetone into the flask, assemble the Soxhlet extraction apparatus and extract the sample for 8 h at a siphon rate of approximately six changes an hour. Adjust the volume of solvent and take aliquots (2–8 μl) for gas chromatography.

Procedure for Plant Samples

Transfer 50 g of a finely chopped sample into the homogeniser container and blend with 100 ml of acetone for 3 min using a loosely fitting cover. Filter using suction and wash the container and filter with 50 ml of acetone. Dilute the extract to 250 ml with water and transfer 50 ml into a 250-ml separating funnel containing 50 ml of water, 10 ml of 5% *m/V* sodium sulphate solution and 20 ml of chloroform. Shake vigorously for 30 s and, after separation of the phases, transfer the lower layer into a small conical flask containing approximately 5 g of anhydrous sodium sulphate and allow to stand for 10 min with occasional swirling. Decant the dried extract into a 150-ml flat-bottomed flask. Repeat this extraction of the aqueous phase with a further 20 ml of chloroform using the same sodium sulphate to dry the extract. After decanting into the first extract, wash the sodium sulphate twice with 5 ml of chloroform. Evaporate the combined chloroform extracts to dryness using a water-bath at 80 °C, exercising much care in the final stages with the application of slight heat. In order to remove the last traces of chloroform, dissolve the residue in a few millilitres of acetone and again evaporate to dryness; repeat the addition and removal of acetone twice more. Dissolve the residue in a convenient volume of acetone for gas chromatography.

Procedure for Nutrient Solution

Transfer 50 ml of sample into a 100-ml separating funnel. Add 10 ml of 5% *m/V* sodium sulphate solution, shake vigorously with 20 ml of chloroform for 30 s and follow the above procedure for plant samples from "... and, after separation of the phases. . . ." The sample to solvent ratio may be 25:1 for nutrient solution, 5:1 for plants and 1:1 for peats and soils.

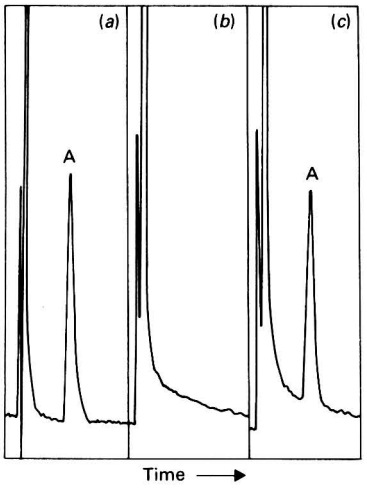

Fig. 1. Gas chromatograms of (*a*) standard containing 8 ng of furalaxyl (A); (*b*) extract from peat compost not treated with furalaxyl; and (*c*) extract of peat in (*b*) after addition of furalaxyl at 20 mg kg^{-1} (2 μl of 1 g per 5 ml). Column temperature 210 °C. Detector temperature 250 °C. Attenuation 50 × 1.

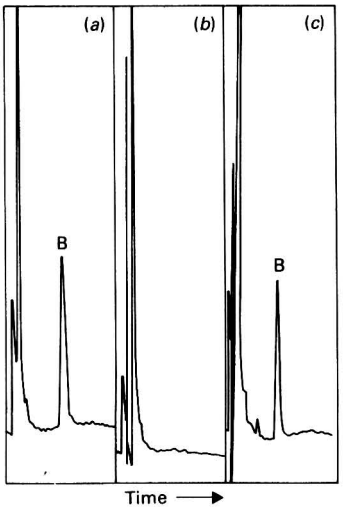

Fig. 2. Gas chromatograms of (*a*) standard containing 6 ng of metalaxyl (B); (*b*) extract from soil A not treated with metalaxyl; and (*c*) extract of soil A after addition of metalaxyl at 20 mg kg^{-1} (6 μl of 1 g per 20 ml). Column temperature 190 °C. Detector temperature 250 °C. Attenuation 50 × 1.

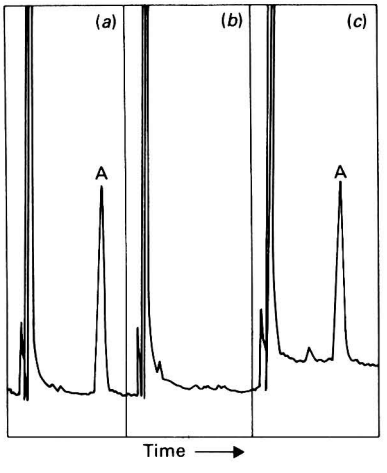

Fig. 3. Gas chromatograms of (*a*) standard containing 8 ng of furalaxyl (A); (*b*) extract from soil B not treated with furalaxyl; and (*c*) extract of soil B after addition of furalaxyl at 10 mg kg^{-1} (4 μl of 1 g per 5 ml). Column temperature 205 °C. Detector temperature 250 °C. Attenuation 50 × 1.

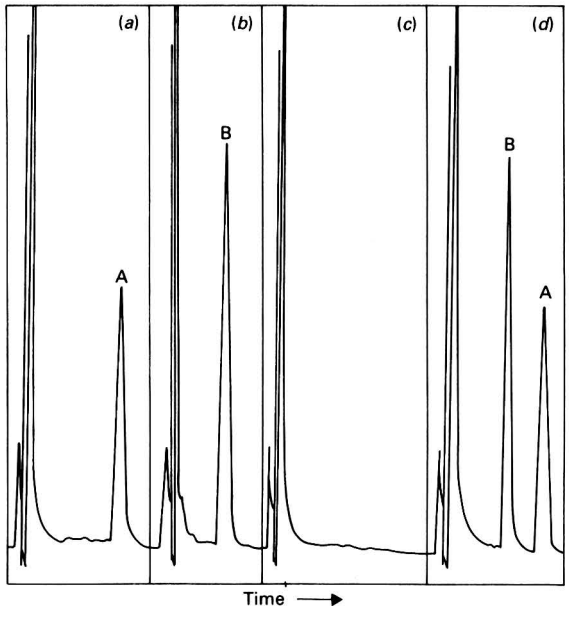

Fig. 4. Gas chromatograms of (*a*) standard containing 8 ng of furalaxyl (A); (*b*) standard containing 8 ng of metalaxyl (B); (*c*) extract of nutrient solution containing neither fungicide; and (*d*) extract of nutrient solution in (*c*) after addition of furalaxyl and metalaxyl at 5 mg kg^{-1} (2 μl of 4 ml of nutrient solution in 5 ml of acetone). Column temperature 205 °C. Detector temperature 250 °C. Attenuation 50 × 1.

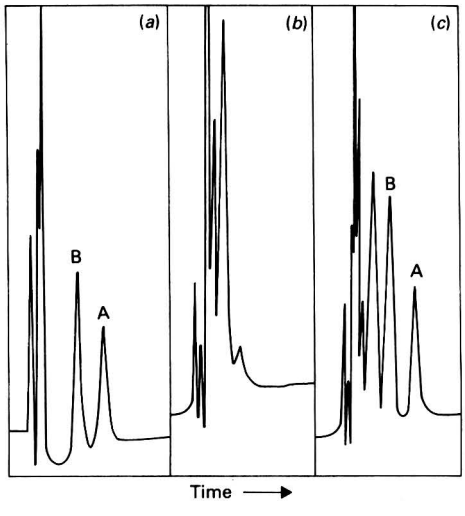

Fig. 5. Gas chromatogram of (*a*) standard containing 4 ng of furalaxyl (A) and metalaxyl (B); (*b*) extract (2 μl of 1 g ml^{-1}) of field-treated lettuce containing 0.3 mg kg^{-1} of metalaxyl; and (*c*) extract as in (*b*) after addition of furalaxyl and metalaxyl at 4 mg kg^{-1} (2 μl of 1 g per 2 ml). Column temperature 210 °C. Detector temperature 250 °C. Attenuation 50 × 1.

Results and Discussion

The recovery of the two fungicides from peat composts and soils was measured by the addition of stock solutions to the air-dried materials and, after thorough mixing and standing for 24 h, extraction with acetone as detailed above; additions to plant samples were made prior to maceration with acetone and, with nutrient solutions, as a direct addition to the aqueous phase. The mean recoveries of duplicate determinations are given in Table I and are illustrated in Figs. 1–5.

TABLE I

RECOVERY OF FUNGICIDES ADDED TO SAMPLES

	Furalaxyl			Metalaxyl		
Sample	Added/ mg kg^{-1}	Found/ mg kg^{-1}	Recovery, %	Added/ mg kg^{-1}	Found/ mg kg^{-1}	Recovery, %
Peat compost	200.00	190.00	95	400.00	313.00	78
	100.00	105.60	106	200.00	200.00	100
	50.00	44.00	88	100.00	88.00	88
	20.00	17.00	85	40.00	40.00	100
	10.00	7.50	75	20.00	21.00	105
	5.00	4.00	80	—	—	—
Soil A (calcareous silty loam; pH 7.6; organic matter 3.57%)	200.00	200.00	100	40.00	30.00	75
	100.00	111.00	111	20.00	16.00	80
	50.00	49.30	99	10.00	8.30	83
	20.00	23.00	115	5.00	4.00	80
	10.00	7.50	75	1.00	1.10	110
	5.00	4.30	86	—	—	—
Soil B (sandy loam; pH 5.7; organic matter 3.82%)	200.00	147.00	74	20.00	17.00	85
	100.00	80.00	80	10.00	7.70	77
	50.00	39.00	78	5.00	4.50	90
	20.00	16.00	80	1.00	0.70	70
	5.00	4.50	90	—	—	—
Nutrient solution	20.00	19.20	96	20.00	19.30	97
	10.00	9.40	94	10.00	9.60	96
	5.00	4.80	96	5.00	4.90	98
	1.00	0.89	89	1.00	0.96	96
	0.50	0.42	84	0.50	0.44	88
	0.20	0.19	95	0.20	0.16	80
	0.10	0.11	110	0.10	0.08	80
Lettuce	20.00	19.40	97	20.00	19.60	98
	4.00	4.20	105	4.00	4.10	103
	2.00	2.00	100	2.00	1.80	90
	0.50	0.49	98	0.50	0.48	96
Hops	—	—	—	5.00	4.90	98

Conclusions

The procedures described are rapid and easy to operate and, although they contain no time-consuming clean-up steps, are free from interferences. Recovery of added fungicides is generally better than 80% with detection limits of 0.5 mg kg^{-1} for peat, 0.01 mg kg^{-1} for soils and plants and 0.02 mg kg^{-1} for nutrient solutions.

The staff of the first author's laboratory, especially Mrs. A. Dray, are thanked for assistance in the preparation of the analytical data and of samples for analysis.

References

1. Norman, R., *Grower*, 1978, October, 658.
2. Maier-Bode, H., and Riedmann, M., *Residue Rev.*, 1975, **54**, 113.

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High-performance Liquid Chromatographic Determination of Four Biogenic Amines in Chocolate

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Some biogenic amines occur in a wide variety of foods including cheese, fish, bakery products, milk products and chocolate. This study was undertaken to analyse and quantify four of the biogenic amines thought to occur in chocolate. Tyramine, tryptamine, 2-phenylethylamine and serotonin (5-hydroxytryptamine) were chosen as the amines of interest. Two high-performance liquid chromatographic (HPLC) systems were used for the final analysis of amine extracts. Both systems employed dual detection, with the first using ultraviolet absorbance at 254 nm and the formation of a post-column *o*-phthaldehyde derivative. The second method used ultraviolet absorbance at 254 nm and the natural fluorescence of the four amines. Thin-layer chromatography (TLC) was performed on all of the extracts to provide further confirmation. All four amines were detected and quantified at varying levels in extracts of several kinds of chocolate and chocolate liquors.

Keywords: *High-performance liquid chromatography*; *biogenic amines*; *chocolate*; *food*

Tyramine, tryptamine, 2-phenylethylamine and serotonin are members of the pressor amine group and tend to cause a rise in blood pressure.[1,2] Many foods contain amines that are members of this pressor amine group including tomatoes[3] and other fruits, as well as fish, meat and poultry products.[4,5,6,7] As early as 1919 Pognic *et al.* suggested that allergies could cause migraines and especially implicated chocolate and the amines found in chocolate.[7] Recent literature contains conflicting information about the presence or absence of these various amines in chocolate.[9–15]

This study was undertaken to determine the levels of four of these amines in various chocolate products and cocoa liquors. Samples were de-fatted and extracted using recognised procedures for biogenic amines.[14,16,17]

The final determination was carried out on one of two HPLC systems. Both used reversed-phase HPLC with dual detection and ultraviolet absorbance at 254 nm as one of their detection modes. The systems differed in that one used the formation of a post-column *o*-phthaldehyde derivative[18] of the biogenic amines while the other used natural fluorescence. These two analytical systems gave comparable results; recovery and precision studies of samples and standards showed the methods to be satisfactory. Results using both HPLC systems are presented for several chocolate and cocoa liquor samples.

Experimental

HPLC System 1

This was a modular system consisting of an M6000A solvent delivery system, Model 440 absorbance detector at 254 nm and U6K universal injector (Waters Associates). The column was Bondapak C_{18} Reversed Phase (Waters Associates). The flow-rate was 1.0 ml min^{-1}. The fluorescence detector was a Gilson, Spectra Glo Filter Fluorimeter equipped with excitation filters (340 nm) and emission filters (418 nm) (Gilson Medical Electronics).

The post-column reaction apparatus consisted of a Milton Roy Mini-Pump (Laboratory Data Control) used to pump the *o*-phthaldehyde solution into a mixing chamber. The pump was equipped with a home-made pulse damper in the form of a standing air column. The pump effluent was passed into one port of a three-port mixing chamber at a flow-rate of 1.0 ml min^{-1}. After mixing, the *o*-phthaldehyde - amine complex was carried into a reaction coil consisting of 10 m of 0.009-in i.d. stainless-steel tubing kept at a constant temperature of 40 °C by immersion in a circulating water-bath.

HPLC System 2

This was a modular system using the same solvent delivery system, ultraviolet detector, injector and HPLC column as the HPLC System 1. The flow-rate was 1.3 ml min^{-1}. The fluorescence detector used was a Varian SF-330 spectrofluorimeter equipped with an HPLC flow cell [excitation wavelength = 285 nm, emission wavelength = 320 nm (cut-off filter)].

Reagents

HPLC mobile phase 1. Acetic acid (0.2 M) at pH 2.8 in HPLC water, the latter was prepared by passing distilled water through ion-exchange and organic absorber cartridges, and finally through a 0.1-μm filter.

HPLC mobile phase 2. An 80 + 20 (*V*/*V*) mixture of 0.2 M potassium dihydrogen phosphate solution adjusted to pH 3.7 with orthophosphoric acid and methanol. This mixture was prepared, de-gassed and passed through a 0.1-μm final system filter.

TLC developing solvent. The solvent system was chloroform - methanol - concentrated ammonia solution (28% *m*/*V*), 12 + 7 + 1.

Boric acid buffer. Boric acid solution (0.4 M) adjusted to pH 10.3 $\pm$ 0.2 with solid potassium hydroxide.

o-Phthaldehyde reagent. This reagent was prepared by dissolving 0.32 g of *o*-phthaldehyde in 100 ml of ethanol and diluting to 1 l with boric acid buffer.

Ninhydrin solution. A mixture of 0.300 g of ninhydrin, 100 ml of butan-1-ol and 3 ml of acetic acid.

Standard solutions. Tyramine and tryptamine (Calbiochem-Behring); 2-phenylethylamine and serotonin (Sigma Chemical Co.). The 2-phenylethylamine was re-distilled prior to use. All standards were prepared in HPLC mobile phase to a final concentration of 0.1 μg μl^{-1}.

Samples. Chocolate samples were of nationally distributed types while chocolate liquor samples were obtained from the Hershey Chocolate Company.

Apparatus

Centrifuge. Capable of 2000 rev min^{-1}.
Oven. Thelco Blue M.
Sorvall Omni-Mixer. DuPont Instruments.
Dual pen recorder.
TLC developing tank.
Silica gel plates. Si-60 (0.25 mm) from EM Labs.

Extraction

The procedure of Kissinger[16] was modified for the extraction of the four compounds. All samples were de-fatted with petroleum spirit (boiling range 36–60 °C) prior to extraction. One gram of de-fatted chocolate or cocoa liquor is mixed with 20 ml of 0.1 N perchloric acid using a Sorvall Omni-Mixer at setting 7 to 10 min. Alternatively this extraction could be carried out by using a wrist action shaker for 45 min. The homogenate is transferred into a centrifuge tube and centrifuged at 2000 rev min^{-1} for 10 min. The supernatant liquid is adjusted to pH 10.3 $\pm$ 0.1 with concentrated ammonia solution and then stored overnight in a refrigerator at −4 °C. The resulting solution is filtered through a Whatman No. 41 filter-paper or its equivalent. The filtrate is saturated with solid sodium chloride and then extracted four times with 5 ml of an ethyl acetate - acetone (2 + 1) mixture. After each extraction the mixture is briefly centrifuged to help separate the two layers. The first three extractions are transferred into a clean test-tube using a Pasteur pipette. The fourth extraction is filtered through Whatman IPS phase-separating paper. The organic extracts are combined and dried with anhydrous sodium sulphate. After decanting, the sodium sulphate is washed with an additional 2 ml of the ethyl acetate - acetone (2 + 1) mixture. The water-free extracts are evaporated to dryness under nitrogen at 20 °C and then dissolved in 1 ml of the HPLC mobile phase.

Analysis

The use of two HPLC systems was a result of the arrival of new equipment that would allow direct measurement of the amines by natural fluorescence[19]; earlier studies used the post-column *o*-phthaldehyde derivative formation HPLC method. Inject 20 μl of the extract and compare with injections of standards. Calculate the concentration in the extract by comparison of the peak heights of the sample and standards. Figs. 1–3 show chromatograms of standards using ultraviolet, post-column derivatisation and natural fluorescence detection, respectively. Figs. 4–6 depict chromatograms of extract using the same methods of detection.

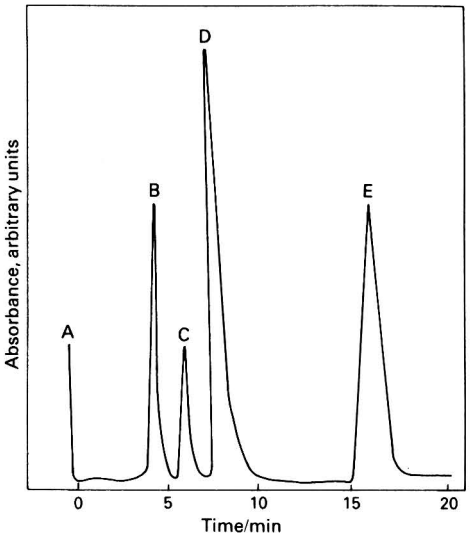

Fig. 1. Chromatogram of standards using ultraviolet detection. A is the injection point. Sample contained 20 μg each of: B, tyramine; C, serotonin; D, 2-phenylethylamine; E, tryptamine. Column, μBondapak C_{18}. Mobile phase, 0.2 M acetic acid. Detector, Waters Associates, Model 440, at 254 nm, 0.02 a.u.f.s.

Thin-layer chromatography. Spot 10 μl of each of the extracts and standards on to Si-60 TLC plates. Develop to 12–14 cm with chloroform - methanol - concentrated ammonia solution (28%) (12 + 7 + 1). Air dry the plates, then spray with ninhydrin solution and heat at 110 °C for 10 min to reveal the spots. Compare the R_F values obtained for the standards with those of the extracts.

Results

Recovery studies were conducted on the matrices of cocoa liquor and milk using the *o*-phthaldehyde post-column reaction and subsequent determination. Spiking for all recoveries was performed by the addition of standard to the perchloric acid extract. Additional recovery studies were carried out on the milk chocolate matrix using native fluorescence detection. The four amines were added at four different levels to the cocoa liquor, three different levels to the whole milk and three different levels to the milk chocolate. Tables I–III show the averages of duplicate determinations.

Tables I–III show good method accuracy using either the *o*-phthaldehyde derivative or natural fluorescence detection methods. Table IV shows the results for five 1-g samples of de-fatted milk chocolate assayed in duplicate using natural fluorescence detection.

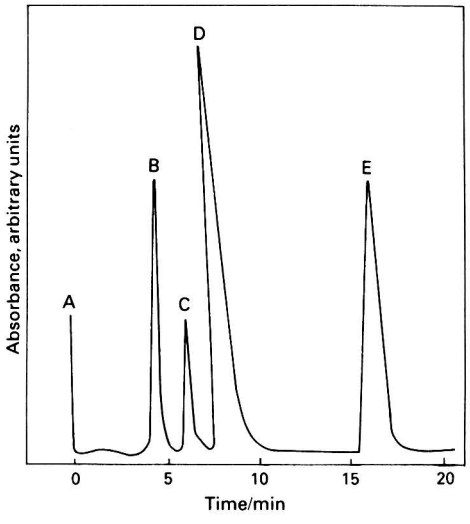

Fig. 2. Chromatogram of standards after post-column formation of *o*-phthaldehyde derivatives using fluorimetric detection. A is the injection point. Sample contained 2 μg each of: B, tyramine; C, serotonin; D, 2-phenylethylamine; E, tryptamine. Column, μBondapak C_{18}. Mobile phase 0.2 M acetic acid. Detector, Gilson Spectra-glo fluorimeter.

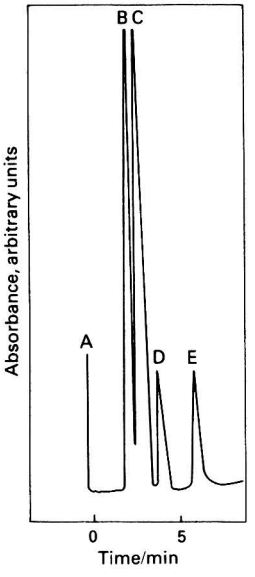

Fig. 3. Chromatogram of standards with detection by means of natural fluorescence. A is the injection point. Sample contained: 0.5 μg tyramine (B); 0.5 μg serotonin (C); 14 μg 2-phenylethylamine (D); 0.54 μg tryptamine (E). Column, μBondapak C_{18}. Mobile phase, 0.2 M potassium dihydrogen phosphate solution, pH 3.7 containing 20% of methanol by volume. Detector, Varian SF-300 with HPLC flow cell.

Tables V and VI give information pertaining to standard and sample precision studies. The ultraviolet data was included only for completeness as the ultraviolet mode was used as an additional confirmatory method and not for quantitative purposes. The lower limits of detection of four of the amines are shown in Table VII.

The average biogenic amine contents of eight selected chocolate liquors are given in Table VIII and the average amine contents of five chocolate products are given in Table IX. These tables show results obtained using both the *o*-phthaldehyde derivative and natural fluorescence methods.

Discussion

The results described indicate the presence of the biogenic amines of interest in chocolate liquor and chocolate products. As shown in Tables VIII and IX, these amines occur at varying levels and in varying ratios. It was not possible to gain information about fermentation patterns from the amine concentrations in the various liquor types, these levels vary as would be expected in natural products. One of the factors that complicates this problem is the tryptamine level. Tryptamine is a likely precursor of the plant growth hormone indole acetic acid.[19] In an attempt to gain further information about the fermentation patterns, similar samples should be analysed during all stages of growth and fermentation. From this information it might be possible to arrive at some meaningful conclusions.

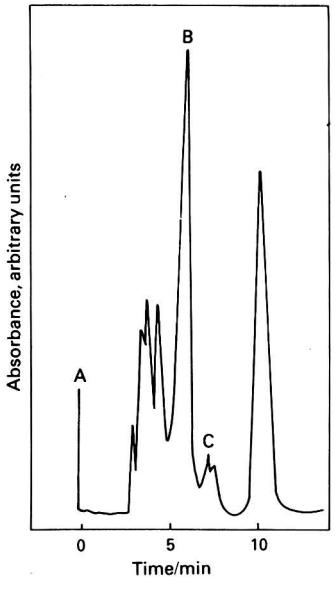

Fig. 4. Chromatogram of cocoa extract using ultraviolet detection. A is the injection point; B, tyramine; and C, serotonin. Column, μBondapak C_{18}. Mobile phase, 0.2 M acetic acid. Detector, Waters Associates, Model 440, at 254 nm, 0.02 a.u.f.s.

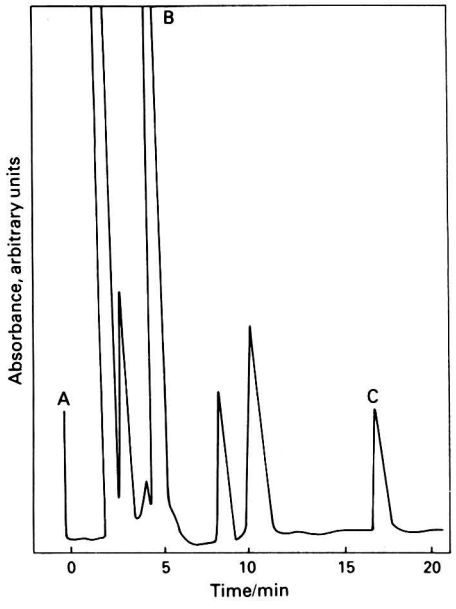

Fig. 5. Chromatogram of cocoa extract after post-column formation of *o*-phthaldehyde derivatives using fluorimetric detection. A is the injection point; B, tyramine; and C, tryptamine. Column, μBondapak C_{18}. Mobile phase, 0·2 M acetic acid. Detector, Gilson Spectra-glo fluorimeter.

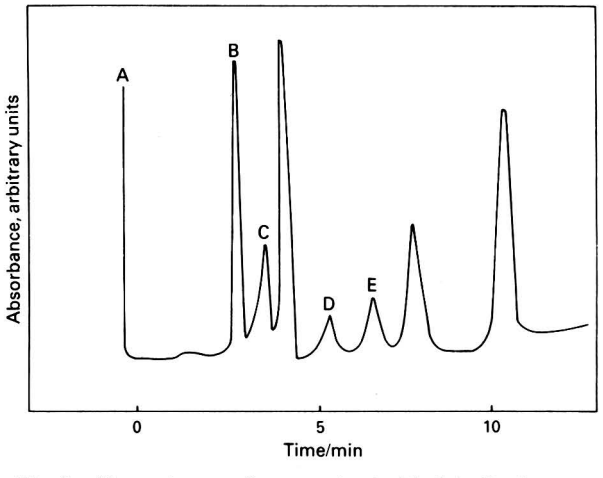

Fig. 6. Chromatogram of cocoa extract with detection by means of natural fluorescence. A is the injection point; B, tyramine; C, serotonin; D, 2-phenylethylamine; and E, tryptamine. Column, μBondapak C_{18}. Mobile phase, 0.2 M potassium dihydrogen phosphate solution, pH 3.7, containing 20% of methanol by volume. Detector, Varian SF 330 with HPLC flow cell.

TABLE I

AVERAGES OF DUPLICATE RECOVERIES FROM COCOA LIQUOR USING *o*-PHTHALDEHYDE DERIVATISATION DETECTION

Sample size = 1 g of de-fatted material in each instance, $n = 2$.

Amine	Amount added/ $\mu g\ g^{-1}$	Amount recovered/ $\mu g\ g^{-1}$	Recovery, %
Tyramine	10	9.56	95.6
	20	18.90	94.5
	50	46.80	93.6
	100	93.60	93.6
			Average = 94.3
Tryptamine	10	9.49	94.9
	20	18.40	92.0
	50	45.50	91.0
	100	88.60	88.6
			Average = 91.6
Serotonin	10	8.96	89.6
	20	19.20	96.0
	50	48.70	97.4
	100	95.80	95.8
			Average = 94.7
2-Phenylethylamine ..	10	8.45	84.5
	20	17.80	89.0
	50	44.50	88.9
	100	92.30	92.3
			Average = 88.7

The analytical data presented in this work are in agreement with those obtained by Kenyhercz and Kissinger who found tyramine levels of 8–11 $\mu g\ g^{-1}$ in cocoa.[14] The data also seem satisfactory when compared with that of Ingles *et al.*[14] where tyramine was determined in one sample at the 5 $\mu g\ g^{-1}$ level by HPLC. However, other reports have suggested that tyramine is absent from cocoa[1–3] so that the literature is confusing concerning even the presence or absence of these compounds in cocoa.

Examination of whole milk extracts indicates the presence of compounds with a primary amino group. This work did not indicate the presence of the four biogenic amines in whole milk extracts.

TABLE II

RECOVERY FROM WHOLE MILK USING *o*-PHTHALDEHYDE DERIVATISATION DETECTION

Amine	Amount added/ $\mu g\ g^{-1}$	Amount recovered/ $\mu g\ g^{-1}$	Recovery, %
Tyramine	20	18.40	92.0
	50	44.90	89.8
	100	96.80	96.8
			Average = 92.9
Tryptamine	20	17.60	88.0
	50	46.80	93.6
	100	91.80	91.8
			Average = 91.1
Serotonin	20	16.30	81.5
	50	44.70	89.4
	100	94.20	94.2
			Average = 87.8
2-Phenylethylamine ..	20	17.80	89.0
	50	42.40	84.8
	100	89.40	89.4
			Average = 87.7

TABLE III

AVERAGE OF DUPLICATE RECOVERIES FROM MILK CHOCOLATE USING NATURAL FLUORESCENCE DETECTION

Sample size = 1 g.

Amine	Amount added/ $\mu g\ g^{-1}$	Amount recovered/ $\mu g\ g^{-1}$	Recovery, %
Tyramine	10	9.78	97.8
	25	23.40	93.6
	50	47.10	95.6
			Average = 95.7
Tryptamine	10	9.61	96.1
	25	24.30	97.2
	50	47.80	95.6
			Average = 96.3
Serotonin	10	9.48	94.8
	25	23.60	94.4
	50	46.55	93.1
			Average = 94.1
2-Phenylethylamine	10	9.34	93.4
	25	24.75	99.0
	50	49.65	99.3
			Average = 97.2

TABLE IV

MULTIPLE MILK CHOCOLATE ANALYSES

Tryptamine could not be detected in any of the samples.

Sample number	Amine content/$\mu g\ g^{-1}$ Tyramine	2-Phenylethylamine	Serotonin
1	12.02,12.02	0.42,0.46	27.10,27.30
2	11.97,12.07	0.44,0.44	27.30,27.10
3	12.03,11.99	0.41,0.47	26.80,27.60
4	12.06,11.98	0.43,0.45	26.92,27.48
5	11.95,12.09	0.40,0.48	27.03,27.37
Mean	12.03	0.44	27.2
Standard deviation	0.045	0.025	0.25
Coefficient of variation, %	0.38	5.68	0.92

TABLE V

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY PRECISION STUDIES ON STANDARDS

Amine	Amount injected/ μg	n	Detection mode	Coefficient of variation, %
Tyramine	20	9	Ultraviolet	0.78
	5	5	Post-column derivatisation	1.44
	5	5	Fluorescence	1.52
Tryptamine	20	9	Ultraviolet	2.27
	5	5	Post-column derivatisation	4.63
	5	5	Fluorescence	3.91
2-Phenylethylamine	20	9	Ultraviolet	7.44
	15	5	Post-column derivatisation	8.74
	5	5	Fluorescence	6.02
Serotonin	20	9	Ultraviolet	2.01
	5	5	Post-column derivatisation	2.97
	5	5	Fluorescence	2.23

TABLE VI

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY PRECISION STUDIES ON SAMPLES

Amine	Amount injected/ μg	*n*	Detection mode	Coefficient of variation, %
Tyramine	20	9	Ultraviolet	2.19
	5	5	Post-column derivatisation	1.53
	5	5	Fluorescence	0.93
Tryptamine	20	9	Ultraviolet	3.17
	5	5	Post-column derivatisation	5.03
	5	5	Fluorescence	4.46
2-Phenylethylamine ..	20	9	Ultraviolet	6.79
	15	5	Post-column derivatisation	6.08
	7	5	Fluorescence	5.58
Serotonin	20	9	Ultraviolet	1.98
	5	5	Post-column derivatisation	2.36
	5	5	Fluorescence	2.92

This study is not yet complete as other amines have been found to occur in chocolate. Kenyhercz and Kissinger,[16] using HPLC with an electrochemical detector, found octopamine, metanephrine and synephrine in chocolate. These amines need to be investigated further and quantified as they are conversion products of tyramine. The other conversion products of tyramine[6,20,21] are methyltyramine, dopamine, hordiene, methyloctopamine and noradrenaline.

These and other amines can be formed by several biochemical pathways, including amino acid decarboxylation, aldehyde amination, phospholipid decomposition and thermal amino acid composition.[6] Other amines found in chocolate are[6] methylamine, butylamine, dimethylamine, isobutylamine, ethylamine, isoamylamine, trimethylamine and triethylamine.

TABLE VII

DETECTION LIMITS FOR FOUR OF THE AMINES

Amine	Detection mode	Lower limit/μg g^{-1}
Tyramine	Post-column derivatisation	1.0
	Natural fluorescence	0.5
Tryptamine	Post-column derivatisation	0.5
	Natural fluorescence	0.5
Serotonin	Post-column derivatisation	2.0
	Natural fluorescence	0.25
2-Phenylethylamine ..	Post-column derivatisation	9.0
	Natural fluorescence	0.25

TABLE VIII

AVERAGE BIOGENIC AMINE CONTENTS OF CHOCOLATE LIQUORS

In each instance the sample size was 1 g and the mean of 2 determinations is shown.

	Amine content/μg g^{-1}							
	Tyramine		Serotonin		Tryptamine		2-Phenylethylamine	
Type	Post-column derivatisation	Natural fluorescence	Post-column derivatisation	Natural fluorescence	Post-column derivatisation	Natural fluorescence	Post-column derivatisation	Natural fluorescence
Costa Rica	7.96	7.96	15.80	16.10	3.54	3.52	N.D.*	4.34
New Guinea ..	14.70	14.70	9.19	9.23	2.88	2.93	N.D.	6.56
Lagos	2.69	2.73	3.96	3.85	0.99	1.04	N.D.	4.38
Equador	8.37	8.42	12.40	12.21	2.41	2.38	N.D.	5.13
Light Lagos ..		2.06		0.92		0.52	N.D.	2.19
Malaysian		2.74		0.18		1.07	N.D.	3.28
Sanchez		0.73		0.79		0.71	N.D.	2.55
Caracas		1.09		3.82		2.04	N.D.	8.02

* N.D. = not detected.

TABLE IX

AVERAGE BIOGENIC AMINE CONTENT OF SOME CHOCOLATE PRODUCTS

In each instance sample size = 1 g and the result given is the mean of 2 determinations.

	Amine content/$\mu g\ g^{-1}$							
	Tyramine		Serotonin		Tryptamine		2-Pheylethylamine	
Product type	Post-column derivatisation	Natural fluorescence	Post-column derivatisation	Natural fluorescence	Post-column derivatisation	Natural fluorescence	Post-column derivatisation	Natural fluorescence
Milk chocolate A ..	11.90	12.02	26.60	27.20	N.D.*	N.D.	N.D.	0.44
Milk chocolate B ..	5.96	6.04	8.51	8.33	N.D.	N.D.	N.D.	2.13
Milk chocolate C ..	3.83	3.76	5.32	5.25	N.D.	N.D.	N.D.	6.60
Milk chocolate D ..	4.27	4.41	1.04	1.02	N.D.	N.D.	N.D.	4.40
Dark chocolate ..	11.90	12.02	8.46	8.64	N.D.	N.D.	N.D.	3.84

* N.D. = Not detected.

The levels in chocolate are low when compared with levels found in other foods. Cheese has been reported as having tryptamine and 2-phenylethylamine levels ranging from below the limits of detection to over 400 $\mu g\ g^{-1}$. Sausage has been reported to contain levels of tyramine ranging from below the limit of detection to over 350 $\mu g\ g^{-1}$, 2-phenylethylamine levels from below the limit of detection to almost 700 $\mu g\ g^{-1}$ and tryptamine levels from below the limit of detection to over 50 $\mu g\ g^{-1}$. Meat and cheese are not the only foods that contain amines.

This work is not a comprehensive study of the amines that occur in chocolate and more investigations need to be made; however, it provides a method for the traction, detection and quantitation of the four biogenic amines at trace levels in chocolate products.

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Standard Atmosphere Generator: a Dynamic System for the Controlled Dilution of Organic Vapours in Air

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An apparatus is described for the production of standard atmospheres of compounds at the concentrations normally encountered in air pollution studies. It is a glass-blown, flow system with continuous syringe injection of the compounds into a vaporiser, prior to their dilution in the main flow line. Statistical analyses of performance tests on a range of compounds show that stable atmospheres are produced within 5 min of start-up. The instrument produces the predicted concentrations of non-polar compounds with a high degree of accuracy, and tests on atmospheres of a relatively involatile compound, 1-methylnaphthalene, reveal no diminution in performance even with concentrations approaching that of its saturated vapour.

Keywords: *Standard atmosphere*; *syringe pump*; *organic vapour*; *air sampling*

For the most accurate assessment of the efficiency of air sampling equipment in collecting many varied pollutants, it is necessary to have an apparatus that can produce standard atmospheres containing the compounds at concentrations similar to those encountered in practice. The apparatus described here, a dynamic system with liquid injection, has been designed to provide standard atmospheres with a minimum of procedure, but with concentrations that can be accurately pre-determined, and without a requirement for lengthy periods of stabilisation.

A dynamic system was chosen in preference to a static system with which inaccuracies arise because of losses to the container walls, and liquid injection was chosen as the means of introduction of the pollutant into the gas flow. Design features specific to this apparatus were vaporisation of the liquid before it mixed with the main air flow, and the incorporation of a smoothing system.

The alternative methods of producing the vapour and controlling its dilution were dismissed because they did not meet the requirements set out above. Some systems are based on the production and dilution of a saturated vapour, but truly saturated vapours can be difficult to obtain. Other procedures depend on diffusion-controlled dilution of a vapour either through a defined orifice[1–3] or across a semi-permeable membrane,[4] but the operator has limited control over the precise concentration that will be produced. In these alternative systems the production of atmospheres containing several components may require separate saturators or diffusors for each vapour.

Standard Atmosphere Generator

The generator is illustrated in Fig. 1. It is essentially a glass-blown construction using 7 mm i.d. tubing for the main flow line. Air is drawn through the apparatus using a Charles Austen, 4N, diaphragm pump, and the necessary flow control is achieved with the combination of a fixed leak and a TF/6/18 Rotaflo valve. The flow-rate is measured in the range 0.005–0.025 m^3 min^{-1} on the Fisher Controls, Series 1100, Rotameter and the incoming air is purified by passage through a 500-ml sintered-glass gas wash-bottle packed with 250 ml of Linde 13X molecular sieve and 250 ml of 30–60-mesh activated charcoal. Pressure can be measured at both ends of the apparatus with a mercury manometer. The Rotameter was calibrated under its operating condition using a gas meter.

The vaporiser section and injection port are constructed from a Pye, Series 104, injection head attached to a 6 mm o.d. × 1 mm i.d. × 50 mm long glass tube, and wound with a 120-W heating cord connected to a variable transformer power supply. Helium carrier gas at 40 ml min^{-1} transports vapours into the main flow line. The organic compounds are injected

using a SAGE, Model 341, syringe pump fitted with SGE or Hamilton gas-tight syringes. The latter has 0.1 mm bore needles and the former 0.2 mm bore needles. The syringe pump and the diaphragm pump are operated from the same power supply so that they can be switched on or off at the same time.

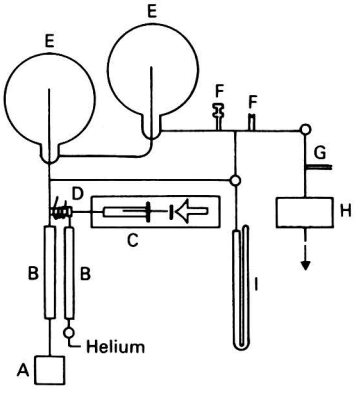

Fig. 1. Standard atmosphere generator: A, air intake filter; B, Rotameter gauges; C, syringe and syringe pump; D, vaporisor; E, 5-l mixing bulb; F, sampling port; G, fixed leak; H, main air flow pump; and I, mercury manometer.

There are two ports for the removal of standard atmospheres from the main flow. The first consists of a 6 mm o.d. × 1 mm i.d. × 50 mm long glass tube fitted with a PTFE coupling (Chemcon Ltd.). The standard atmosphere can be withdrawn continuously from this port using an electric pump. The 1-mm constriction is small enough to permit the port to be opened during operation of the apparatus with little effect (less than 2.5% diminution) on the main flow. When not in use, the PTFE union is plugged with a 6 mm o.d. glass rod. The second port is intended for the withdrawal of syringe samples and consists of a 40-mm length of 6 mm i.d. glass tubing terminated with a Thermogreen, half-hole type, cylindrical septum (Supelco Ltd.).

With the arrangement shown in Fig. 1, the apparatus produces atmospheres at pressures slightly less than ambient. It can be operated equally well at pressures above ambient by connecting the air pump to the inlet filter, instead of the generator outlet, so that it blows air through the apparatus.

Principles of Operation

The operation of the apparatus can be divided into four functions: injection, vaporisation, dilution/mixing and smoothing.

Injection

The syringe pump moves the syringe plunger by a series of pulses, each corresponding to a movement through 6.9×10^{-3} mm. The flow-rate can be varied either by changing the pulse rate (from a minimum of 1.3 up to 3000 min^{-1}) or by varying the size of the syringe (from 25 μl to 50 ml).

Vaporisation

When the temperature of the vaporiser is just below the boiling-point of the injected liquid, vaporisation will take place rapidly at the tip of needle, or on the walls of the chamber if the syringe flow-rate is very high. At temperatures above the boiling-point of the liquid, vaporisa-

tion may occur within the bore of the needle. This second mode of operation is necessary to ensure the vaporisation of very high boiling components when a mixture of compounds is injected.

Fractionation during vaporisation, with the less volatile species tending to remain in the liquid phase and the gas phase concentration correspondingly less than predicted, is not a major problem as it is self-compensatory with time, there being rapid enrichment of such compounds in the immediate vicinity of the liquid front, and their rates of vaporisation increasing to equal their rate of injection.

Dilution/mixing

A simple T-junction suffices as the preliminary mixing stage. The efflux velocity from the narrow bore of the vaporiser is 0.1 m s^{-1} (for a carrier gas flow-rate of 40 ml min^{-1}) and the air velocity in the main flow line falls in the range 0.2–1.1 m s^{-1}.

The atmosphere subsequently passes through Drechsel heads fitted into round-bottomed flasks and the velocity of the incoming air induces rapid mixing.

Smoothing

The mixing process could occur as adequately in small flasks as in the two 5-l flasks chosen for this apparatus. However, these large flasks have the advantage of greatly increasing the volume of the mixing system and enable it to smooth any irregularities in concentration introduced during injection and vaporisation. At its slowest rate the syringe pump produces pulses with a time interval of 0.78 min and this is comparable to the retention times for the 10-l mixing volume, which are in the range 0.4–2.0 min.

Procedures for Using the Generator

Purging

Switch on the vaporiser heater to give a temperature of up to 250 °C, switch on the diaphragm pump to give a main flow-rate of 0.025 m^3 min^{-1}, and switch on the carrier gas flow to give a flow-rate of 40 ml min^{-1}. Leave the apparatus in this condition for about 10 min to purge it of previously adsorbed material.

Conditioning

Fill the syringe with the liquid to be injected, ensuring there are no entrained air bubbles. Switch on the pumps (pre-set to produce the required atmosphere) and insert the needle 30 mm through the injection head. Leave the system running for at least 10 min before using the standard atmosphere. To change the concentration when the apparatus is already running, make the appropriate adjustment to the syringe flow-rate or the main flow-rate and permit at least 10 min for the system to stabilise.

The predicted concentration for the standard atmosphere, C_p, is given by the expression

$$C_p = \frac{fc}{F} \text{ mg m}^{-3}$$

where f μl min^{-1} is the flow-rate from the syringe, c mg μl^{-1} (or g ml^{-1}) is the concentration in the liquid and F m^3 min^{-1} is the main flow-rate. For pure liquids, c is equal to the density in grams per millilitre.

Note: In the tables of results that follow, the measured concentrations are referred to by the symbol C_m.

Test Procedure and Apparatus

Chemicals

Nitrobenzene-d_5 (perdeuteronitrobenzene) was obtained from Koch-Light Laboratories Ltd. Halothane (1,1,1-trifluoro-2-chloro-2-bromoethane) was supplied by ICI. All other chemicals were of analytical-reagent grade.

Sample Tubes

The sample tubes (see Fig. 2) had a 12.5 mm o.d. × 57 mm long stainless-steel tube welded at each end to short tubes, 6.3 mm o.d. × 12.5 mm long (G. N. Instrumentation Consultancy Ltd.). The body of the tube was packed with 1 g of 60–80-mesh Tenax GC and plugged with silanised glass-wool. The sorption characteristics of these tubes have been described previously.[5,6]

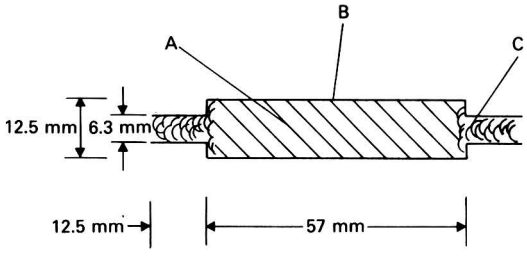

Fig. 2. Adsorption tube: A, 60–80-mesh Tenax GC; B, stainless-steel body; and C, silanised glass-wool.

Sampling and Analysis

Three methods of sampling were used. Atmospheres containing halothane were drawn through a Carlo Erba gas sampling valve via a 1.6 mm o.d. PTFE tube. The sample loop volume was 0.5 ml. Suction was provided by a Charles Austen Dymax 2A diaphragm pump, regulated by a needle valve, and the pressure in the sampling line was monitored on a mercury manometer. Direct injection was made on to a 2 m × 3 mm i.d. column packed with 5% OV-101 on 80–100-mesh Supelcoport, and fitted to a Carlo Erba flame-ionisation detector. The carrier gas flow-rate was 15 ml min^{-1} (101 kN m^{-2}, 20 °C) and the column temperature was constant at 60 °C. This was a variation of the method of MacDonald and MacKenzie.[7] For comparison, 1-μl portions of standard solutions of halothane in carbon tetrachloride were injected through the septum injection port of the gas chromatograph. At least five injections of each solution were made.

Some atmospheres were sampled on adsortion tubes by connecting the tube directly to the PTFE union and drawing off the standard atmosphere at 100–200 ml min^{-1} using an electric pump for large volumes, or a gas-tight syringe for volumes of less than 500 ml. Before sampling, the tubes were loaded with the appropriate internal standard (2 μg of anisole for hydrocarbon analysis, or 0.1 μg of nitrobenzene-d_5 for nitrobenzene-d_0 analysis) according to the method of Brookes.[6] The samples were heat desorbed into a liquid nitrogen cooled trap using a GN concentrator (G. N. Instrumentation Consultancy Ltd.) and flash desorbed on to a 30 m × 0.25 mm i.d. SE-30 WCOT gas-chromatographic column. For the hydrocarbon analysis this was programmed from 20 to 110 °C at 4 °C min^{-1} and the peaks were monitored using a VG Organic MM16F mass spectrometer in its integrated ion mode of detection. The same column was programmed from 60 to 100 °C at 5 °C min^{-1} for nitrobenzene analysis using the mass spectrometer in its selective ion mode to monitor the nitrobenzene-d_0 and -d_5 parent ions. Calibration graphs for these analyses were obtained by making injections of known amounts of the compounds of interest on to adsorption tubes, together with the appropriate internal standard, and analysing the tubes in the above manner. All of the analyses were carried out within 36 h of the preparation of the standard solutions and standard atmospheres.

For continuous monitoring a 3.1 mm o.d. PTFE tube was connected between the PTFE union and a Carlo Erba flame-ionisation detector. With the air pump blowing air through the apparatus at a rate of 0.025 m^3 min^{-1}, an exit pressure of 0.9 kN m^{-2} above ambient was obtained, and this caused a small portion (30 ml min^{-1}) of the standard atmosphere to flow directly into the detector. The output was recorded on a chart recorder and data for statistical analysis were obtained by measuring the response at regular 0.5- or 1-min intervals.

Test Results

All of the work reported here was taken from data obtained when producing standards with concentrations in the range from ambient to the Threshold Limit Value[8] or sometimes in the region of the Odour Threshold Value.[9]

The time required for the apparatus to produce a stable atmosphere of a volatile compound was determined whilst monitoring a halothane atmosphere continuously for 60 min. Details of the statistical analysis of the detector response measured at regular 1-min intervals are given in Table I. The two values for the mean responses measured for the periods 5–20 min and 45–60 min after start-up showed no difference when compared in an unpaired *t*-test at the 0.05 level of significance, indicating that stabilisation had effectively occurred within 5 min of start-up.

TABLE I

STATISTICAL ANALYSIS OF HALOTHANE, 1-METHYLNAPHTHALENE AND NITROBENZENE ATMOSPHERES, SHOWING STABILISATION

Compound*	Predicted concentration/ mg m^{-3}†	Air flow-rate/ m^3 min^{-1}†	Syringe flow-rate/ μl min^{-1}	Period of data analysis/ min (after start-up)	Number of data	Mean response ± S.E./mm
Halothane	77	0.0259	1.07	5–20	16	91.7±0.3
	77	0.0259	1.07	45–60	16	92.5±1.5
1-Methylnaphthalene	15.2	0.0259	0.386	5–20	16	100.2±0.8
	15.2	0.0259	0.386	45–60	16	101.0±0.5
	61.1	0.0259	1.55	5–20	16	435±3
	61.1	0.0259	1.55	45–60	16	438±2
Nitrobenzene	18.0	0.0259	0.386	5–20	16	37.3±0.8
	18.0	0.0259	0.386	29–44	16	37.5±0.7
	36.5	0.0259	0.785	5–20	16	211±2
	36.5	0.0259	0.785	20–35	16	209±2

* The pure compounds were injected with the following vaporiser temperatures: halothane, 100 °C; 1-methylnaphthalene, 220 °C; and nitrobenzene, 190 °C.
† At 102.2 kN m^{-2} and 20 °C.

The accuracy with which the apparatus produced atmospheres at pre-determined concentrations was measured for a succession of standard atmospheres of halothane in the range 20–280 mg m^{-3} (20 °C and 101.3 kN m^{-2}) by varying the syringe flow-rate and holding the main flow-rate constant. The syringe was filled with pure halothane. Halothane peak heights resulting from the gas-chromatographic analysis of the standards are plotted with respect to time in Fig. 3, which also contains the procedural information. Each sample represented the mean concentration produced by the generator over a 0.5-s period. On the two occasions when a freshly charged injector syringe was inserted into the vaporiser, the halothane concentration exceeded the steady-state level. This phenomenon occurs as a result of the rapid vaporisation of halothane in the dead space of the needle when it is inserted.

The mean values of the peak heights for each steady state are plotted in Fig. 4 with respect to the amount of halothane injected on to the gas-chromatographic column, the amount injected being calculated from the predicted concentration, which is also shown on the horizontal co-ordinate. In addition, Fig. 4 shows the results for the liquid standards, which were injected directly on to the gas-chromatographic column and analysed in the same way as the gas standards. For each set of data the line of regression, the standard errors (S.E.) of the constants and the correlation coefficients (R) were calculated. Describing the line of regression by an equation of the form

$$y = ax + b$$

where y represents peak height and x the amount of halothane injected, the data given in Table II were calculated.

TABLE II

STATISTICAL ANALYSIS OF HALOTHANE DATA FOR THE LINE OF REGRESSION $y = ax + b$

Standards injected	$a \pm$ S.E./ mm ng^{-1}	$b \pm$ S.E./ mm $\times 10^2$	Number of measurements	Correlation coefficient, R
Gas standards	4.46 ± 0.05	+0.13 ± 0.12	41	0.998
Liquid standards	4.66 ± 0.11	−0.27 ± 0.32	32	0.992

An unpaired t-test on the values of a and b for the two sets of data showed no significant difference at the 0.05 level of significance.

Tests were also carried out on a relatively involatile compound. Standard atmospheres of 1-methylnaphthalene were produced by varying the rate of injection of the pure liquid and holding the main flow-rate constant. Sampling via a gas sampling valve or a glass syringe

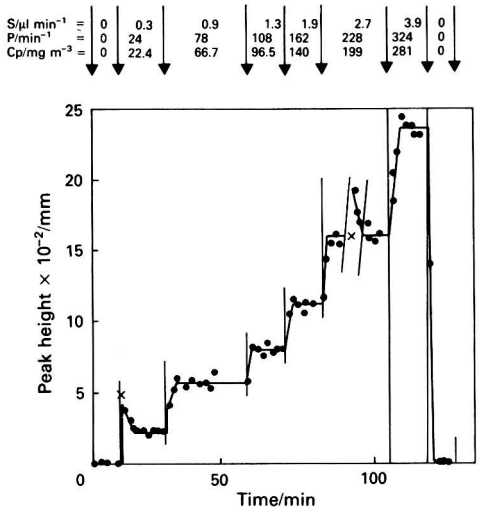

Fig. 3. Stabilisation of halothane atmospheres with respect to time for a series of different flow settings. S, Syringe flow-rate; P, syringe pulse-rate; C_p, predicted concentration (101.3 kN m^{-2} and 20 °C). At points × on the graph a freshly charged syringe was inserted into the vaporiser. Vaporiser temperature, 100 °C (boiling-point of halothane, 50 °C); main flow-rate, 0.025 m^3 min^{-1}; sampling period, 0.5 s.

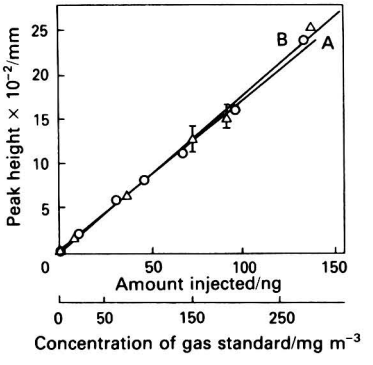

Fig. 4. Mean gas-chromatographic GC responses (± 2 × standard error) for the injection of halothane gas standards (○, A) and liquid standards (△, B) and plotted against the amount of halothane injected. For gas standards this was calculated from the predicted concentration, which is also shown on the graph. In all but two instances the error bars were smaller than the size of the point.

proved to be unsuitable. The former method gave gas-chromatographic peaks with extensive tailing and the latter evidenced losses of the compound to the walls of the syringe. Continuous monitoring was the only satisfactory procedure available. The results and procedural information for the stabilisation studies are given in Table I. In each of the two atmospheres tested, the t-test showed no difference at the 0.05 level of significance between the mean responses measured near the beginning and at the end of each run. The amospheres were generated on different occasions and no correlation tests were carried out on the data because of variation in gas-chromatographic sensitivity.

To test specifically for correlation a series of 1-methylnaphthalene atmospheres were generated in the range 3.18–124 mg m^{-3} (102.2 kN m^{-2}, 20 °C), again by varying the syringe flow-rate and holding the air flow-rate constant. Each atmosphere was allowed 5 min to stabilise and the detector response was measured at regular 0.5-min intervals over the subsequent 10-min period. The statistical analysis of the data is given in Table III.

TABLE III

STATISTICAL ANALYSIS OF 1-METHYLNAPHTHALENE DATA FOR THE LINE OF REGRESSION $y = ax + b$

Predicted concentration/mg m^{-3}*	3.18	7.03	15·2	29.5	61.1	124
Air flow-rate/m^3 min^{-1}*	0.0259	0.0259	0.0259	0.0259	0.0259	0.0259
Syringe flow-rate/μl min^{-1}† ..	0.0806	0.179	0.386	0.749	1.55	3.14
Mean response ± S.E./mm ..	20.5±0.5	51.0±0.5	112±1	214±1	438±2	908±4
Total number of measurements ..			126			
Correlation coefficient			0.9996			

* At 102.2 kN m^{-2} and 20 °C.
† Pure 1-methylnaphthalene injected at 220 °C.

Further confirmation of the performance of the instrument was obtained from quantitative measurements of standard atmospheres containing mixed hydrocarbons at predicted individual concentrations of 0.0535 mg m^{-3} (101.3 kN m^{-2}, 20 °C), which were produced on two separate occasions by injection of a methanolic solution of the hydrocarbons. The results of the gas chromatography - mass spectrometry of adsorption tube samples of these atmospheres are given in Table IV.

In order to test the apparatus for the production of atmospheres containing high-boiling polar compounds, a number of measurements were made using nitrobenzene. Table I gives the results of statistical analyses of two nitrobenzene atmospheres, which were monitored continuously using the flame-ionisation detector. In both instances the *t*-test demonstrated stabilisation within 5 min of start-up, but the detector response showed a greater degree of variation compared with the atmospheres of other compounds. Quantitative measurements were also carried out. The difficulties in the production of atmospheres of compounds such as nitrobenzene apply equally to their sampling and gas-chromatographic analysis. These analytical problems were minimised by sampling on adsorption tubes with nitrobenzene-d_0 for the standard atmosphere and nitrobenzene-d_5 as the internal standard. The results presented in Table V were obtained with standard atmospheres produced on four separate occasions. Pure nitrobenzene-d_0 was injected for the first three atmospheres and a methanolic solution was injected to produce the fourth. The results show a much greater degree of variation than those obtained for other compounds.

Discussion

All of the continuous monitoring tests reported in Table I demonstrated the stabilisation of each standard atmosphere within 5 min of start-up. With 1-methylnaphthalene the test was acutely sensitive because the degree of variation about each mean value was very small, and great care was needed to eliminate interferences from outside contamination of the air supply, as the normal variations would have considerably reduced the sensitivity of these tests. It may be that the minimal variation exhibited by 1-methylnaphthalene was a function of its comparative involatility in that it permitted a smoother vaporisation process.

TABLE IV

ANALYTICAL RESULTS AND OPERATING CONDITIONS FOR HYDROCARBON ATMOSPHERES

Operating conditions: main flow-rate = 0.010 m^3 min^{-1} at 99.3 kN m^{-2}; liquid flow-rate from the 100-μl SGE syringe = 1.05 μl min^{-1}; syringe solution = 0.0005 mg μl^{-1} of each hydrocarbon in methanol; syringe pulse rate = 77 min^{-1}; injection temperature = 100 °C; conditioning time = 15 min; sampling time = 60 min; sample volume = 0.00952 m^3 at 101.3 kN m^{-2} and 20 °C.

Parameter	Test	*o*-Xylene	*p*-Xylene	Trimethylbenzene	Naphthalene
Boiling point/°C (101.3 kN m^{-3}) ..	—	144	138	165	218
Predicted concentration, (C_p)/mg m^{-3}*	Both tests	0.0535	0.0535	0.0535	0.0535
Measured concentration, (C_m)/mg m^{-3}*	First test	0.0543	0.0545	0.0539	0.0513
	Second test	0.0560	0.0572	0.0538	0.0513
Deviation, $(C_p - C_m)/C_p$, % ..	First test	+1.4	+1.9	+0.7	−4.0
	Second test	+4.7	+6.9	+0.6	−4.0

* At 101.3 kN m^{-2} and 20 °C.

For the purposes of the quantitative and correlative measurements on halothane, the onset of stabilisation was estimated by visual inspection of the data in Fig. 3. These estimates agreed with that expected from the detailed analysis reported in Table I. The degree of variation about each mean value was very small and in Fig. 4 the 95% confidence limits for the gas standards data were too narrow to be represented. These observations, together with the high correlation and the comparability with liquid standards further confirmed that stabilisation effectively took place within 5 min of start-up.

TABLE V

ANALYTICAL RESULTS AND OPERATING CONDITIONS FOR NITROBENZENE ATMOSPHERES

Parameter	Atmosphere number 1	2	3	4
Main flow-rate/m^3 min^{-1}*	0.0243	0.0243	0.0243	0.0238
Inlet pressure/kN m^{-2}	98.6	98.6	98.6	98.6
Syringe type	H†	H†	H†	S†
Syringe liquid flow-rate/μl min^{-1}	0.00765	0.00765	0.00765	1.05
Nitrobenzene concentration in the syringe/mg μl^{-1}	1.20‡	1.20‡	1.20‡	0.0005§
Syringe pulse rate/min^{-1}	1.3	1.3	1.3	77
Vaporisor temperature/°C	190	190	190	190
Conditioning time/min	60	65	90	15
Sampling time/min	0.5	0.5	0.5	15
Sample volume/m^3*	0.0001	0.0001	0.0001	0.0020
Predicted concentration, C_p/mg m^{-3}*	0.377	0.377	0.377	0.022
Measured concentration, C_m/mg m^{-3}*	0.372	0.45	0.46	0.018
Deviation, $(C_m - C_p)/C_p$, %	−1.3	+19	+22	−18

* At 101.3 kN m^{-2} and 20 °C.
† H = 50-μl Hamilton syringe; S = 100-μl SGE syringe.
‡ The density of pure nitrobenzene.
§ Solution in methanol.

The correlation coefficients for halothane and methylnaphthalene indicated a highly reproducible and predictable performance in the production of atmospheres from compounds of very different volatility. The quantitative tests supported this conclusion. Calibrations of the gas-chromatographic analyses were made with liquid standards administered to the gas chromatography and gas chromatography - mass spectrometry systems either by direct syringe injection, which allowed the rapid production of a statistically significant number of data, or via adsorption tubes with an internal standard, which permitted very accurate indi-individual measurements.

The halothane measurements in Fig. 4 and Table II were calibrated by direct syringe injection. The statistical treatment revealed high individual correlation coefficients and small standard errors, a situation in which the application of the *t*-test to the two sets of data was especially critical; even so, the test showed no significant difference between the gas-chromatographic responses to the gas and liquid standards. Adsorption tubes were used for the performance tests with mixed hydrocarbon atmospheres (Table IV) and of nitrobenzene atmospheres (Table V). For hydrocarbons the differences between the measured concentrations were small and within the experimental error of the analytical procedure. In particular, naphthalene, which is relatively involatile, showed deviations of only 4% in both tests. Nitrobenzene, a compound of similar volatility to naphthalene but more polar, had much greater deviations of up to 22% in the four tests. The poor performance of the apparatus for nitrobenzene was not unexpected in view of the physical properties of this substance and the likelihood of it having irregular sorption characteristics on the glass surfaces of the instrument. An apparatus constructed from PTFE might overcome this problem.

The performance of the equipment and the stability of the atmospheres might have been expected to decrease for any compound as concentrations approached that of its saturated vapour. Extrapolation of the vapour pressure data for 1-methylnaphthalene at 244 and 107 °C[10] leads to a value of 60 mg m^{-3} at 20 °C, compared with the concentrations of 61 and 124 mg m^{-3} in Tables I and III. The errors in this extrapolation are very large, but the

concentrations of 1-methylnaphthalene in this study were certainly close to, and may have exceeded, that of the saturated vapour without showing any loss of correlation or stability. If the concentration of the saturated vapour was exceeded, the atmosphere must have existed either in a state of supersaturation or with partial formation of an aerosol.

Conclusion

This apparatus was designed for the production of standard atmospheres of both pure compounds and mixtures, at pre-determined concentrations, with a high degree of accuracy and a minimum of procedure. For all of the compounds tested the atmospheres have been observed to stabilise within 5 min of start-up and the apparatus met all of the above requirements for non-polar compounds. For a compound that was both polar and high boiling the accuracy of the apparatus in achieving pre-determined concentrations was diminished. There was no evidence of any diminution in performance with decreasing volatility of the non-polar compounds or with the production of concentrations close to that of the saturated vapour pressure.

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Comparison of Some Porous Polymers as Adsorbents for Collection of Odour Samples and the Application of the Technique to an Environmental Malodour

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A range of Chromosorbs and Tenax-GC have been compared with regard to their efficiency as adsorbents for volatile odorous compounds using a very simple model system. When desorption was accomplished by solvent elution using acetone, Chromosorb 103 provided the best recoveries of those investigated (between 90 and 95%). In situations where thermal lability of trapped compounds is not a problem, use of Tenax-GC and thermal desorption at about 250 °C would be recommended on the basis of this survey, during which this approach consistently provided recoveries greater than 96% from small sample volumes. The latter procedure was applied to the analysis of an industrial malodour from an animal rendering factory, and using gas chromatography - mass spectrometry over 35 compounds (92% of the total odour sample) were positively identified. In particular, a range of alkylthiophenes may be characteristic of this particular odour.

Keywords: *Odour analysis*; *porous polymer adsorbents*; *Chromosorbs*; *Tenax-GC*; *animal rendering odour*

Many industrial and related processes give rise to objectionable malodours, which frequently are allowed to escape into the environment to the discomfort of the local community. It is likely that legislation will be enforced to control such pollution, but in order to enact this adequately it is necessary to identify as far as possible the particular compounds responsible for the objectionable odours. Further, with this information it should then be more feasible to devise and introduce rational abatement procedures for the problem compounds.

From an analytical point of view it is necessary to develop procedures whereby representative samples of the offensive odours can be collected in a form suitable for examination by the technique ideally suited to the analysis of complex mixtures of volatile components, namely gas chromatography - mass spectrometry. However, the compounds of interest are present in the atmosphere in relatively small amounts so some form of concentration of the odorous air sample is usually necessary before analysis. Frequently sampling and concentration can be achieved in one step by some trapping procedure. There are three main methods: cryogenic trapping,[1–3] adsorption on charcoal[4,5] or adsorption on a porous polymer.[6–11] Of these, the first two methods suffer disadvantages not exhibited by the third. For example, with cryogenic trapping the nature of the coolant can be problematical. Although liquid oxygen has been used successfully[2] it is a hazardous material for routine use. If liquid nitrogen is used then liquid air will also be condensed and cause difficulties. Further, whatever the coolant, considerable amounts of water will be condensed from the air sample as ice, with the possibility of the formation of solid blockages in the trap. The main problem with charcoal as an adsorbant is that it is often difficult, if not impossible, to effect complete desorption for analysis, particularly of polar compounds. Porous polymers, on the other hand, can usually be readily and completely desorbed either simply by heat or by elution with an appropriate solvent. Equally, as they are hydrophobic they do not retain large amounts of water at ambient temperatures, although in some instances sufficient can be retained to cause some difficulties. For these and other reasons, porous polymers are a good choice as potential trapping materials for odorous air samples. However, there are many such commodities on the market and clearly it is necessary

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to evaluate these with regard to their efficiency and suitability for the analysis in question. This paper therefore partly describes a survey undertaken to assess the performance of some porous polymers as adsorbents, using a model system consisting of a simple mixture of volatile, odorous compounds.

Having determined a suitable adsorbent in this manner, and at the same time optimum experimental conditions for its use, this system was then applied to a real analytical problem, namely the offensive odour associated with the process of hot rendering of animal by-products. Little previous work has been reported in this particular area, although Doty *et al.*[12] conducted a preliminary survey of the problem as long ago as 1972.

Experimental

Adsorbent Traps

The porous polymer adsorbents examined were a selection of Chromosorbs (Phase Separations Ltd., Queensferry, Clwyd, UK) and Tenax-GC (Field Instruments, Twickenham, Middlesex, UK). All were 60–80 BSS mesh. Traps were prepared by packing a glass collection tube (200 × 4 mm i.d.) to a length of 75 mm with porous polymer, and were conditioned before use at the maximum recommended temperature for that particular adsorbent for 1 h in a flow of helium.

Evaluation of Adsorbents Using a Model System

The model system consisted of equal volumes of pentanal, pyridine, butyl acetate and diallyl disulphide. A glass tube (400 × 8 mm i.d.) was connected to the adsorbent trap using a PTFE sleeve, and an exact aliquot of the standard mixture (2 μl) was introduced into the horizontal sample tube via a vertical inlet protected by means of a silicone-rubber septum. A flow of dry nitrogen (variable within the range 50–750 ml min^{-1}) was passed over the liquid sample and through the trap at ambient temperature for various lengths of time (initially mainly 15 or 30 min). In some instances a second trap of Chromosorb 103 was connected in series beyond the test trap, again by means of a PTFE sleeve. Initially the traps were desorbed by elution with acetone (exactly 500 μl) and 1 μl of the eluate was then analysed. Thermal desorption is described later.

Collection of Odorous Air Samples

At first, samples were collected both by on-site trapping and by on-site collection of the atmosphere in poly(vinyl fluoride) (Tedlar; Du Pont de Nemours International S.A., Geneva, Switzerland) bags of about 30-l capacity. Subsequently it was found that samples stored in bags were unchanged after a period of up to 1 week, so thereafter only collection in Tedlar bags was used as this was much more convenient for replicate analyses. The bags were fitted with PTFE valves and sampling lines (Production Techniques Ltd., Fleet, Hampshire, UK) and were mounted in plastic carboys for portability. The carboy was evacuated on site by means of a battery-operated pump so that samples could be drawn into the bag. Samples were taken from the effluent pipes of the animal rendering cookers, where possible after condensers which removed the bulk of unwanted water. Odorous air (2 l) from the bag was passed through a trap of Tenax-GC at ambient temperature at a rate of 500 ml min^{-1}. A second trap in series beyond the first cooled to −78 °C in a solid carbon dioxide - acetone bath could be used to collect highly volatile, gaseous organic components that passed through the main trap. Adsorbed odour samples collected in this manner could be stored unchanged for a few days at room temperature provided that both ends of the trap were securely stoppered.

Analysis by Gas Chromatography

A Pye-Unicam 104 instrument with a heated flame-ionisation detector was used. The columns employed were 1.5 or 5.5 m × 4 mm i.d. glass tubes packed with PEG 20M (10%) or PEG 6000 (5%) on Diatomite C, acid-washed and HMDS-treated (100–120 BSS mesh). The column temperatures employed were 90 °C isothermal for the examination of the model system and programmed from 70 to 175 °C at 2.5 °C min^{-1} for the odorous air samples. The flow-rate of the carrier gas (helium or nitrogen) was always 30 ml min^{-1}. Solvent desorbed samples (1 μl) were injected directly into the column in the normal manner. Thermal desorption of Tenax-GC traps was achieved as follows. The trap was connected at the beginning of

the gas-chromatographic column using a suitable valving system and a carrier gas by-pass line (effectively a sample loop). The trap was then flushed with helium carrier gas at ambient temperature for 5–10 min, as it was found that if Tenax-GC was heated above 200 °C in the presence of oxygen some degradation occurred, and this caused the appearance of spurious peaks on the chromatograms. The gas flow was then stopped (*i.e.*, passed directly through the gas-chromatographic column) and the trap heated as rapidly as possible to 260 °C using a heating coil. The gas flow (30 ml min^{-1}) was then re-diverted through the trap, and the sample thus rapidly introduced on to the column as a discrete band. The chromatogram was then developed. Total odour samples thermally desorbed in this manner from traps back into Tedlar bags were found to possess the same odour as the original odorous air samples.

Analysis by Gas Chromatography - Mass Spectrometry

An AEI MS 30 double-beam, double-focusing instrument was used, fitted with an integrated Pye-Unicam 104 gas chromatograph connected via a heated membrane separator interface. The mass spectrometer was linked on-line to an AEI DS 50 data processing system equipped with the facility for double-beam accurate mass measurement. Broadly the same gas-chromatographic conditions as already summarised (including sample introduction) were employed. Relevant mass spectrometric operating parameters were as follows: ionisation potential, 70 eV; ionisation current, 500 μA; source temperature, 200 °C; resolving power, 1 500; and scan speed, 3 s per decade (repetitive throughout run).

Results and Discussion

Probably the most widely used porous polymer adsorbents for similar work (*e.g.*, collection of food aroma volatiles) are the Chromosorbs, Tenax-GC and, to a lesser extent, the Porapaks. In this project a range of Chromosorbs and Tenax-GC were evaluated using a model system consisting of a mixture of arbitrarily selected volatile, odorous compounds (pentanal, b.p. 102–103 °C; pyridine, b.p. 115–116 °C; butyl acetate, b.p. 126–127 °C; diallyl disulphide, b.p. 174 °C). The components covered a range of compound type and polarity, and are typical of those which might be expected in environmental odour samples.

An empty, horizontal glass tube was connected to a trap of the adsorbent under examination and 2 μl of the sample mixture were injected into the tube. Trapping was then accomplished by allowing a stream of dry nitrogen (variable, but typically about 250 ml min^{-1}) to sweep over the sample mixture at ambient temperature and through the adsorbent trap for various lengths of time. Solvent elution from the trap was then employed, using acetone. Preliminary experiments on the Chromosorbs indicated that Chromosorb 103 was probably the most efficient and it provided excellent recoveries of all components of the mixture. Detailed results for this adsorbent are given in Table I, and it can be seen that there was good reproducibility on replicate determinations. Improved collection was obtained using a sampling (nitrogen sweep) time of 30 min compared with 15 min. Longer periods did not provide any further advantage. The improved recovery between 15 and 30 min was not due to less complete evaporation of the test mixture from the glass tube during the shorter time. Other experiments showed that at least 96% of all components were evaporated within 5 min at a flow-rate of 500 ml min^{-1} (see results for thermal desorption from Tenax-GC tubes, discussed later).

TABLE I

PERCENTAGE RECOVERIES OF COMPONENTS OF A SAMPLE MIXTURE FROM A CHROMOSORB 103 TRAP USING ACETONE AS ELUENT AND A SAMPLING FLOW-RATE OF 250 ml min^{-1}

Component	Sampling time/min	
	15	30
Pentanal	89, 85, 87	94, 94, 91
Pyridine	87, 80, 80	92, 90, 92
Butyl acetate	86, 84, 84	94, 94, 94
Diallyl disulphide	80, 85, 83	90, 93, 93

Similar preliminary assays using Tenax-GC and the same sampling rates (250 ml min^{-1}) gave poorer results overall (about 50% at 30 min and 80% at 15 min). However, with this adsorbent, clearly efficiency was inversely proportional to the length of these sampling times, in contrast to the results for the Chromosorbs. Hence the optimum time was likely to be even less than 15 min, and with Tenax-GC increasing sampling periods beyond a certain point was counter-productive. Presumably the initially adsorbed sample was being desorbed and eluted in the nitrogen stream with time. Obviously, sampling time is a most important operational parameter when using Tenax-GC.

A more detailed comparative survey of the adsorbents in question was then carried out as follows. The same system as already described was used, except that a second trap containing the known high-efficiency Chromosorb 103 was placed beyond the first sample trap to ascertain any loss of sample volatiles by incomplete adsorption. This also provided a valuable means of developing optimum sampling times, nitrogen flow-rates, etc., for various samples, as any circumstances that permitted sample components to "break through" the main trap into the second Chromosorb 103 trap were clearly unacceptable. Table II gives recoveries of the components from the same sample mixture from all the porous polymer adsorbents examined under conditions of flow-rate, sampling time, etc., optimised for that particular adsorbent (*i.e.*, in all of these instances quoted, no components were detected in the second, reference trap). The optimum sampling time for the Chromosorbs was about 30 min and did not vary with the particular type, whereas that for Tenax-GC was about 5 min. The nitrogen flow-rate was not so critical, but about 200 ml min^{-1} was appropriate for the Chromosorbs and about 500 ml min^{-1} for Tenax-GC.

It can be seen from Table II that, for any particular adsorbent, recoveries of all components of the test mixture were virtually the same, within experimental error, except for Chromosorb 102 with which the recovery of diallyl disulphide was distinctly low and that of pyridine was extremely poor. As neither of these components was collected in the second trap, the problem here is incomplete elution, and it is possible that in these instances there is some interaction between solute and adsorbent. Whatever the reason, these results illustrate the necessity to assess adsorbents carefully before embarking on a real analytical problem. Clearly Chromosorb 102 is suspect for this type of project. With this exception, the recoveries for particular components were similar for the other three Chromosorbs and Tenax-GC, and at an acceptably high level of about 90% or just below. Chromosorb 103 would seem to be consistently slightly the best.

As no components were detected in the second trap in these experiments, the weakness of the procedure, although slight, is the solvent elution. Clearly the alternative, thermal desorption, could have disadvantages in dealing with thermally susceptible components, but as this could not be a problem with regard to the animal rendering sample for analysis in this project, this method of elution was also assessed on samples trapped as already described. The particular procedure adopted was similar to that of Cropper and Kaminsky.[13] Thermal desorption possesses some advantages in being simpler and less liable to quantitative inaccuracies. However, using this method of elution the Chromosorbs were much less satisfactory owing to their

TABLE II

PERCENTAGE RECOVERIES OF COMPONENTS OF A SAMPLE MIXTURE FROM VARIOUS POROUS POLYMER ADSORBENTS UNDER INDIVIDUAL OPTIMISED TRAPPING CONDITIONS USING ACETONE AS ELUENT

	Recovery, %*				
	Chromosorb				
Component	101	102	103	105	Tenax-GC
Pentanal	88	84	93	87	87
Pyridine	84	54	91	83	87
Butyl acetate	88	82	94	83	84
Diallyl disulphide	85	75	92	83	84

* Recoveries are averages of four determinations. Typical standard deviations for the figures quoted range from 0.866 to 3.55%.

relatively low thermal stability. At temperatures at which they did not degrade to produce an unacceptably high interfering background, the efficiency of desorption was very poor (in particular, chromatographic performance was bad). Solvent elution is hence the better procedure for these adsorbents. Tenax-GC, on the other hand, has a high thermal stability and reputedly it does not degrade below 350 °C. However, desorption temperatures of about 260 °C were adequate to provide almost quantitative elution. Thus, using Tenax-GC traps with relatively short sampling times (5 min), a flow-rate of 500 ml min^{-1} and thermal desorption, it was possible to obtain recoveries for all components in the sample mixture consistently and reproducibly better than 96%. Individual results obtained were as follows (the figures given are the averages of four determinations rounded to whole numbers, and standard deviations are given in parentheses): pentanal, 96% (1.32); pyridine, 98% (0.78); butyl acetate, 98% (1.41); and diallyl disulphide, 97% (0.50). On the basis of these results, this system was then adopted as the best for the analysis of the odorous air samples obtained from factories concerned with the hot rendering of animal by-products. It should be emphasised that although this system is entirely appropriate for this particular project, Chromosorb 103 and solvent elution would be the procedure of choice for the examination of odorous samples and volatiles produced by other than thermal means.

Although Tenax-GC has a very high affinity for organic compounds, it was found that when the odorous air sample was pumped through the single trap using the optimised conditions just described, a few gaseous, organic components were not retained. However, these compounds were readily collected by incorporating in series beyond the main trap a second, identical Tenax-GC trap but which was cooled to −78 °C in a carbon dioxide - acetone bath. This arrangement then retained all volatile components in that none was eluted from a third trap (sub-ambient), and the combined eluates from the two traps possessed the same aroma as the original sample. The second trap merely contained the expected collection of gases that emanate from the majority of such industrial processes, namely ammonia, hydrogen sulphide, methanethiol and trimethylamine. These can usually be prevented from escaping into the atmosphere from the factory by "good housekeeping".

Examination of the contents of the main trap by routine gas chromatography showed them to be very much more complex than those of the second trap, consisting of at least 60 components, and clearly these were more specific to the particular sample in question. The majority of these components were identified by gas chromatography - mass spectrometry using an AEI MS 30 instrument equipped with a DS 50 data processing system. Details of identities are given in Table III together with the Kováts retention indices of the gas-chromatographic peaks and the approximate concentration in parts per million of each component in the original total odour sample. The latter were assessed using the TIC monitor of the gas chromatograph - mass spectrometer and suitable reference standards, and were corrected to allow for the over-all recovery of the analytical procedure. Initially identities of unknown components were suggested from their mass spectra on the basis of knowledge of fundamental fragmentations. These assignments were then confirmed by comparison with literature spectra. In all instances where positive identifications are quoted in Table III, agreement with literature spectra was nearly perfect and within experimental (*i.e.*, instrumental) variability. None of the compounds identified is particularly unusual chemically, and mass spectra are not quoted here as all appear in the existing literature. The background subtraction facility and the retrospective single-ion monitoring facility of the mass spectrometer data system were extensively employed in evaluating the results, and were particularly valuable in assigning some alkanals and aliphatic hydrocarbons that were not resolved by gas chromatography - mass spectrometry (see Table III).

A number of series of compounds can be recognised in Table III. Thus a range of straight-chain aliphatic hydrocarbons from C_7 to C_{14} inclusive was identified in the odour sample. A similar collection of alk-1-enes, but with a few unspecified, was also detected. In general the former were produced in greater amounts than the latter. A range of straight-chain alkanals from C_4 to C_8 inclusive, and of alkylbenzenes up to hexyl, were also identified. Some of the latter were produced in large amounts, but a significant contributor to the specific odour would probably be 3-methylbutanal at 8.1 p.p.m. Much more important to the characteristic odour, however, would be the interesting range of alkylthiophenes from C_3 to C_6 inclusive. Although in general they were formed in only trace amounts, such compounds do have very low odour threshold values. For example, the threshold in water for a C_2-substituted thiophene is

1.3×10^{-3} p.p.m.,[14] compared with 5.0×10^{-3} p.p.m. for hydrogen sulphide and 2.0×10^{-3} p.p.m. for methanethiol.[15] Alkylthiophenes have been previously identified in certain heat-treated foods, *e.g.*, propyl- and butylthiophenes in coffee[16] and ethyl-, butyl-, pentyl- and octylthiophenes in boiled beef.[17,18] Particularly with respect to the latter occurrence, their liberation on the hot rendering of animal by-products would not, therefore, be unexpected. However, numerous other compounds have been determined in cooked meat aroma that were not detected in the factory odorous air sample, so clearly the alkylthiophenes have special significance here. The origin of such a series is intriguing but no explanation can yet be offered.

Table III

Analysis of the volatile composition of animal rendering odour

Component	Kováts retention index	Approximate abundance in odour sample, p.p.m.*	Component	Kováts retention index	Approximate abundance in odour sample, p.p.m.*
Unknown	650	0.6	Octanal	1290	0.6
Heptane	700	5.5	Tridecane	1300	0.6
Hept-l-ene	735	0.4	1,3,5-Trimethylbenzene	1320	tr
Octane	800	5.5	Butylbenzene	1345	2.8
An octene	820	0.3	Unknown	1355	0.5
An octene	830	0.3	A tridecene	1360	0.3
Butanal	840	0.4	Unknown	1370	0.1
An octene	875	0.4	2-Butylthiophene	1380	tr
Nonane	900	0.3	Unknown	1385	0.4
3-Methylbutanal	910	8.1	Tetradecane	1400	1.3
Non-l-ene	925	tr	A tridecene	1410	tr
Benzene	930	0.6	Dimethyl trisulphide	1415	0.3
Pentanal	970	0.6	Unknown	1415	0.1
Decane	1000	0.6	Pentylbenzene	1440	2.1
Dec-l-ene	1045	tr	Unknown	1445	0.6
Toluene	1060	2.8	2-Pentylthiophene	1465	tr
Dimethyl disulphide	1070	1.3	Unknown	1475	0.5
Hexanal	1070	1.3	Decan-2-one	1490	0.6
Undecane	1100	0.6	Hexylbenzene	1510	2.8
A xylene	1120	0.6	C_8 unsaturated aldehyde ($C_8H_{13}CHO$)	1520	0.6
An undecene	1140	tr	2-Pentylpyridine	1540	0.5
A xylene	1150	2.0	Unknown	1550	0.6
A xylene	1170	tr	2-Hexylthiophene	1560	tr
Heptanal	1185	2.0	C_8 unsaturated aldehyde ($C_8H_{13}CHO$)	1570	0.4
Dodecane	1200	1.3	Unknown	1600	0.5
Ethylbenzene	1225	11.3	C_8 unsaturated aldehyde ($C_8H_{13}CHO$)	1610	tr
Unknown	1225	1.3	C_9 unsaturated aldehyde ($C_9H_{17}CHO$)	1630	0.4
Unknown	1240	0.5	$C_{10}H_{20}O$ isomer	1675	tr
C_{12} branched-chain hydrocarbon	1250	0.5	Unknown	1730	0.6
C_{12} branched-chain hydrocarbon	1260	0.4			
Styrene	1270	0.6			
2-Propylthiophene	1270	tr			

* tr = trace.

Of the 61 components detected in the trapped sample of odorous air (Table III), 36 were positively identified with a further 13 being partially characterised. Overall, about 92% of the total odour sample was fully identified and the remaining 8% was distributed over about 25 minor components, many of which were characterised to some extent. As the odour sample was representative of animal rendering odour and as about 92% of it was positively identified, it is reasonable to claim that the majority of compounds that contribute to animal rendering odour have now been determined.

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Simultaneous Determination of Trace Metals in Sea Water Using Dithiocarbamate Pre-concentration and Inductively Coupled Plasma Emission Spectrometry

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A method based on dithiocarbamate pre-concentration and inductively coupled plasma emission spectrometry is described for the simultaneous determination of cadmium, copper, iron, molybdenum, nickel, vanadium and zinc in sea water. The metals are extracted from 500 g of sea water with ammonium tetramethylenedithiocarbamate - diethylammonium diethyldithiocarbamate in chloroform and back-extracted into nitric acid; the sea water concentration factor is 250 or 500. Advantages of the method include high precision, simplicity of calibration and a detection capability in the nanograms per litre range. The method has been applied to Japan Sea, Pacific Ocean and Atlantic Ocean samples.

Keywords: Trace metals; sea water; dithiocarbamate pre-concentration; inductively coupled plasma emission spectrometry

The National Institute for Environmental Studies, Japan, is involved in studies to determine background levels of selected pollutants for monitoring purposes and one objective is the determination of trace metals in sea water.

Graphite furnace atomic-absorption spectrometry (GFAA), an extremely sensitive analytical technique, has found widespread use in sea water analysis and an extensive review of the subject is available.[1] Invariably, as indicated in recent reports,[2–6] when utilising the graphite furnace technique, some form of pre-concentration is required for quantification of the extremely low levels of trace metals in open-ocean water. In a study on cadmium, copper, nickel and zinc by Bruland *et al.*,[2] for example, the minimum metal concentrations determined for a vertical sea water profile (from the surface to a depth of about 3000 m; 37°05'N, 123°22'W) were 15 ng l^{-1} of cadmium (upper value 118 ng l^{-1}), 92 ng l^{-1} of copper (upper value 240 ng l^{-1}), 228 ng l^{-1} of nickel (upper value 693 ng l^{-1}) and 7 ng l^{-1} of zinc (upper value 628 ng l^{-1}). The analytical results, among the lowest reported in the literature, were considered to reflect the total metal content of sea water, *i.e.*, the dissolved, colloidal and particulate fractions.

A report has recently been published[7] describing the application of inductively coupled plasma (ICP) emission spectrometry with ultrasonic nebulisation, and ion-exchange pre-concentration to sea water analysis. The ICP technique lacks the sensitivity of GFAA but provided relatively high pre-concentration factors are achieved, the sensitivity limitation can be circumvented and the analytical advantages of the ICP technique such as (*i*) a simultaneous multi-element capability, (*ii*) a relative absence of matrix interference and (*iii*) a wide dynamic range, can be realised for sea-water analysis. In the afore-mentioned ICP study,[7] a 100-fold pre-concentration of sea water allowed quantification of manganese, iron, zinc, copper and nickel in a relatively unpolluted coastal water sample. A multi-element pre-concentration

procedure based on co-precipitation and flotation with indium hydroxide, and offering a 240-fold concentration factor, has also been used in conjunction with ICP emission spectrometry for the determination of heavy metals in water and artificial sea water.[8]

Of the pre-concentration procedures developed for GFAA, the solvent extraction method of Bruland *et al.*,[2] based on metal dithiocarbamate formation, is considered suitable for ICP analysis for several reasons. The dithiocarbamate system is applicable to many trace elements, the extraction process eliminates the bulk of the sea-water salts and problems related to the nebulisation of extracts having high dissolved solids contents are therefore avoided. Furthermore, a relatively high concentration factor, a basic requirement for the ICP analysis of sea water, was demonstrated in the study by Bruland *et al.*[2] Briefly, the reported procedure involved a double extraction of acidified sea water (250 g) using chloroform and combined ammonium tetramethylenedithiocarbamate (APDC) - diethylammonium diethyldithiocarbamate (DDDC) followed by back-extraction of the metal carbamates into nitric acid. Subsequent sample evaporation - oxidation and semi-quantitative volumetric transfer gave a 200-fold sea-water concentrate.

In this paper a modified dithiocarbamate extraction procedure in combination with ICP emission spectrometry is evaluated for the simultaneous determination of cadmium, copper, iron, molybdenum, nickel, vanadium and zinc in sea water. Method development considerations are discussed with attention focused on maximising the sea-water concentration factor, minimising the potential matrix interference effects in the sea-water concentrate and minimising the number of steps for sample processing. The analytical results for open-ocean samples indicate that the ICP method offers a high detection capability, together with good precision and reliability.

Experimental

Purification procedures and sample processing steps were performed in a class 1000 clean-room and as much as possible at a laminar-flow clean-air bench. Pre-cleaned PTFE laboratory equipment (Nalgene FEP, Nalge Co., New York) was used unless otherwise stated, for the preparation and storage of purified reagents and for sample processing steps. Miniature PTFE beakers with tight fitting caps (4-ml capacity) (Sanai Co., Nagoya, Japan) were used in the final stage of sample processing. The pre-cleaning procedure for laboratory equipment was similar to that reported by Patterson and Settle.[9] Much information regarding reagent purification, amounts of reagents, shaking and standing times for extraction, etc., was obtained from the references already cited.

Reagents and Purification Procedures

Water. A quartz sub-boiling still utilising Millipore water as the input was used. The product was stored in a 2-l pre-cleaned PTFE bottle .

Nitric acid. Sub-boiling distilled nitric acid was prepared in an all-PTFE still of similar design to that reported by Mattinson.[10] The nitric acid feed was atomic-absorption grade (Kanto Chemical Co., Tokyo, Japan).

Chloroform. Spectroscopic grade chloroform (400 ml; Wako Pure Chemical Industries, Osaka, Japan) was extracted 3-times with nitric acid (100 ml, 2 M; Kanto, atomic-absorption grade) in a 500-ml separating funnel with fresh nitric acid being used for each extraction. The chloroform was next washed 3 times with high-purity water and then transferred into a PTFE bottle. When not in use, the PTFE bottle was placed in a black polyethylene bag and stored in a refrigerator. Usually 2 l of purified chloroform were prepared at one time, this solvent being stable for about 3 weeks.

APDC - DDDC solution. APDC (0.5 g, Wako) and DDDC (0.5 g, Wako) were weighed into a 50-ml measuring cylinder and dissolved in 50 ml of water . The solution was then transferred into a 250-ml separating funnel and extracted using 10 ml of purified chloroform. The procedure was repeated a further 5 times using fresh chloroform. The purified extracting reagent was stored in a pre-cleaned 100-ml PTFE bottle. A sea-water extraction experiment indicated that the solution was stable for at least 24 h.

Ammonium acetate buffer. Equal volumes (500 ml) of glacial acetic acid (Kanto EL, high-purity grade) and 25% ammonia solution (Kanto, atomic-absorption grade) were mixed slowly over a 2-h period, in a polypropylene container placed in a water-bath. The acetate

buffer solution was then purified as follows. Purified APDC - DDDC solution (5 ml) was added to 400 ml of the buffer solution and the mixture extracted 6 times with separate 40-ml portions of purified chloroform. The purified buffer solution was stored in a pre-cleaned 2-l PTFE bottle.

Standard solutions. Multi-element standard solutions were prepared from single element stock solutions (1 000 μg ml^{-1}) by appropriate dilution. High-purity metals or oxides were used to prepare the stock solutions.

Sea water. Synthetic sea water prepared from analytical-reagent grade chemicals[3] and Japan Sea water were used for method development. Open-ocean samples, obtained from the Atlantic Ocean and the Pacific Ocean, were acidified on collection to approximately pH 2 by the addition of 1 ml of nitric acid (sub-boiling distilled) to 1 l of sea water. The Pacific sample was obtained during the Hako Maru cruise (KH 80.2, April 26th–June 18th, 1980; University of Tokyo Oceanographic Institute) at station 10 (32°01'N, 144°60'E), from a depth of 4500 m. An all-stainless-steel sampler of Niskin design was used for collection and the sample was stored in a pre-cleaned 5-l polyethylene container. The Atlantic water, made available to participants attending the Intergovernmental Oceanographic Commission's inter-calibration workshop, Bermuda Biological Station, January 10–26th, 1980, was obtained from a high-volume holding tank (polyethylene lined, 500-l capacity) containing sub-surface water that had been sampled about 2 miles off Bermuda (32°22'N, 64°44'W) utilising an all-PTFE pump with polyethylene tubing. Approximately 20 l of sea water were withdrawn from the tank into a pre-cleaned polypropylene container.

Pre-concentration Procedure

Weigh accurately about 500 g of acidified sea water (pH about 2) into a 1-l beaker on a pan balance. Transfer into a 500-ml separating funnel and add 1.4 ml of acetate buffer, 2 ml of APDC - DDDC solution and 10 ml of chloroform. Shake the mixture on a mechanical shaker for 3 min and then allow 5 min for the phases to separate and drain off the chloroform layer into a 125-ml separating funnel. Add a further 10 ml of chloroform, shake for 3 min, allow to stand for 5 min and then combine the two chloroform extracts. Add 0.20 ml of concentrated nitric acid to the chloroform extracts, shake for 1 min and then allow to stand for 5 min. Add high-purity water (about 2 ml), shake for 1 min and then allow to stand for 5 min, discard the chloroform layer and drain the aqueous layer into a 4-ml PTFE beaker, rinse the separating funnel with about 2 ml of high-purity water, adding the washings to the PTFE beaker. Then evaporate the extract to dryness using an infrared lamp on a clean-air bench and dissolve the residue in 0.3 ml of concentrated nitric acid. Continue to heat the nitric acid solution under the lamp to a small volume (about 50 μl) and then add water (exactly 1.0 or 2.0 ml). The solution is then assayed directly from the PTFE beaker.

Instrumentation and Calibration

Inductively coupled plasma. A Jarrell-Ash Atomcomp MKII direct-reading emission spectrometer with pneumatic nebulisation (cross-flow type nebuliser) was used. The wavelengths for the elements programmed into the polychromator and instrument detection performance under normal plasma operating conditions are listed in Table I. The values for the principal plasma operating parameters (*i.e.*, RF power 1.1 kW, observation height 18 mm and sample uptake rate 1.0 ml min^{-1}) were determined from a previous optimisation study.[11]

Standardisation of the instrument was based on a two-point calibration procedure, using a multi-element solution containing cadmium, copper, iron, molybdenum, sodium, nickel, vanadium and zinc (1 μg ml^{-1} of each in 0.4 M nitric acid) as the high standard and distilled water (zero metal concentration) as the low standard. The sample volume of both the 1 and 2 ml concentrates permitted the acquisition of 3 independent intensity measurements and the print-out in concentration units therefore consisted of three values. For the 1-ml extract, however, none of the sample remained after these measurements. A sample pre-rinse time of about 20 s was used. Standardisation of the instrument was usually repeated after the assay of each set of sea-water extracts to minimise the effects of instrument drift. Distilled water and a check standard were also included in the analysis sequence.

Atomic-absorption spectrometer. An IL atomic-absorption instrument (IL 151) with a graphite furnace attachment, Model 555, was used. The atomic-absorption wavelengths and operating conditions for the graphite furnace atomiser were those recommended by the manufacturer.

TABLE I

ANALYTICAL WAVELENGTHS AND DETECTION LIMITS FOR ICP INSTRUMENTATION

See text for principal plasma operating conditions.

Element	Wavelength/nm	Detection limit*/μg l^{-1}
Cadmium	228.8†	1
Copper	324.8	1
Iron‡	259.9	3
Molybdenum	202.0	4
Nickel‡	231.6†	8
Vanadium‡	292.4	3
Zinc	213.9	1

* Detection limit is defined as the concentration that corresponds to an emission signal equivalent to twice the standard deviation of the background noise.
† Refers to 2nd order.
‡ Refers to ion line.

Results and Discussion

Comments on the Pre-concentration Procedure

The solvent extraction procedure of Bruland *et al.*[2] was considered suitable for ICP analysis but was modified for a number of reasons. To compensate for the poorer sensitivity of the ICP technique relative to GFAA, a procedure offering, at maximum, a 500-fold concentration was devised. This was achieved by concentrating 500 g of sea water to 1 ml. The sample volume of 1 ml represents the minimum acceptable volume for ICP analysis using commercially available nebulisers. A 2-ml sea-water extract however, may be preferable (see Pre-concentration Procedure) as the sample volume is then not exhausted by ICP analysis and further data may be obtained by other techniques, *e.g.*, GFAA. Unless otherwise stated the results in this section are based on a 250-fold concentration factor.

An additional step, taken to ensure the maximum ICP detection capability, was to minimise the nitric acid concentration in the final sea-water extract. The ICP detection sensitivity is known to deteriorate in solutions of high nitric acid concentration and this difficulty was noted by McLaren *et al.*[7] in a sea-water analysis study utilising an ion-exchange pre-concentration procedure. In the present study only a slight deterioration in detection performance was noted for sea-water extracts 0.8 M in nitric acid (the approximate acid strength for the 1-ml concentrate). No significant deterioration was found for the solution 0.4 M in nitric acid (the approximate acid strength for the 2-ml concentrate). As indicated later for the assay of an open-ocean sample the agreement between data for the 1- and 2-ml concentrates confirmed the absence of acid interference.

An important feature of this method is that ICP analysis is performed directly from the PTFE vessels used to receive the sea-water extracts after back-extraction. The elimination of an additional sample transfer step reduces the possibility for analyte loss, volumetric errors and contamination. It should be noted that evaporation of sea-water extracts to small volumes is time consuming (at least 6 h) relative to solvent extraction and ICP analysis, but the evaporation can be conveniently performed overnight and unattended. The formation of the 50-μl droplet, following oxidation of the residue, is rapid (about 30 min) and reproducible and this, together with the final volumetric step (the addition of 1 or 2 ml of high-purity water), ensures good reproducibility for the procedure. In a normal working day up to 24 sea-water samples may be processed to the evaporation stage.

Effect of pH on Extraction Efficiency

The combined APDC - DDDC extracting reagent was first proposed by Kinrade and Van Loon[12] for the pre-concentration of trace elements from natural waters, primarily because of its ability to complex many metals and also because there is a broad pH range over which the extraction process is equally efficient. In recent applications to sea water pre-concentration the method of Danielsson *et al.*[3] utilised pH 5 for the extraction of cadmium, cobalt, copper, iron, nickel, lead and zinc whereas Bruland *et al.*[2] used pH 4 for cadmium, copper, nickel and zinc. For this study the effect of pH on extraction efficiency was investigated as information

on molybdenum and vanadium, utilising the combined extracting reagent, is not documented. As can be seen in Fig. 1 the extraction efficiency was constant over the pH range 2–6 for many metals, with the curves for cadmium, copper, iron, nickel and zinc being similar to those reported by Danielsson *et al.*[3] For molybdenum and vanadium, particularly the former, extraction efficiency was sensitive to pH with the curves exhibiting maxima at about pH 4. Based on these findings precise control of the pH of the medium is required to obtain quantitative data for molybdenum and vanadium and for this work pH 4.1 ± 0.1 was utilised.

Matrix Interference

It has been demonstrated that the ICP technique does not suffer appreciably from matrix interference. The deterioration in detection performance for solutions of high acid content is well known and has already been discussed for nitric acid. The possibility of stray light and nebuliser-related effects associated with extracts containing high concentrations of alkali and alkaline-earth metals was considered, but was not found to be significant. Fortunately the extraction process removes the bulk of the sea-water salts and the concentrations of sodium, calcium and magnesium for the 250-fold sea-water extracts were typically in the ranges 0.1–60, 0.05–3 and 0.2–12 μg ml^{-1}, respectively. In routine analysis it is recommended that the multi-standard solution contains an appropriate concentration of sodium (or calcium or magnesium), *e.g.*, 1–10 μg ml^{-1} to check the level of salt carry-over.

Reports in the literature[13,14] have indicated that plasma background shifts occur when solutions containing organic matter are nebulised. The occurrence of such phenomena due to retention of residual chloroform in the sea-water extracts was considered to be unlikely as oxidation of the residues was carried out. In an attempt to detect such interference the pre-concentration procedure was performed with omission of the oxidation step, *i.e.*, sea-water extracts were evaporated directly to 50 μl before the final volumetric addition. The analytical data for sample and blank solutions by the above technique and by the recommended procedure were indistinguishable and background shifts were not observed. The oxidation step in sample processing is recommended, however, as the addition of nitric acid was found to improve the reproducibility of droplet formation.

Method Sensitivity and Blank

The method sensitivity for the elements based on the respective ICP lower limits for quantification (LLQ) and a 500-fold concentration factor are presented in Table II. A compilation of the trace element concentrations in sea water is also given for comparison purposes. Intended as a rough guide, it can be seen from Table II that the sensitivity of the proposed ICP method may be sufficient for quantifying at the lower end of the concentration scales. As indicated in recent sea-water analysis studies,[2–7] however, trace element concentrations in unpolluted water may be significantly lower than those indicated in Table II. The present discussion assumes that the analytical blank levels do not represent significant fractions of the total analytical signals. Minimisation and control of the blank levels are essential for the achievement of reliable determinations of trace metals in sea water on a routine basis.

TABLE II

METHOD SENSITIVITY FOR ELEMENTS AND APPROXIMATE SEA-WATER CONCENTRATIONS

Element	Method sensitivity*/ ng l^{-1}	Sea-water concentration†/ ng l^{-1}
Cadmium	10	70
Copper	10	600–2000
Iron	30	100–62000
Molybdenum	60	2100–18800
Nickel	80	1100–4000
Vanadium	30	200–4000
Zinc	10	100–50000

* Based on 500-fold concentration factor and ICP lower limit for quantification (LLQ). The LLQ is taken as 5 times the detection limit, *e.g.*, LLQ for cadmium is 5 μg l^{-1}.

† See reference 15.

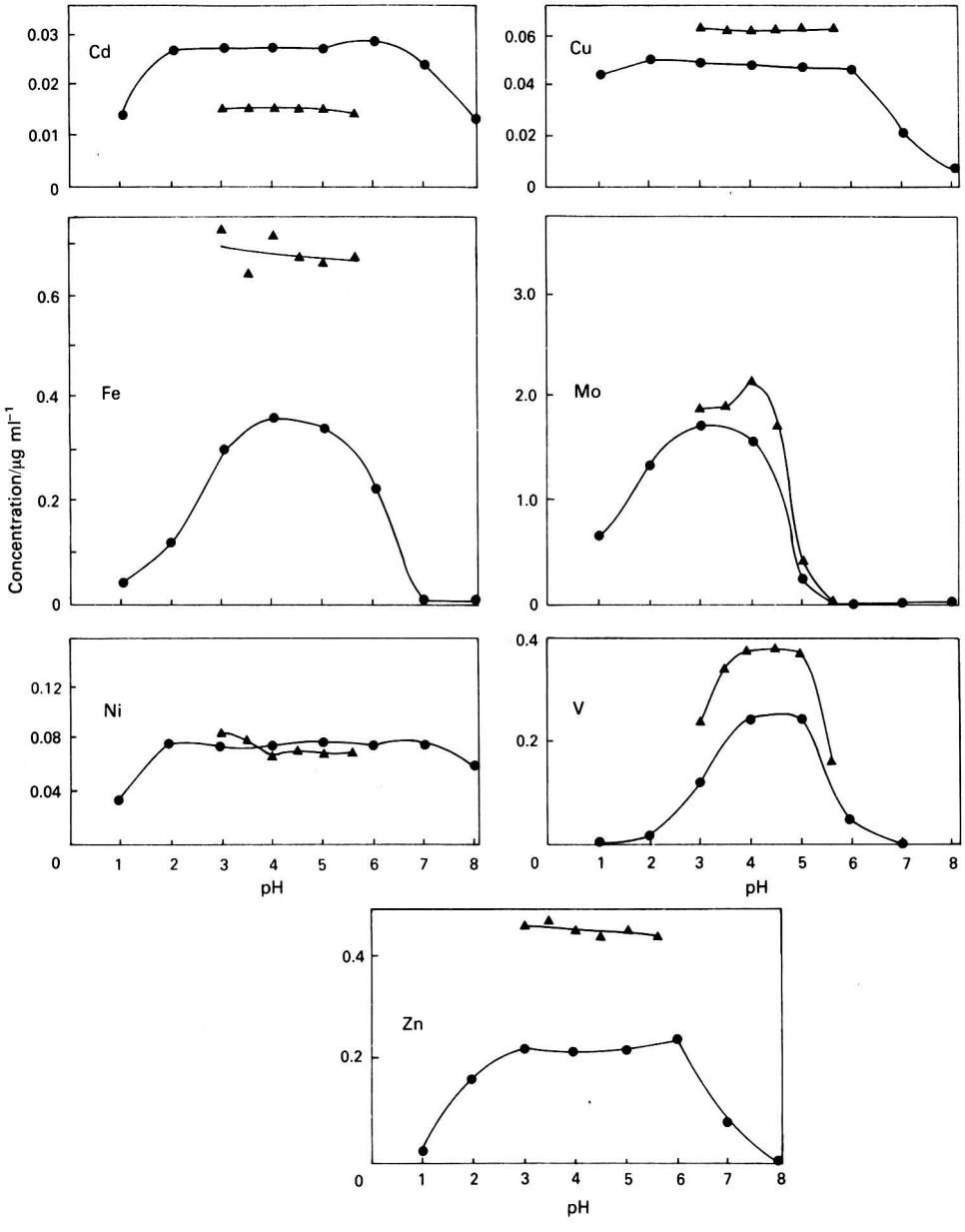

Fig. 1. Effect of pH on extraction efficiency. ▲, Japan sea water; ●, synthetic sea water: the solution was enriched with multi-element solution before extraction; actual concentrations for Cd, Cu, Fe, Ni and Zn were 10 times greater than indicated; buffer, 1 M sodium phosphate solution (3 ml). Solution pH was adjusted by addition of HNO_3 or NH_3 solution.

Blank extracts were obtained by performing the entire procedure (equivalent to a 250-fold concentration) in the absence of a sea-water sample and ICP analysis indicated that the contamination levels were below the respective LLQ except with iron. Therefore, the more sensitive graphite furnace technique was employed and the analytical results for the metal blank levels are given in Table III. The molybdenum and vanadium blanks were not detected by GFAA and contamination levels for the remaining elements, except iron, were generally insignificant in relation to sea-water concentrations (see Table VII). The high iron blank represented a significant fraction of the analytical signals and in an attempt to obtain reliable data, a subtraction of the blank was performed. A lower limit for iron determinations in sea water is considered to be about 500 ng l^{-1}, where the contamination level accounts for about 20% of the ICP signal. Experiments indicated that iron contamination originated primarily from the chloroform and nitric acid used in the sample processing. With respect to the purification of nitric acid a report[16] has revealed that the PTFE sub-boiling still can be a source of iron contamination and all-quartz apparatus would be preferable.

TABLE III

METHOD BLANK AS DETERMINED BY GRAPHITE FURNACE ATOMIC-ABSORPTION SPECTROMETRY

Values refer to blank sample concentrates. To obtain method blank in terms of an equivalent sea-water concentration divide data by 250. Uncertainty limits expressed as standard deviation. Number of replicate analyses, 12.

Element	Concentration/$\mu g\ l^{-1}$
Cadmium	0.3 ± 0.1
Copper	0.4 ± 0.1
Iron*	23 ± 8
Molybdenum	<1.0
Nickel	3 ± 1
Vanadium	<1.0
Zinc	0.3 ± 0.1

* By ICP emission spectrometry.

Precision and Accuracy

Method precision was determined by performing replicate analyses of a Japan Sea sample ($n = 8$) and a synthetic sea water ($n = 6$). The results are given in Table IV together with those for instrumental measuring precision. The values for the latter were derived from analysis of a multi-element solution with a composition similar to the Japan Sea water concentrate. The data in Table IV indicate high precision for the procedure, the relative standard deviations

TABLE IV

METHOD AND INSTRUMENTAL PRECISION

Element	Japan Sea water		Synthetic sea water		Instrumental RSD, %	Multi-element solution concentration/ $\mu g\ ml^{-1}$
	Concentration/$\mu g\ l^{-1}$	RSD*, %	Concentration/$\mu g\ l^{-1}$	RSD*, %		
Cadmium	0.016	31	0.076	8.4	12	0.01
Copper	0.308	5.8	0.948	3.8	2.0	0.1
Iron	1.26	7.6	14.0	3.3	1.5	0.2
Molybdenum	7.76	4.6	<4.0		1.2	0.2
Nickel	0.236	10	2.35	3.2	5.8	0.05
Vanadium	1.46	1.1	<3.0		1.3	0.5
Zinc	1.42	2.1	7.84	3.1	0.9	0.2

* RSD = relative standard deviation.

(RSD) being as good as or better than those obtained using methods based on GFAA. For certain metals (*e.g.*, cadmium and nickel) whose concentrations in the Japan Sea concentrate were extremely low, determinations were performed at, or close to, the respective LLQ and therefore ICP measuring precision contributed significantly to the total method precision (*cf.*, corresponding data for the synthetic sea water).

For a critical assessment of accuracy there is no substitute for a sea-water standard reference material (SRM) certified for elemental composition but, unfortunately, such a standard has yet to be prepared. The accuracy of the procedure was therefore tested by performing a spike

recovery experiment and by analysing SRM 1643a, Trace Elements in Water (simulated fresh-water standard, US National Bureau of Standards). For the former, two 500-g aliquots of the Japan Sea sample were spiked with multi-element solution (0.5 ml; see Table V for composition) just before extraction. Element recoveries, as indicated in Table V, were essentially 100% except for molybdenum. An additional experiment indicated that recoveries for a single extraction using only 10 ml of chloroform were approximately 10% lower than the values listed in Table V.

TABLE V

RECOVERY RESULTS FOR SPIKE ADDITION TO JAPAN SEA SAMPLE

Element	Original* concentration/ $\mu g\ ml^{-1}$	Amount added†/ $\mu g\ ml^{-1}$	Amount found*/$\mu g\ ml^{-1}$		Recovery, %
			1	2	
Cadmium	0.004	0.0125	0.017	0.017	100
Copper	0.077	0.125	0.205	0.202	101
Iron	0.315	0.25	0.532	0.542	95
Molybdenum	1.94	2.5	4.16	4.02	92
Nickel	0.059	0.125	0.185	0.196	104
Vanadium	0.365	0.5	0.830	0.846	97
Zinc	0.355	0.5	0.849	0.855	100

* Values for original and found refer to sea-water concentrates. To obtain concentrations for Japan Sea sample divide original value by 250.

† 0.5 ml of the multi-element solution added to two 500-g aliquots of sea water; multi-element solution composition Cd 0.05 $\mu g\ ml^{-1}$, Cu 0.5 $\mu g\ ml^{-1}$, Fe 1.0 $\mu g\ ml^{-1}$, Mo 10 $\mu g\ ml^{-1}$, Ni 0.5 $\mu g\ ml^{-1}$, V 2 $\mu g\ ml^{-1}$ and Zn 2 $\mu g\ ml^{-1}$.

In a further attempt to assess accuracy, SRM 1643a was analysed after appropriate dilution. The SRM water, which contains trace elements at the nanogram per gram level and may be analysed directly by ICP emission spectrometry, was diluted 100-fold (10 ml to 1 l in a polypropylene calibrated flask) with sub-boiling distilled water and acidified to pH 2. The samples were then processed in the normal manner and the results are presented in Table VI. The results for direct analyses were in good agreement with the certificate values except

TABLE VI

ANALYTICAL RESULTS FOR TRACE ELEMENTS IN WATER SRM 1643A

Means ± 95% confidence levels.

Element	Certificate* value/ $ng\ g^{-1}$	Amount found/$ng\ ml^{-1}$	
		Direct†	After dilution (100-fold) and pre-concentration‡
Cadmium	10±1	10±2	10±1
Copper	18±2	19±2	19±2
Iron	88±4	90±2	106±15
Molybdenum	95±6	99±6	82±8
Nickel	55±3	51±14	54±8
Vanadium	53±3	48±2	44±2
Zinc	72±4	61±4	60±4

* To convert to nanograms per millilitre, multiply by specific gravity of SRM; the specific gravity at 23 °C is 1.017 g ml^{-1}.

† Total number of measurements, 18.

‡ Results derived from analyses performed on two separate occasions: number of replicates (*i.e.*, of 500-g aliquots), 6; total number of measurements, 18.

for zinc and therefore the accuracy of the prepared multi-standard solution (see Experimental) was confirmed. The disparity in zinc concentrations was significant at the 95% confidence level, the relative error being 15%.

An independent ICP analysis also failed to reach agreement with the certified zinc value. The pre-concentration data were generally satisfactory but particularly so for cadmium, copper and nickel. Based on the previous recovery study the relatively low results for molybdenum (relative error 14%) and zinc were not unexpected.

A blank correction was not performed for iron in order to assess the magnitude of the error arising from contamination. The relatively poor precision for the iron determination is also revealed by the magnitude of the uncertainty limits. High precision was obtained for the remaining elements; it should be noted that the ICP measuring precision was the limiting factor rather than the precision associated with pre-concentration (*cf.*, uncertainty limits for direct and pre-concentration data).

Analysis of Open-ocean Samples

The analytical results for a deep (4500 m) Pacific Ocean sample and a sub-surface Atlantic Ocean sample are given in Table VII. In view of the low metal concentrations expected, concentration factors of 250 and 500 were employed for the latter sample. The nickel concentration for the Atlantic Ocean sample was such that determinations for the 250-fold concentrate were close to the LLQ (42 μg ml^{-1} compared to 40 μg ml^{-1}) and improved measurement precision was realised for the 500-fold concentrate. Even with the adoption of the maximum concentration factor, the cadmium concentration for the Atlantic sample was below the ICP detection capability and GFAA was required for quantification, the cadmium value being 4 ng l^{-1}. Agreement in the results for both sea-water concentrates confirmed the reliability of the final volumetric step in the procedure. Further, as mentioned earlier, although the nitric acid concentration for the 500-fold concentrate was approximately double that for the 250-fold concentrate the good agreement in data confirmed the absence of acid interference.

TABLE VII

TRACE METAL CONCENTRATIONS FOR OPEN-OCEAN SAMPLES

See Experimental for sample description. Number of replicate analyses, 3; total number of measurements, 9. Results are in micrograms per litre. The values in parentheses are standard deviations.

		Atlantic	
Element	Pacific	Concentration factor of 250	Concentration factor of 500
Cadmium	0.096 (0.004)	<0.02	<0.01
Copper	0.348 (0.012)	0.392 (0.007)	0.390 (0.012)
Iron	1.05 (0.10)	0.544 (0.011)	0.546 (0.026)
Molybdenum ..	8.36 (0.58)	7.80 (0.36)	7.48 (0.25)
Nickel	0.612 (0.032)	0.168 (0.018)	0.164 (0.006)
Vanadium ..	1.41 (0.04)	1.33 (0.01)	1.30 (0.02)
Zinc	2.30 (0.04)	0.556 (0.007)	0.544 (0.016)

The data for the Pacific and Atlantic samples reveal a similarity in concentration levels for certain elements, and for cadmium, copper and nickel the relatively low concentrations are of similar magnitude to those reported in the study of Bruland *et al.*[2] The agreement in the results for molybdenum and vanadium is an interesting finding considering the differences in geographical location and sample depth. Molybdenum and vanadium concentration levels for Japan Sea samples (sub-surface, 800 m and 3000 m) were also similar to the data in Table VII. In addition, it is of interest to note that a recent study[17] on molybdenum and based on β-naphthoin oxime pre-concentration and neutron activation analysis reported the molybdenum concentration for Atlantic Ocean samples to be within a narrow range of 7.31–7.95 μg l^{-1}. An earlier study[18] for the North East Atlantic Ocean reported dissolved molybdenum to be within the concentration range 9.1–13.0 μg l^{-1}, whereas that of vanadium was 0.83–1.57 μg l^{-1}.

Unfortunately in this study, sampling bottles and storage containers for the Pacific and Atlantic samples were dissimilar (see Experimental) and, therefore, caution is required in evaluating data, particularly for zinc and iron, elements prone to contamination.[1] Finally, it should be mentioned that the present method has been used in a recent trace metal intercalibration study* designed to determine the optimum sampling procedure for deep-ocean water and it is hoped that much needed information on sampling and storage will be provided when the official document is published.

* Trace Metal Intercalibration Exercise of Intergovernmental Oceaongraphic Commission, Bermuda, January 10–26th, 1980.

Conclusion

The present study has attempted to determine background levels of selected trace metals in open-ocean water, utilising dithiocarbamate pre-concentration and ICP emission spectrometry. Similarities for trace metal concentrations were demonstrated for Pacific and Atlantic Ocean samples, and cadmium, copper, nickel and zinc found at the nanogram per litre levels are consistent with recent independent analyses based on atomic-absorption spectrometry. Analytical merits for the ICP method included simultaneous multi-element analysis, high precision, simplicity of calibration and high detection capability. The method blank was significant for iron and further work is needed to reduce the iron contamination. Although accuracy of the method has been tested, the possibility of contamination and/or adsorption losses associated with the sampling and storage of sea water should be kept in mind. Future work is to be directed at accumulating data for coastal and open-ocean areas and it is hoped that such studies can indicate the extent and trends of heavy-metal pollution.

We are grateful to S. Fujiwara and Y. Nojiri (Chemistry Department, University of Tokyo) for the collection of Japan Sea and Pacific Ocean samples. M. Nishikawa (Analysis Section, NIES) deserves mention for his constant support during the analysis programme. Discussion at the trace metal analysis workshop of the Intergovernmental Oceanographic Commission was invaluable and the assistance and co-operation given by participants and staff of the Bermuda Biological Station is acknowledged.

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Kinetic Parameters and Current Efficiencies for Manganese(III) Generation from Manganese(II)

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Electrogeneration of manganese(II) at platinum, gold and glassy carbon was investigated by rotating disc electrode voltammetry. Anodic charge-transfer kinetic parameters were determined and used to compute generation current efficiencies. The maximum value obtained was 99.91% at a generating current density of 8.95 mA cm^{-2}, in sulphuric acid. The most appropriate electrode material is gold.

Keywords: Manganese(III); rotating disc electrode; charge-transfer kinetic parameters

Manganese(III) has been used as a coulometrically generated titrant with success.[1] Kinetic studies for the reduction of manganese(III) in sulphuric[2,3] and perchloric acid[4] using platinum have been reported. Determinations of current efficiencies in the past were performed either by titrimetry[5–7] or by graphical methods,[5,8,9] and the maximum reported value at platinum is 99.9% at a generating current density of 3.1 mA cm^{-2}, As it is the anodic reaction that is actually involved in coulometric titrations with manganese(III), we decided to study this reaction directly.

Neither a complete survey of experimental parameters nor the use of glassy carbon has been carried out in the past. The aim of this work was to determine charge-transfer kinetic parameters in sulphuric acid at three electrode materials, for both background and manganese anodic reactions. These parameters were used to calculate current efficiencies.

Experimental

The reagents and instrumentation used are described elsewhere.[10] The rotating disc electrode (R.D.E.) assembly with interchangeable tips has been described previously.[11]

The determination of conditional potentials was performed by the coulometric extrapolation method.[12]

The treatment of electrode surface is described in a previous paper.[12]

Computation of current efficiencies was performed by means of a FORTRAN IV program.

Results and Discussion

Kinetic Parameters

Conditional potentials were used as reference points to express conditional charge-transfer kinetic parameters. A perfect fit with the Nernst equation and no significant difference between the extrapolated values, as shown in Table I, confirmed that the measured zero current potentials were true equilibrium potentials.

TABLE I

CONDITIONAL POTENTIALS OF THE Mn(II) - Mn(III) COUPLE *versus* S.C.E. IN 7.5 M SULPHURIC ACID

Electrode	Glassy carbon	Platinum	Gold
Conditional potential/V	1.2498	1.2482	1.2499

Although the anodic oxidation of manganese(II) is complicated by adsorption - disproportionation at the electrode surface,[10] this can be overcome in 7.5 M sulphuric acid.

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Fig. 1 shows typical voltammograms obtained at a glassy carbon R.D.E. These can be described by simple mass transfer - charge transfer theory. The main equations are

$$\frac{1}{i} = \frac{1}{I} + \frac{K}{\omega^{\frac{1}{2}}} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

and

$$\text{Log } I = \text{Log } nFACk^{o\prime} + \frac{\beta nF}{RT}(E - E^{o\prime}) \quad .. \quad .. \quad .. \quad (2)$$

where i A is the current for a particular electrode potential, ω rad s^{-1} the R.D.E. rotational frequency, K the potential-dependent constant, I A the current corrected for diffusion, E V the electrode potential *vs.* S.C.E., $E^{o\prime}$ V the conditional potential *vs.* S.C.E., $k^{o\prime}$ the conditional charge-transfer rate constant, β the anodic charge-transfer coefficient, A cm^2 the electrode surface area, C mol l^{-1} the bulk concentration of manganese(II) and n, F, R and T are as in the Nernst equation.

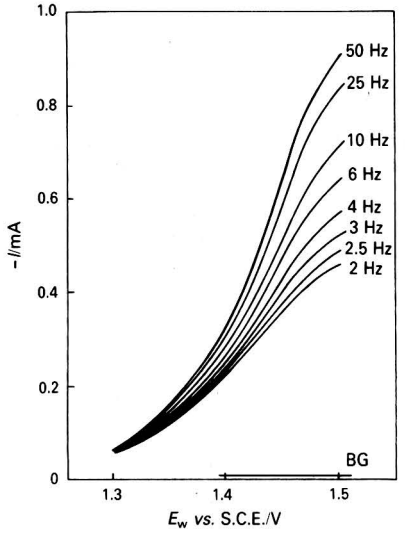

Fig. 1. Current - potential graphs for the oxidation of manganese(II) at glassy carbon rotating disc electrode. Solution: 0.02 M $MnSO_4$ + 7.5 M H_2SO_4. Electrode area: 0.5 cm^2.

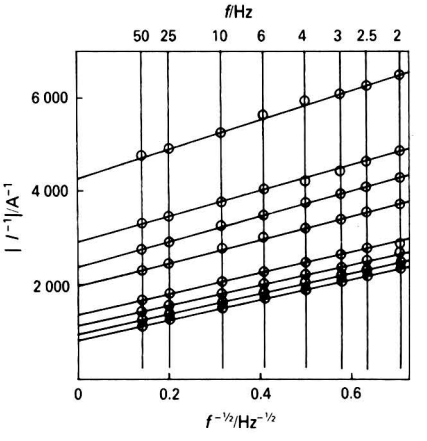

Fig. 2. Mass extrapolation-out graph following equation (1). Each line corresponds to a different electrode potential. f: $\omega^{-1}(2\pi)$ is R.D.E. spinning frequency in Hz.

If equation (1) is applied to the set of voltammograms in Fig. 1, the plot of $1/i$ *versus* $1/\omega^{\frac{1}{2}}$ gives I from the intercept. A different value of I can be obtained for each electrode potential E, as illustrated in Fig. 2.

A graph of I *versus* E using equation (2) gives a straight line. The charge-transfer kinetic parameters $k^{o\prime}$ and β are obtained from the intercept and slope and are given in Table II.

TABLE II

CHARGE-TRANSFER KINETIC PARAMETERS FOR Mn(II) OXIDATION IN 7.5 M SULPHURIC ACID

Parameter	Electrode: Glassy carbon	Platinum	Gold
β	0.39	0.47	0.38
$k^{o\prime}$/cm s^{-1}	3.4×10^{-5}	3.5×10^{-5}	1.1×10^{-4}

Table III gives the background reaction kinetic parameters, obtained by stationary electrode voltammetry.

TABLE III

CHARGE-TRANSFER KINETIC PARAMETERS FOR BACKGROUND REACTION IN 7.5 M SULPHURIC ACID

Parameter	Electrode		
	Glassy carbon	Platinum	Gold
β	0.06	0.13	0.16
$k^{o\prime}$/cm s^{-1}	6.0×10^{-22}	1.4×10^{-23}	1.8×10^{-26}

The electrode material has no marked effect on the manganese oxidation reaction rate; the $k^{o\prime}$ sequence is gold > platinum $\approx$ carbon.

The effect of electrode material is more marked on the background kinetic parameters. The charge-transfer rate constants follow the sequence carbon > platinum > gold, whereas the charge-transfer coefficients decrease in the order gold > platinum > carbon. The net effect is that the reaction rate follows the sequence platinum > carbon > gold, as confirmed by the observed overpotentials.

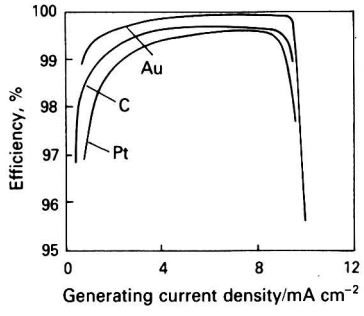

Fig. 3. Current efficiency plots for the generation of manganese(III) from manganese(II). Effect of electrode material. Solution: 0.1 M $MnSO_4$ + 7.5 M H_2SO_4. Mass-transfer rate constant: $k_m = 10^{-3}$ cm s^{-1}.

Current Efficiencies

Current efficiency graphs[12] were prepared for a 0.1 M solution of manganese(II) (this is a nearly saturated solution) in 7.5 M sulphuric acid, and are shown in Fig. 3. The current efficiencies are highest at gold, followed by glassy carbon and platinum. This difference can be attributed to the marked effect of the electrode material on the oxygen evolution overpotential or background reaction rate.

The maximum current efficiencies (% Eff) found are summarised in Table IV.

TABLE IV

MANGANESE(III) GENERATION CURRENT EFFICIENCIES

0.1 M $MnSO_4$ + 7.5 M H_2SO_4; mass-transfer rate constant, $k_m = 10^{-3}$ cm s^{-1}.

	Electrode		
	Platinum	Glassy carbon	Gold
% Eff	99.60	99.68	99.91
Current density/mA cm^{-2}	7.63	7.07	8.95

Conclusions

The use of a high sulphuric acid concentration (7.5 M) allows the complete suppression of manganese(III) adsorption - disproportionation at the electrode surface. Simple charge transfer - mass transfer theory can be used to determine charge-transfer kinetic parameters in this medium. The values found are in close agreement with reported values from cathodic polarisations.[13]

Gold and glassy carbon proved to be the best electrode materials for manganese(III) generation. The highest current efficiency is obtained at gold, being very similar to reported values[5] at platinum but a higher generating curent density can be used at gold (8.95 instead of 3.1 mA cm^{-2}).

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Generation of Chlorine at Glassy Carbon. Study of Kinetic Parameters and Current Efficiencies by Rotating Disc Electrode Voltammetry

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A glassy carbon electrode is used for the anodic generation of chlorine. The reaction mechanism determined allows the use of approximate current - potential relationships for calculating charge-transfer kinetic parameters. The values found are interpreted by the condition of the electrode surface and adsorbed chlorine. Generating current efficiencies were calculated using the determined kinetic parameters. A maximum of 99.99% was found for a current density of 58.4 mA cm^{-2}. Electrode surface attack was detected at potentials above 1.7 V *versus* S.C.E.

Keywords: *Glassy carbon*; *chlorine generation*; *rotating disc electrode*; *charge-transfer kinetic parameters*

Electrogenerated chlorine has been used in coulometric determinations of pharmaceuticals and organic compounds,[1] iodine,[2] arsenic(III),[3–5] thallium(I)[6,7] and iron(II).[8] Kinetic data can be found for the reduction of chlorine[9,10] and oxidation of chloride[11,12] at platinum and graphite[13–15] electrodes. Generating current efficiencies determined using different carbon electrodes have been reported.[16,17] Other workers have studied the chlorine - chloride electrode at platinum,[18,19] gold[20] and platinised titanium.[21]

Considering that platinum and gold are chemically attacked by chlorine, graphite is oxidised[16] and absorbs chlorine,[15] glassy carbon, which appeared very promising for anodic reactions,[22] was chosen as the working electrode material. The aim of this work was the determination of charge-transfer kinetic parameters at glassy carbon and the evaluation of the chlorine generation current efficiencies.

Experimental

Reagents, apparatus, instrumentation, electrode surface conditioning and the determination of conditional potentials have been described elsewhere.[23,24]

Rotating disc electrode voltammetry was used for the determination of charge-transfer kinetic parameters by working at a fixed rotating speed. The temperature was 20 °C.

Results and Discussion

The anodic oxidation of chloride at glassy carbon involves two charge-transfer steps, the second being the rate-determining step[24]:

$$Cl^- \rightleftharpoons Cl_{ads} + e$$

$$Cl_{ads} + Cl^- \longrightarrow Cl_2 + e$$

Approximate current - potential relationships derived previously[24] were used to describe the experimental voltammograms. For the cathodic reaction

$$I = 2FAk^{o\prime}[Cl_2]_s \exp\left[-\frac{\alpha F}{RT}(E - E^{o\prime})\right]$$

and for the anodic reaction

$$-I = 2FAk^{o\prime}[Cl^-]_s \exp\left[\frac{\beta F}{RT}(E - E^{o\prime})\right]$$

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where $k^{o\prime}$ is the conditional charge-transfer rate constant, α and β are cathodic and anodic charge-transfer coefficients, respectively, $E^{o\prime}$ is the conditional potential for the system Cl_2 - Cl^-, s denotes surface concentrations, A is the electrode surface area, I and E are observed current and potential, respectively, and R,T and F are as in the Nernst equation.

Charge-transfer Kinetic Parameters

Voltammograms obtained for the cathodic reduction of chlorine and for the anodic oxidation of chloride at different bulk concentrations are shown in Figs. 1 and 2. These can be described by the approximate relationships mentioned above by calculating surface concentrations from the limiting current observed in the former instance and using bulk concentrations in the latter. Charge-transfer kinetic parameters were obtained from the following equations. For cathodic scans:

$$\text{Log}\ \frac{I}{1\text{-}I/I_{LC}} = \text{Log}\ (2FA[Cl_2]_b k^{o\prime}) - \frac{\alpha F}{2.3\ RT}(E - E^{o\prime})$$

and for anodic scans

$$\text{Log}\ |I| = \text{Log}\ (2FA[Cl^-]_b k^{o\prime}) + \frac{\beta F}{2.3\ RT}(E - E^{o\prime})$$

where b denotes bulk concentrations and I_{LC} is the cathodic limiting current observed.

A plot of the left-hand side against E gives a perfect straight line. Charge-transfer coefficients obtained from the slopes and charge-transfer rate constants obtained from the intercepts are given in Tables I and II.

Charge-transfer Coefficients

Both the cathodic and anodic coefficients are far from the ideal value of 0.5 and do not add up to unity. The cathodic coefficient, α, does not change with concentration (or time), whereas the anodic coefficient, β, increases with concentration (or time). These low values can be

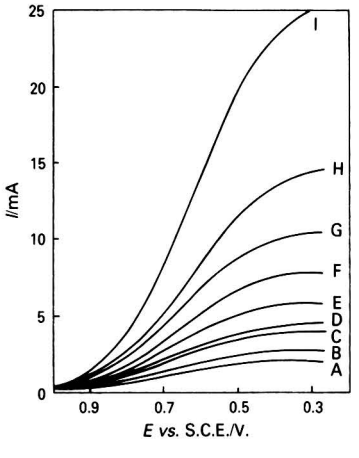

Fig. 1. Voltammograms for the reduction of chlorine at glassy carbon rotating disc electrode. Supporting electrolyte: 1 M sulphuric acid. Electrode area: 0.5 cm². Rotating frequency: 10 Hz. Chloride concentration: 0.0799 M. Chlorine concentrations: (A) 0.0031; (B) 0.0041; (C) 0.0063; (D) 0.0070; (E) 0.0093; (F) 0.0120; (G) 0.0160; (H) 0.0230; and (I) 0.0400 M.

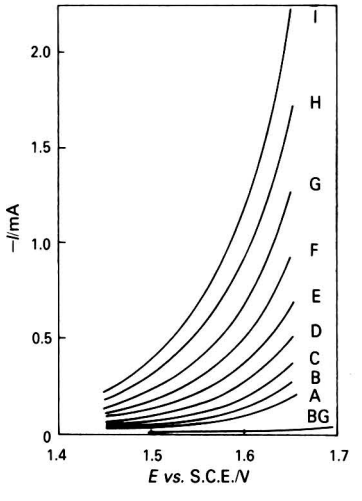

Fig. 2. Voltammograms for the oxidation of chloride at glassy carbon rotating disc electrode. Supporting electrolyte: 1 M sulphuric acid. Electrode area: 0.5 cm². Rotating frequency: 10 Hz. Chlorine concentration: 0.0060 M. Chloride concentration: (A) 0.0316; (B) 0.0418; (C) 0.0556; (D) 0.0739; (E) 0.0975; (F) 0.1290; (G) 0.1690; (H) 0.2200; and (I) 0.2860 M.

TABLE I

CHARGE-TRANSFER KINETIC PARAMETERS FOR THE REDUCTION OF CHLORINE AT GLASSY CARBON WITH CHANGING BULK CONCENTRATION

$[Cl_2] \times 10^3$/M	α	$k^{o\prime} \times 10^4$/cm s^{-1}
3.1	0.23	1.0
4.1	0.23	1.1
6.3	0.25	0.75
7.0	0.25	0.75
9.3	0.25	0.63
12.0	0.26	0.46
16.0	0.25	0.48
23.0	0.24	0.47
40.0	0.24	0.40

interpreted by the position of the activated complex with respect to the electrode surface. Fig. 3 shows a potential energy diagram with respect to distance from the electrode surface for both cathodic and anodic reactions. The lower the cathodic charge-transfer coefficient, α, the further is the activated complex from the electrode surface.

On the other hand, the lower the anodic charge-transfer coefficient, β, the closer is the activated complex to the electrode surface.

TABLE II

CHARGE-TRANSFER KINETIC PARAMETERS FOR THE OXIDATION OF CHLORIDE AT GLASSY CARBON WITH CHANGING BULK CONCENTRATION

$[Cl^-] \times 10^3$/M	β	$k^{o\prime} \times 10^6$/cm s^{-1}
3.2	0.17	1.65
4.2	0.19	1.25
5.6	0.21	0.94
7.4	0.22	0.73
9.8	0.24	0.56
12.9	0.26	0.40
16.9	0.27	0.36
22.0	0.28	0.32
28.6	0.30	0.26

A reasonable assumption is to consider the activated complex as a polarised chlorine molecule, ^+Cl–Cl^- (Fig. 3). This would form at a greater distance from the electrode surface in the cathodic reaction because only the long-distance electrostatic interaction with the free electrode surface is required. When the anodic reaction is involved, the activated complex forms closer to the electrode surface as the formation of a bond with the adsorbed chlorine atoms at the inner Helmholtz plane is required.

The increase of β with time (or concentration in Table II) is interpreted as being due to ageing of the electrode surface. The formation of carbon oxides at a glassy carbon surface at these high electrode potentials has been reported.[16] These oxides, by a simple steric effect, would increase the distance between the activated complex and the electrode, thus increasing the value of β.

As the two reactions are studied several hundred milivolts apart, under different electrode surface conditions, we cannot expect to obtain $\alpha + \beta = 1$.

Charge-transfer Rate Constants

Values determined from the anodic scans are about 100 times smaller than those obtained from the cathodic polarisations. Again, the difference is due to the surface condition. An oxide-free surface would favour electron exchange, whereas an oxide-covered surface should hinder and slow down the charge-transfer reaction.

Kinetic data reported at graphite[15] give an anodic charge-transfer coefficient between 0.38 and 0.46, being temperature dependent, and an exchange current density of 0.12 mA cm^{-2}, which cannot be compared with our charge-transfer rate constant unless it is assumed that $\alpha + \beta = 1$.

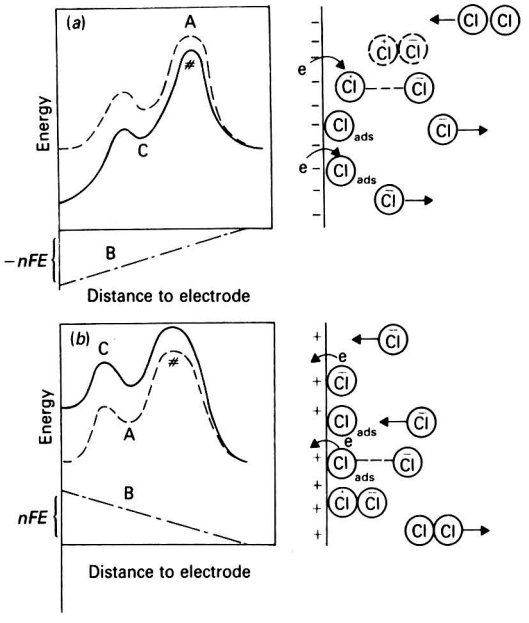

Fig. 3. Potential energy diagram and symbolic reaction mechanism for cathodic (*a*) and anodic (*b*) direction in the system Cl_2/Cl^- at glassy carbon electrode. The addition of chemical (A) and electrical (B) contribution results in the electrochemical (C) activation energy profile. The formation of activated complex $\overset{+}{Cl} - \bar{Cl}$ is denoted by #.

Generation Current Efficiencies

Current efficiencies were determined by computing the different currents involved at a particular electrode potential: the current due to chloride oxidation, the current due to oxygen evolution and the residual current. The general procedure is described elsewhere.[23] The maximum values found are given in Table III.

A graph of current efficiency *versus* generating current density is shown in Fig. 4.

High current efficiencies are possible in the generation of chlorine. The concentration of the generating reagent plays a fundamental role. Higher concentrations of chloride could be obtained if hydrochloric acid were used instead of sodium chloride in sulphuric acid, overcoming the problem of limited sodium sulphate solubility at chloride concentrations above 2 M.

TABLE III

GENERATION OF CHLORINE AT A GLASSY CARBON ELECTRODE

Electrolyte, 1 M H_2SO_4; $k_m = 10^{-3}$ cm s^{-1}.

NaCl concentration/ M	Maximum current efficiency, % Eff	Current density/ mA cm^{-2}	Practical current efficiency, % Eff	Practical current density/mA cm^{-2}
0.1	99.92	9.9	99.89	2.5
0.5	99.98	49.4	99.98	12.1
2.0	99.99	198.0	99.99	58.4

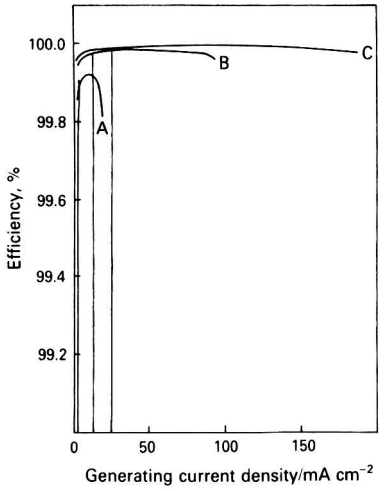

Fig. 4. Generation of chlorine at glassy carbon rotating disc electrode. Graph of current efficiencies *versus* generating current density. $k_m = 10^{-3}$ cm s^{-1}; 1 M sulphuric acid. Sodium chloride concentration: (A) 0.1; (B) 0.5; and (C) 1.0 M. Vertical lines indicate current density corresponding to an electrode potential of 1.7 V *versus* S.C.E., selected as the maximum practical generating current density that can be used.

Glassy carbon proved not to be inert. Visible attack of the electrode surface was observed at high current densities or electrode potentials above 1.7 V *versus* S.C.E. The vertical lines in Fig. 4 show the maximum current densities to be used in order to keep the electrode potential under this value. This upper limit does not affect the current efficiencies but reduces the generating current density to be used, as shown in the last two columns in Table III.

Conclusions

Chlorine can be anodically generated at glassy carbon with a current efficiency very close to 100%. This makes it useful as a high-potential coulometric intermediate. The electrode material imposes a limitation on the generating current density. The risk of electrode oxidation is present at potentials above 1.7 V *versus* S.C.E. Hence electrogenerated chlorine at glassy carbon is a suitable coulometric intermediate for ordinary analytical work, but could not be recommended for high-precision coulometry.

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Potentiometry of Alkoxylates

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The response characteristics of barium ion-selective electrodes to various alkoxylates have been investigated in aqueous and aqueous - ethanol test solutions in the presence and absence of barium ions and in the presence of sodium and potassium salts.

The observed potentiometric response is an increase in the e.m.f. of the barium ion-selective electrode of up to about 100 mV, according to the amount of added alkoxylate in the 2×10^{-5}–10^{-3} M range. The response is linear with log[alkoxylate], but is characterised by a break in the linearity, which is attributed to the critical micelle concentration (CMC) of this class of non-ionic surfactants.

The alkoxylates studied include nonylphenoxypoly(ethyleneoxy)ethanols (NPs) of the Antarox CO series, namely, CO-430, -630, -730, -850, -880 and -890; octylphenoxypoly(ethyleneoxy)ethanols (OPs), namely, Antarox CA-620 and Triton X-100; a sorbitan-9-octadecanoate poly(ethyleneoxy)ethanol, namely, Tween 80; alkylpoly(ethyleneoxy)ethanols, namely, Dobanol 25–7, Lutensol AO7 and Synperonic 7; a fluoroalkyl poly(ethyleneoxy)ethanol, namely, Monflor 51; poly(ethylene glycol) (PEG)1540; and poly(propylene glycol) (PPG)1025. CMCs were characterised in all instances except for PEG 1540, where the increases in the e.m.f. values were smaller than for the other materials studied.

Anionic surfactants gave small decreases in e.m.f., but the cationic surfactant, benzyldimethylhexadecylammonium chloride, gave a large increase in e.m.f., characteristic of a cationic response.

Exposure to alkoxylates reduced the lifetime of the barium ion-selective electrodes.

Keywords: Non-ionic surfactants; alkoxylates; poly(alkylene glycols); ion-selective electrodes; critical micelle concentration

Alkoxylates may be regarded as acyclic polyethers with the $-[CH(R)CH_2O]-$ repeating unit. They include the following compounds: poly(ethylene glycols) (PEGs), $HO(CH_2CH_2O)_nH$; poly(glycol dimethyl ethers) (glymes), $CH_3O(CH_2CH_2O)_nCH_3$; poly(propylene glycols) (PPGs), $HO[CH(CH_3)CH_2O]_nH$; and alkylphenoxypoly(ethyleneoxy)ethanols, $RC_6H_4O(CH_2CH_2O)_nH$, R = alkyl. The materials have various applications. For example, the low relative molecular mass PEGs are solvents and can be used as blenders to alter the humectant properties of simple glycols and glycerine[1] as they are less hygroscopic. Alkylphenoxypoly(ethyleneoxy)ethanols and many related materials are important non-ionic surfactants[2] and variation in the number of ethyleneoxy units can produce useful changes in wetting, detergency, emulsification, solubility and foam properties.[2,3]

The tetraphenylborate (TPB) salts of complexes between alkaline earth metal cations and certain alkoxylates, especially nonylphenoxypoly(ethleneoxy)ethanol (NP) and PPGs have been exploited for their response in ion-selective electrodes.[4–11] These have various applications as metal ion sensors, and a PVC matrix-membrane barium ion-selective electrode based on the TPB salt of the barium complex of Antarox CO-880 ($\bar{n} = 30$ in this NP) as the sensor and 2-nitrophenyl phenyl ether as solvent mediator may be used as an indicator electrode in the potentiometric titration of sulphate with barium ions.[7,12–15] Another interesting feature of this electrode is the observation[16] of its response to the presence of Antarox CO-880 and other alkoxylates in solution. This paper describes studies concerning this response of the barium ion-selective electrode.

Experimental

Materials and Reagents

Many of the alkoxylates and other materials used were gifts: members of the Antarox series of non-ionic surfactants [GAF (Great Britain) Ltd., Manchester]; Monflor 51 (ICI, Runcorn); poly(ethylene glycol) 1540 (Union Carbide, Southampton); and Synperonic 7,

Dobanol 25–7, Lutensol A07, sodium dodecylbenzene sulphonate and sodium nonylbenzene sulphonate (Unilever Ltd., Port Sunlight). The remaining alkoxylates and other surfactants were purchased: Triton X-100, poly(propylene glycol) 1025, sodium dodecyl sulphate and benzyldimethylhexadecylammonium chloride (BDH Chemicals) and Tween 80 and sodium stearate (Hopkins and Williams Ltd.). All other materials and reagents were of analytical-reagent grade.

Assembly and Operation of Electrochemical Cells

PVC matrix-membrane barium ion-selective electrodes, with 1 M barium chloride as the inner reference solution and fabricated as described previously[7] from the TPB salt of the barium complex of Antarox CO-880 and 2-nitrophenyl phenyl ether, were used as indicator electrodes in potentiometric cells in conjunction with a low-leak (approximately 0.01 $cm^3\ h^{-1}$) calomel reference electrode (Corning No. 476 107 but with the inner filling solution changed to saturated potassium chloride). A fresh membrane was fitted to the barium solution ion-selective electrode for each set of alkoxylate additions. The electrodes were left standing in water for 15–20 h before use.

Following the addition of aliquots (usually 0.1 cm^3) of polyalkoxylate solutions (usually 10^{-2} M) and other surfactants to 25 cm^3 of de-ionised water or barium-containing solutions, the cell e.m.f.s were recorded using a high-impedance millivoltmeter (Corning, Model 112) reading to 0.1 mV and used in conjunction with a Servoscribe, Model RE 4541, potentiometric recorder. The data used for summarising the alkoxylate response were normally from the second runs of alkoxylate additions, the first runs being used for guidance only.

Results

Table I summarises the main features of the reponses by barium ion-selective electrodes to various alkoxylates. For convenience, the inflection points (critical micelle concentrations, CMC) of the e.m.f. *versus* log[alkoxylate] graphs of this study are also recorded in Table I.

TABLE I

NUMERICAL DATA ON ALKOXYLATES $[RO(CR'H.CH_2O)_nH]$

Trade name of alkoxylate	Hydrophobe, R [R′ is H except for PPG(1025)]	$\bar{n}$	Average relative molecular mass	CMC* /10^{-4} M
Antarox CO-430	Nonylphenol	4	396	0.65
Antarox CO-630		9	617	0.92
Antarox CO-730		15	880	1.1
Antarox CO-850		20	1100	1.7
Antarox CO-880		30	1540	2.2
Antarox CO-890		40	1980	4.1
Antarox CA-620	Octylphenol	7	514	6.0
Triton X-100		9–10	603–647	8.0
Tween 80	Sorbitan-9-octadecanoate	20	1286	4.0
Dobanol 25–7	$C_{12}H_{25}$–$C_{15}H_{31}$	7	495–537	3.1
Lutensol AO7	$C_{13}H_{27}$–$C_{15}H_{31}$	7	508–537	2.7
Synperonic 7		7	508–537	2.5
Monflor 51	C_8F_{15}	23	1790	2.3
PEG 1540	—	35	1540	(1.6)†
PPG 1025	(R′ is CH_3)	17	1025	0.80

* See Fig. 4.

† The break in e.m.f. increase *versus* log[PEG 1540] graph [Fig. 4(*f*)] is for a change to a greater slope with increasing PEG 1540 concentration rather than a change to a smaller slope normally observed for surfactants.

Fig. 1 typifies the e.m.f. response on the addition of aliquots of Antarox CO-880 to water. The response occurred in each instance within 2–3 s of the addition of the Antarox CO-880, although a further 5–15 min were required for e.m.f.s to reach the full equilibrium values (steady readings to $\pm$ 1 mV for at least 10 min) shown by the data points in Fig. 1.

Fig. 2 summarises the range of responses of ten barium ion-selective electrodes, expressed as an e.m.f. increase over the initial value observed when water alone is in the cell, *versus*

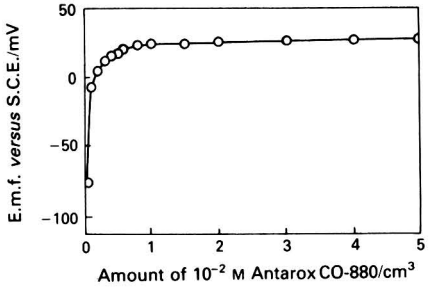

Fig. 1. Cell e.m.f. changes (barium ion-selective electrode *versus* saturated calomel electrode) on adding aliquots of 10^{-2} M Antarox CO-880 to 25 cm^3 of water.

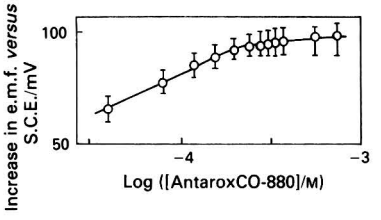

Fig. 2. Increases in cell e.m.f.s for 10 barium ion-selective electrodes *versus* log([Antarox CO-880]/M).

log[Antarox CO-880]. The break in the graph corresponds to the CMC of the non-ionic surfactant which, for the ten experiments summarised here, fell within the range 2.5×10^{-4} M (standard deviation 0.24×10^{-4} M). Similar responses were observed when Antarox CO-880 solution was added to solutions of barium chloride (Fig. 3). Here, the CMC breaks occurred at slightly lower Antarox CO-880 concentrations when the barium chloride concentration was increased.

Use of Barium Ion-selective Electrodes for Replicate Experiments

For the experiments summarised in Figs. 1–3, fresh membranes were used in the barium ion-selective electrodes for each set of Antarox CO-880 additions, and readings for the second runs in each instance as described under Experimental are shown; this was not a serious hindrance because of the ease of membrane replacement.

The need for using fresh electrode membranes was enforced by the otherwise poor reproducibility of the effects produced by Antarox CO-880. Thus, attempts to replicate e.m.f. increases by the addition of separate aliquot sequences of 10^{-2} M Antarox CO-880 to fresh samples of water or barium chloride standards, and using the same membrane in the barium ion-selective electrode, led to e.m.f. increases that were about 25 mV smaller for the second than for the first runs, and increases that were about 15 mV smaller for the third than for the second runs. Thus, changes in equilibrium e.m.f.s on addition of Antarox CO-880 are greater than the spread of about 15 mV observed in several second run measurements with barium ion-selective electrodes having new membranes (Fig. 2).

Changes in liquid junction potentials may also contribute to such spreads in e.m.f.s, but the reproducibility in the pattern of the e.m.f. values on changing the barium electrodes suggests that any liquid junction effect is subsidiary.

Exposure of any one electrode membrane to more than four or five successive runs was not possible because of the reduction in normal electrode lifetimes brought about by repeated exposure to the Antarox CO-880 non-ionic surfactant. Nevertheless, it was found that conditioning the electrodes by standing them in 7.5×10^{-3} M Antarox CO-880 solution for 20 h permitted duplication to within 1–2 mV between the experiments, although these were limited by the electrode lifetime. However, the increases in e.m.f. observed in the post-conditioning experiments were approximately 40 mV less than for the pre-conditioning stage.

Because of the reasonable reproducibility of the effect of adding Antarox CO-880 to water on electrode response and the fact that the general structures of the graphs obtained were similar to those of conditioned electrodes, fresh electrode membranes were used used for the barium ion-selective electrode for each set of surfactant solution additions in the experiments discussed here and readings for the second runs of alkoxylate additions noted. This was also justified by the occurrence of the CMC breaks at the same point despite the conditioning state of the electrode.

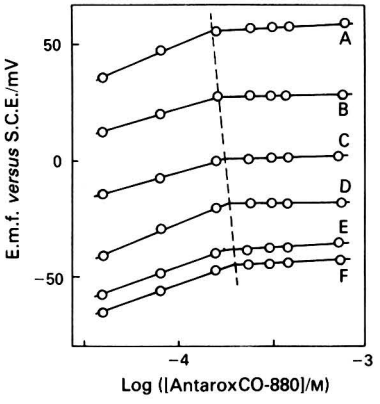

Fig. 3. Increases in cell e.m.f.s on adding 10^{-2} M Antarox CO-880 to various barium chloride solutions: A, 10^{-1}; B, 10^{-2}; C, 10^{-3}; D, 10^{-4}; E, 10^{-5}; and F, 10^{-6} M.

The general effect of Antarox CO-880 on the barium ion response of the barium ion-selective electrodes was to shorten the linear calibration range, lengthen response times and increase the calibration slopes. Also, the normal lifetime of 28–30 d for electrodes used only in solutions of barium salts was considerably reduced, to just 5–7 d, by exposure to Antarox CO-880. Allowing the electrode to stand for a day in solutions of barium chloride after exposure to Antarox CO-880 will lessen the effects, but will not completely reverse them. Table II summarises a typical pattern obtained on calibrating barium ion-selective electrodes with barium chloride solutions after electrode exposure to Antarox CO-880.

TABLE II

TYPICAL EFFECT OF EXPOSURE TO ANTAROX CO-880 ON BARIUM ION RESPONSE OF BARIUM ION-SELECTIVE ELECTRODES

Calibration was with barium chloride solutions after calibrations of the kind shown in Fig. 1 with Antarox CO-880 solutions: A, calibration 1 with $BaCl_2$; B, expose to Antarox CO-880; C, calibration 2 with $BaCl_2$; D, expose to Antarox CO-880; E, calibration with $BaCl_2$; F, expose to Antarox CO-880, etc.

Calibration with $BaCl_2$ solutions	Sequence of exposure to Antarox CO-880	Electrode slope to Ba^{2+}/ mV decade^{-1}	Lower limit of linear calibration range/M	Response time/ min
First	None	26.0	10^{-5}	<2 at 10^{-2} M <10 at 10^{-6} M
Third	Second	27.5	10^{-4}	~ 10 at 10^{-2} M ~ 30 at 10^{-6} M
Several	Several	32 to 33	10^{-4} and with loss of linearity at upper end of range	>10 at 10^{-2} M >30 at 10^{-6} M

Effect of Other Alkoxylates and Surfactants on Barium Ion-selective Electrode Response

Fig. 4 summarises the various e.m.f. increases obtained on adding aliquots of 10^{-2} M alkoxylate solutions to water, solutions of alkali metal salts [Fig. 4(*a*)] and ethanol - water [Fig. 4(*b*)].

The effect of adding various anionic surfactants to the water is minimal compared with that of adding alkoxylate non-ionic surfactants (Fig. 5). A cationic surfactant, illustrated here by benzyldimethylhexadecylammonium chloride (*i.e.*, cetyldimethylbenzylammonium chloride, CBAC), gives a much larger increase in e.m.f. than do the non-ionic surfactants (Fig. 5,

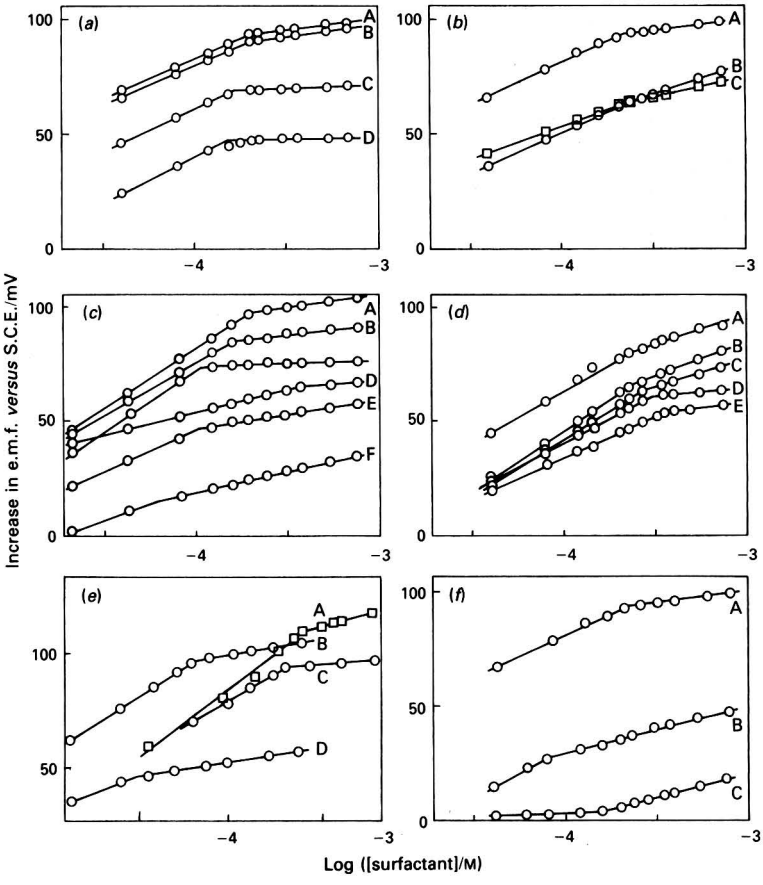

Fig. 4. Summary of increases in cell e.m.f.s for various alkoxylates. (*a*) Effect of adding aliquots of 10^{-2} M solution of Antarox CO-880 to aqueous electrolyte solutions: A, water; B, 10^{-1} M sodium chloride; C, 1 M potassium chloride; D, 1 M sodium nitrate. (*b*) Effect of adding 10^{-2} M Antarox CO-880 to ethanol - water: A, water; B, 30% ethanol - water; and C, 15% ethanol - water. (*c*) Effect of adding 10^{-2} M of various Antarox NPs to water: A, CO-880; B, CO-850; C, CO-730; D, CO-890; E, CO-630; and F, CO-430. (*d*) Effect of adding 10^{-2} M Monflor 51 (A), alkylpoly(ethyleneoxy) ethanols (B, Synperonic 7; C, Lutensol 407; D, Dobanol 25–7) and Tween 80 (E) to water. (*e*) Effect of adding 10^{-2} M OPs to water: A, Triton X-100; B, Antarox CO-880 (for comparison); C, Antarox CA-620; and D, Antarox CO-630 (for comparison). (*f*) Effects of adding 10^{-2} M alkoxylates with no hydrophobes to water: A, Antarox CO-880 (for comparison); B, PPG 1025; and C, PEG 1540.

curve A), but in this instance the electrode function is completely destroyed, for after just the first experiment there is no response to either barium ions or to alkoxylate. The break in the graph (1.2×10^{-4} M) occurs at a lower level than expected from the normal CMC value (approximately 10^{-3} M) of the CBAC.

Discussion

General Features of Response by Alkoxylates

There are many instances whereby ion-selective electrodes respond to species that are very different from that for which the electrode was designed. An example is the response of a

copper(II) electrode to several complexing agents,[17,18] which include EDTA, ethylene glycol bis(β-aminoethyl ether)-*NNN'N'*-tetraacetic acid (EGTA), nitrilotriacetic acid (NTA), humic and fulvic acids. It is, therefore, not entirely surprising that the barium ion-selective electrodes with a sensor based on a barium - alkoxylate complex should respond to alkoxylates. However, the pre-CMC slopes of the responses (approximately 30 mV per decade) for Antarox CO-880 is not Nernstian, bearing in mind that the stoicheiometry of the barium - Antarox CO-880 complex requires that each molecule of Antarox CO-880 complexes 2.5 barium ions.[9,19] However, Fig. 4(*c*) shows an underlying trend for the pre-CMC slope to increase with the average number, $\bar{n}$, of ethoxylate groups in the NP molecule (Table I). The pattern is broken for Antarox CO-890, but with such a large value of $\bar{n}$, namely 40, the relatively large hydrophilic nature may overshadow any effect that the hydrophobe may have in establishing an equilibrium with the electroactive barium at the electrode membrane surface.

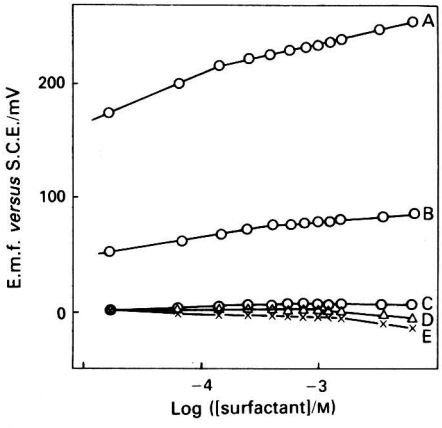

Fig. 5. Changes in cell e.m.f.s on adding various ionic surfactants (sodium salts for anionics) to water. A, Benzyldimethylhexadecylammonium chloride (cationic surfactant, CBAC); B, Antarox CO-880 (for comparison); C, sodium nonylbenzene sulphonate and sodium stearate; D, sodium dodecyl sulphate; and E, sodium dodecylbenzene sulphonate.

Moderate amounts of electrolyte (10^{-1} M) have relatively little effect on the magnitude of the increase in e.m.f. [Figs. 3 and 4(*a*)]. Larger amounts (1 M) reduce the extent of the increase, possibly owing to the cation complexing some of the alkoxylate [Fig. 4(*a*)] for it has been shown[9,19] that sodium and potassium complex Antarox. The presence of such high levels of electrolyte in solution may, by itself, alter the balance of membrane - solution equilibria.

Ethanol also markedly reduces the extent of e.m.f. increases brought about by added Antarox CO-880 [Fig. 4(*b*)]. This may be looked upon as being caused by the alkoxylate favouring the bulk solution, thus having a lesser effect on the membrane - solution equilibrium.

There is no definite pattern whereby individual alkoxylates may be picked out from the response by barium ion-selective electrodes to them (Fig. 4), although a number of interesting groupings occur. Thus, Synperonic 7, Lutensol AO7 and Dobanol 25–7 fall closely together [Fig. 4(*d*)], in keeping with their similar molecular character and small differences in the hydrophobe (Table I).

Antarox CA-620 and Triton X-100 have slightly different hydrophobes from that of Antarox CO-630 (octylphenol in place of nonylphenol), but the number of ethoxylate groups ($\bar{n} = 7$ or 9) is similar in the three instances. These two types yield similar e.m.f. increases at lower concentrations of alkoxylate [Fig. 4(*e*), curves B, C and D], although the octylphenyl hydrophobe produces CMC breaks at higher concentrations.

Tween 80 with its sorbitan-9-octadecanoate hydrophobe produces lower e.m.f. increases [Fig. 4(*d*), curve E] than the corresponding Antarox CO-850 [Fig. 4(*c*), curve B].

The fluorinated material, Monflor 51, is unique among the range studied as it has a fluorinated hydrophobe, but it is interesting that it falls into a similar response pattern [Fig. 4(*d*), curve A] to Antarox CO-850 [Fig. 4(*c*), curve B], which has a nonylphenyl hydrophobe; each has a similar number of ethyleneoxy groups, namely, 23 for Monflor 51 and 20 for Antarox CO-850.

Poly(propylene glycol) 1025 and poly(ethylene glycol) 1540 do not have hydrophobes and consist entirely of alkoxy groups. They may, therefore, be regarded as hydrophilic and hence favour the solution phase; the extent of the e.m.f. increases produced by these two materials when added to water is less than for similar alkoxylates having hydrophobic ends [Fig. 4(*f*), curves B and C compared with curve A].

CMC Breaks

An interesting characteristic of ion-selective electrode response to anionic and cationic surfactants is the inflection occurring in the e.m.f. *versus* log[surfactant] calibration graph at the CMC.[20–29] Inflections are also featured in the non-ionic surfactant data of this study, and as the effect arises from the tendency for surfactants to form micelles in solution, it is reasonable to assume that these breaks in the e.m.f. *versus* log[alkoxylate] graphs relate to the CMCs.

The CMC tends to be higher in ionic surfactants than in the non-ionic materials of about equivalent hydrophobic chain lengths. For example, sodium dodecyl sulphate has a CMC of 8×10^{-3} M, but Lutensol AO7 with a hydrophobic chain of 13 carbon atoms (Table I) has a CMC of 1.1×10^{-4} M. This is because in non-ionic types there is little or no electrical charge resisting aggregation.[30] In ionic surfactants, micelle formation is ascribed to a balance between hydrocarbon chain attractions and ionic repulsion, but with non-ionic types the hydrocarbon chain attractions are opposed by hydration and space requirements of the ethyleneoxy chain.[31]

Non-ionic surfactants do not show such well defined CMCs as their ionic counterparts because of an uneven distribution of ethyleneoxy units in the hydrophilic chains and there is normally a concentration range over which micelles gradually appear.[32] Nevertheless, the inflections observed here in the e.m.f. *versus* log[alkoxylate] graphs are quite definite and it is interesting to compare literature CMC data with such breaks (Table III). The agreements are reasonable and the electrochemical values also compare well with those calculated (Table III) from the formula derived by Hsiao *et al.*[30]:

$$\ln (10^6 \times \mathrm{CMC}) = A\bar{n} + B \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (1)$$

where A and B are constants for a particular hydrophobic group and $\bar{n}$ is the average number of

Table III

Critical micelle concentrations of Antarox CO-type non-ionic surfactants

Surfactant Antarox No.	Critical micelle concentration/10^{-4} M: Experimental result (this work) Mean	Experimental result (this work) Standard deviation	Literature value	Calculated value [equation (1)]
CO-890	4.6	0.38	—	4.5
CO-880	2.5	0.24	2.5–3.0* 1.85†	2.6
CO-850	1.4	0.26	1.35–1.75* 1.40†	1.5
CO-730	1.1	0.056	1.0–1.30* 1.10†	1.11
CO-630	0.83	0.074	—	0.80
CO-430	0.60	0.055	—	0.60

* From reference 30.
† From reference 31.

ethyleneoxy units in the molecule. For a nonylphenol, $A = 0.056$ and $B = 3.87$. From comparisons of data in Table III it seems likely that the observed inflections occur at the CMC.

The CMCs for the non-ionic surfactants emanating from this study are generally around 2×10^{-4} M (Table I and Figs. 2 and 4), but those for the lower members of the Antarox CO series and poly(propylene glycol) 1025 fall below 10^{-4} M while those for the OPs are much nearer to 10^{-3} M. Poly(ethylene glycol) 1540 does not show an inflection characterising a CMC.

The gradual fall in CMC of Antarox CO-880 in barium chloride solutions of increasing concentration (from 2.0×10^{-4} M in 10^{-6} M barium chloride solution to 1.6×10^{-4} M in 10^{-1} M barium chloride solution) may arise from the clustering of the hydrophilic ethyleneoxy units during complexation, thus promoting hydrocarbon attractions for micelle formation. The lower CMCs of the alkoxylate in 1 M sodium nitrate and potassium chloride solutions follow the same reasoning.

Effect of ionic surfactants

As noted above, the effect of anionic surfactants is negligible compared with the increases noted for the alkoxylates (Fig. 5). Also, the nature of the response is different, in that here there is a decrease in the e.m.f. This is akin to the e.m.f. lowering brought about by anionic surfactants for calcium ion-selective electrodes,[33] although the magnitude of the effect here is smaller than that noted for the phosphate ester sensor system of the calcium electrodes.

The effect of CBAC cationic surfactant (Fig. 5) is to give a response characteristic of the cationic surfactant types of ion-selective electrodes. The CBAC cation can be expected to interact with the tetraphenylborate of the electrode membrane in order to reflect this cationic response, but in so doing the electrode is destroyed.

Conclusion

Alkoxylates give rise to an interesting potentiometric response in water, electrolyte solutions and in ethanol - water. The e.m.f. increases shown by barium ion-selective electrodes will be detrimental to the normal applications of such electrodes in analytical potentiometry in the event of any of these materials being present in samples on analysis. However, the observed effect can have added analytical significance for the analysis of small amounts of non-ionic surfactants in solution in water. This is the subject of a continuing study, but meanwhile, the breaks observed in potentiometric responses provide an alternative method for measuring the CMCs of non-ionic surfactants.

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Analytical Methods Committee

REPORT PREPARED BY THE ESSENTIAL OILS SUB-COMMITTEE

Application of Gas - Liquid Chromatography to the Analysis of Essential Oils

Part VIII.* Fingerprinting of Essential Oils by Temperature-programmed Gas - Liquid Chromatography Using Methyl Silicone Stationary Phases

Problems of obtaining reproducible results in the "fingerprinting" of essential oils by temperature-programmed gas - liquid chromatography have been examined and reported in Part VII of this series. That report was concerned both with the general problems and with the specific use of a polar stationary phase, *i.e.*, Carbowax 20M. This report is concerned with the use of non-polar stationary phases of the methyl silicone type and the application of the method of column standardisation described in Part VII.

A collaborative study with methyl silicone stationary phases and a specification of "*g*-pack values" for the column packing has resulted in the production of a method that yields reproducible relative retention indices for the test substances limonene, acetophenone, linalol, naphthalene, linalyl acetate and cinnamyl alcohol and has been applied with satisfactory results to oils of bergamot, Jamaican ginger, Nigerian ginger, West Indian nutmeg and East Indian nutmeg. A recommended method is given for the reproducible temperature-programmed gas - liquid chromatographic fingerprinting of essential oils when methyl silicone stationary phases are used.

Keywords: *Essential oils analysis*; *temperature-programmed gas - liquid chromatography*; *methyl silicone stationary phases*

The Analytical Methods Committee has received and approved for publication the following report from its Essential Oils Sub-Committee.

Report

The constitution of the Sub-Committee responsible for the preparation of this Report was: Mr. A. M. Humphrey (Chairman), Mr. C. J. Brett, Mr. E. Cummings, Dr. D. Farley, Mr. W. S. Matthews, Mr. P. Metson, Miss D. M. Michalkiewicz, Mr. M. Milchard, Mr. D. A. Moyler, Mr. A. Osbiston, Mr. R. G. Perry, Mr. J. Ridlington, with (the late) Dr. N. W. Hanson and Mr. J. J. Wilson (from September 1979) as Secretaries.

Introduction

This report is a logical extension and addition to the Part VII[1] report in this series and the introduction and references given in that report apply equally in this paper. The major change concerns the use of non-polar stationary phases, rather than Carbowax 20M, which was an example of the polar type of phase. The use of six test compounds and their determined relative retention indices under closely defined operating conditions is continued, as is the mathematical calculation of the "*g*-pack value" for the packed columns that are prepared by the absorption coating method.

The original work by van den Dool on the determination of the *g*-pack values for columns used only two stationary phases: Carbowax 20M and SE-30 as examples of non-polar types. However, in this work it was recognised that several different non-polar methyl silicone stationary phases are in common use and they differ in their degree of polymerisation, temperature stability, viscosity and purity. The non-polar character of columns prepared from different examples of these phases is similar and any particular choice of phase

* For details of Part VII of this series, see reference list, p. 455.

can be made on the basis of other desirable criteria. For example, it is thought that the lower viscosity members of the class are easier to coat on to the support and give slightly improved separations when operated at low temperatures, whilst rubber-like members of the class have a higher temperature stability. These aspects have not been investigated by the Sub-Committee who have used only specific members of methyl silicone stationary phase group, *viz.*, SE-30 and OV-101.

Experimental

The upper temperature limits of the methyl silicone stationary phases used by the collaborating members were significantly higher than that for Carbowax 20M and it was found satisfactory to use an upper temperature of 275 °C and to adjust the elution of the C_{24} alkane to coincide with this temperature during the temperature programme, *i.e.*, to arrange for a relative retention index at 275 °C (R.R.I.275) of 2400.

The effect of stationary phase loading was investigated and was found to have a negligible effect on the *g*-pack value of the column. With Chromosorb W HP supports, the stationary phase loading was as high as 20% in order to minimise the effect of active sites; the corresponding loading on Gas-Chrom Q, to compare with the results reported in Part VII, was 16%.

The column efficiency was determined as before, by measuring the separation of a closely eluting pair of compounds and using the peak/valley ratio method of expression (see Fig. 2, Part VII[1]). In this instance, limonene and acetophenone form such a pair when run on non-polar columns; the degree of separation of these two components in the test mixture [Netherlands Committee (N.C.) mixture] was used as a measure of column efficiency in all of the Sub-Committee's investigations.

The effects of carrier-gas flow-rate, column length and inner diameter, programming rate and starting temperature were studied in detail and, on the basis of the results, a full collaborative trial was arranged in which the operating conditions were specified in a manner very similar to those given in the Appendix for the standardisation of the chromatographic system.

Results

The results of the collaborative study using the N.C. test mixture with the proposed standard procedure (Appendix I) are given in Table I. The mean values, standard deviation and relative standard deviations for the relative retention indices are given in Table II. These results were considered by the Sub-Committee to be satisfactory.

A collaborative trial using five essential oils (bergamot, Jamaican ginger, Nigerian ginger, West Indian nutmeg and East Indian nutmeg) produced a series of chromatograms, which, when reduced photographically to similar dimensions, were compared subjectively by the Sub-Committee. Each set of chromatograms for each oil was considered to show minimum variations and the Sub-Committee was satisfied that the chromatographic procedure was satisfactory.

Discussion

Much of the relevant discussion on the general aspects of the method is given in Part VII,[1] but some further discussion on aspects concerning the specific use of non-polar stationary phases is desirable. These phases will elute a range of compounds in an order approximately relating to their boiling-points, and polarity effects, which play a significant part in determining the order of elution from more polar stationary phases, are minimal; hence the need to ensure that the methyl silicone columns in use continue to be free from any polarity, which is very important.

Non-polar columns are very susceptible to oxidation and this will be reflected by an increase in the *g*-pack value. Use of oxygen-free carrier gas and a leak-free system will prolong the life of the columns, as will a thorough flushing of the columns with the carrier gas prior to heating.

The order of elution and relative retention indices of the test compounds are different from those found when using Carbowax 20M and this is reflected in the changes made to the check/calculation table (Table III) and the two compounds used for measuring the resolution.

TABLE I

RESULTS OF COLLABORATIVE STUDY OF RECOMMENDED METHOD (APPENDIX I)

	Laboratory										
	1	2	3A	3B	4	5	6	7A	7B	8A	8B
g-pack	1.003	1.006	1.004	1.004	0.998	1.003	1.005	1.004	1.002	1.001	1.001
R.R.I.275	2350	2375	2403	2409	2364	2400	2400	2380	2384	2464	2390
Resolution, %	95	98	40	56	100	100	99	97	98	100	100
Time/min	101	103	100	100	100	101	101	100	103	100	100
Column inner diameter/mm	4	4	2	2		4	4	4	4	3.4	3.4
Material	Glass	Glass	Glass	Stainless steel		Glass	Glass	Glass	Glass	Glass	Glass
Flow-rate/ml min^{-1}	41	45	6	7		40	23	46	45	40	40
Stationary phase	OV-101	SE-30	OV-101	OV-101	SE-30	OV-101	SE-30	OV101	SE-30	OV-101	OV-101
Support	Chromosorb W HP	Chromosorb W	Chromosorb W HP	Chromosorb W HP		Chromosorb W HP	Chromosorb WAW-DMCS	Chromosorb W HP	Chromosorb W HP	Chromosorb W HP	Gas-Chrom Q
Test compounds: relative retention indices—											
Limonene	1027	1029	1028	1028	1024	1026	1028	1027	1026	1026	1024
Acetophenone	1040	1046	1042	1043	1038	1041	1044	1041	1044	1037	1037
Linalol	1084	1088	1087	1088	1085	1086	1087	1086	1085	1083	1083
Naphthalene	1171	1178	1173	1174	1169	1173	1176	1172	1169	1167	1167
Linalyl acetate	1241	1243	1242	1243	1236	1241	1242	1241	1242	1241	1242
Cinnamyl alcohol	1276	1285	1281	1282	1277	1277	1282	1279	1286	1276	1275
n-Alkanes: elution temperature/°C—											
C_{10}	117	124	121	121	116	116	120	120	116	112	115
C_{12}	145	152	149	149	145	146	148	148	145	140	143
C_{14}	172	178	176	176	172	172	175	174	172	167	171
C_{16}	197	208	200	200	198	196	199	199	196	191	196
C_{18}	219	224	221	221	221	218	220	221	220	216	219
C_{20}	239	243	241	240	242	238	240	241	241	235	240
C_{22}	258	261	259	258	260	257	258	259	259	255	259
C_{24}	275	277	275	274	275	275	275	277	277	275	276
Test compounds: elution temperature/°C—											
Limonene	121	128	125	125	120	120	124	124	120	116	118
Acetophenone	122	131	127	127	122	122	126	126	123	118	120
Linalol	129	136	133	133	128	129	133	132	129	124	127
Naphthalene	141	149	145	146	140	142	145	144	141	135	139
Linalyl acetate	150	158	155	155	150	151	154	153	151	146	149
Cinnamyl alcohol	155	163	160	160	155	156	159	158	157	150	154

TABLE II

SUMMARY OF RESULTS IN TABLE I: MEAN RELATIVE RETENTION INDICES AND STANDARD DEVIATIONS

	Mean relative retention index	Standard deviation	Relative standard deviation, %
Limonene	1027	1.63	0.16
Acetophenone	1041	2.99	0.29
Linalol	1086	1.80	0.17
Naphthalene	1172	3.55	0.30
Linalyl acetate	1241	1.90	0.15
Cinnamyl alcohol	1280	3.80	0.30

Furthermore, it is essential to include the C_8 alkane in the reference mixture, as the test compounds elute at an earlier stage in the programme. The fundamental change in elution pattern that is noted when comparing non-polar columns and Carbowax 20M columns is the reason why fingerprinting is often desirable using two columns with different stationary phases.

The use of the absorption coating technique has again been shown to be superior to the slurry coating technique when applied to non-polar stationary phases, although the solutions of SE-30 at the concentrations required are rather viscous. For this reason OV-101 may be preferred. The high level of stationary phase loading is recommended in order to minimise the tailing of polar compounds on residual active sites of the support. This high level of loading does not appear to have an adverse effect on stationary phase bleed at high temperatures; an upper temperature limit of 280 °C gives a very low bleed and columns may be operated at this temperature for very long periods without any significant changes in their performance characteristics.

The collated results of the collaborative trial show excellent agreement, but the aromatic compounds show slightly greater variabilities of relative retention indices than the non-aromatic compounds. This is an effect noted in Part VII when using Carbowax 20M and is attributed to the greater temperature dependence of relative retention indices for aromatic compounds. The effect has also led to a wider acceptable range for the relative retention indices of the aromatic compounds. The variations found in the relative retention indices and elution temperatures on the methyl silicone columns are attributed to the greater effect caused by small changes of the *g*-pack value. This is indicated by the result from Laboratory 2 where all the results are higher than the average. To a lesser extent, similar trends are also indicated by the other laboratories, which shows that low *g*-pack values are accompanied by lower relative retentions and elution temperatures.

APPENDIX I

Recommended Method for the Reproducible Fingerprinting of Essential Oils by Temperature-programmed Gas Chromatography Using Methyl Silicone Stationary Phases

Preparation of the Packing

The packing will be coated with methyl silicone stationary phase (typically OV-101, SE-30, OV-1 or SP-2100) at a loading dependent upon the type of support. A high quality, acid-washed and silanised support with a mesh size of 80–100 or preferably 100–120 should be chosen. Proprietary products such as Chromosorb W HP (see Note) and Gas-Chrom Q have been found to be satisfactory. These two supports must be coated with 20% and 16% of methyl silicone stationary phase, respectively.

NOTE—
A range of proprietary calcined Celite supports is available under the description Chromosorb, and if the same volume of methyl silicone stationary phase were used for coating, a number of the impregnated supports might not be sufficiently free-flowing to give a satisfactory packing.

TABLE III

METHOD OF TABULATING RESULTS FOR GLC COLUMN CHARACTERISATION USING NON-POLAR COLUMNS

LABORATORY: COLUMN CODE: DATE:

Chromatographic parameters:

g-pack	1.003
R.R.I.275	2400
Resolution	100%
Stationary phase	OV-101
Support	Chromosorb W HP
Particle size	80–100
Loading	20%
Length	2 m
I.D.	4 mm
Material	Glass
Injection temperature	150 °C
Starting temperature	75 °C
Finishing temperature	275 °C
Programming rate	2 °C min^{-1}
Programming time	101 min
Carrier-gas flow-rate	40 ml min^{-1}

Elution temperatures:

C_8	90 °C
C_{10}	116 °C
Limonene	120 °C
Acetophenone	122 °C
Linalol	129 °C
Naphthalene	142 °C
C_{12}	146 °C
Linalyl acetate	151 °C
Cinnamyl alcohol	156 °C
C_{14}	172 °C
C_{16}	196 °C
C_{18}	218 °C
C_{20}	236 °C
C_{22}	257 °C
C_{24}	275 °C

Calculation of *g*-pack value:

Test compound	Relative retention index	*Y* factor	*X* factor	*Z* factor
Limonene ..	1026	$\frac{\text{R.I.} \times 0.14 + 2}{136.23} = 1.0691$	$1.0584 \times Y = 1.1315$	$1.05844^2 \times Y = 1.1976$
Acetophenone	1041	$\frac{\text{R.I.} \times 0.14 + 2}{120.14} = 1.2297$	$1.3350 \times Y = 1.6417$	$1.33500^2 \times Y = 2.1917$
Linalol ..	1086	$\frac{\text{R.I.} \times 0.14 + 2}{154.24} = 0.9987$	$1.0218 \times Y = 1.0205$	$1.02188^2 \times Y = 1.0427$
Naphthalene ..	1173	$\frac{\text{R.I.} \times 0.14 + 2}{128.16} = 1.2970$	$1.3361 \times Y = 1.7329$	$1.3361^2 \times Y = 2.3153$
Linalyl acetate ..	1241	$\frac{\text{R.I.} \times 0.14 + 2}{196.28} = 0.8954$	$0.8797 \times Y = 0.7876$	$0.8797^2 \times Y = 0.6929$
Cinnamyl alcohol ..	1277	$\frac{\text{R.I.} \times 0.14 + 2}{134.17} = 1.3474$	$1.4993 \times Y = 2.0201$	$1.4993^2 \times Y = 3.0288$
		$\Sigma Y = 6.8373 \times 1.07977 = 7.3827$ Minus	$\Sigma X = 8.3343 \times 2.88734 = 24.0640$ Plus	$\Sigma Z = 10.4690 \times 1.49758 = 15.6782$ Minus

g-pack value $= f_2\Sigma X - f_1\Sigma Y - f_3\Sigma Z =$ **1.003**

Weigh the support (16 g of Chromosorb W HP or 16.8 g of Gas-Chrom Q) into a 100-ml beaker. Weigh the stationary phase (4 g for the Chromosorb W HP or 3.2 g for the Gas-Chrom Q) into a 250-ml conical flask and add 12 ml of chloroform. Stopper the flask and swirl until solution is complete. With the silicone rubber stationary phases the solutions may be noticeably viscous. Add the appropriate support from the beaker using a powder funnel. Re-stopper and rotate the flask gently until an even distribution is obtained. The packing at this stage should be particulate and free-flowing. If the packing appears to be damp and the particles coalesce to any extent, this is indicative of a low-absorbing support and the preparation must be repeated using a smaller volume of solvent. Allow the flask and contents to stand overnight and then transfer the damp, but free-flowing, packing into a

flat open dish in a fume cupboard. Allow the solvent to evaporate whilst gently turning over the packing with a spatula, avoiding vigorous action, which may break down the particles. When the solvent has evaporated sieve the packing and reject any "fines" or coarse lumps, then store the packing ready for use.

Smaller or larger amounts of packing may be prepared using *pro rata* amounts, and different supports may be used with the appropriate stationary phase loadings and volumes of solvent as determined by experiment.

Packing the Columns

For the most reliable results the column must be glass; for some purposes stainless steel will be satisfactory. The column length must be 2 m; the internal diameter is not critical but should be known so that the optimum carrier-gas flow-rate can be estimated. Clean the column with solvent and dry it in a current of air. Plug one end with silanised glass-wool and attach it to a vacuum line (water-pump or similar). Fit the open end of the column with a funnel and add small amounts of the packing at intervals. Between each addition, tap the column gently with a metal spatula until no more settling takes place. When the column is full plug the open end with silanised glass-wool. Pack and operate the columns in pairs.

Conditioning the Columns

Connect the columns to the gas chromatograph at the injector end only and adjust the carrier-gas flow-rate to the appropriate value. Programme the temperature of the oven from 75 to 280 °C at the lowest rate available and leave the columns at 280 °C overnight with the carrier gas flowing. Cool the oven and examine the packing for signs of settling; if this has been severe (greater than 10 mm) unplug the column and top up with fresh packing. Repeat the conditioning. The columns are then ready for use.

Gas-chromatographic Conditions

It is very important that the oven conditions and settings are checked before use. It is not uncommon to find that the temperature calibrations require adjustment and these should be set at 75 and 275 °C using a reliable thermometer as a reference. Any variations at temperatures between these limits are likely to be small and may be ignored. The programme rate is also very important and should be adjusted to 2 °C min^{-1} using a stop-watch to time the complete programme from 75 to 275 °C. A tolerance of 100 $\pm$ 3 min for the run is permissible.

The carrier-gas flow-rate must be controlled by a mass flow controller; pressure control is not suitable. The actual flow-rate will depend on the internal diameter of the column.

The septum must be changed regularly and the maintenance of the correct carrier-gas flow-rate must also be checked at intervals. Some types of flow controllers drift with time and others do not re-set accurately after a temperature-programmed cycle. If a pressure gauge is fitted between the flow controller and the injection port it will give an indication of any change in the flow.

Preparation of Test Mixtures

Prepare a mixture of equal masses of the even carbon numbered n-alkanes from C_8 to C_{24}.

Prepare a mixture of 1.00 parts of limonene, 1.37 parts of linalol, 1.60 parts of linalyl acetate, 1.40 parts of acetophenone, 1.13 parts of naphthalene and 1.80 parts of cinnamyl alcohol (N.C. mixture).

Prepare a mixture of 55% *m/m* of the N.C. mixture and 45% *m/m* of the hydrocarbon mixture. Each n-alkane is then 5% and each N.C. component is then approximately 10% of the total mixture.

Test Chromatogram

Set up the gas chromatograph, with the prepared columns, for temperature-programmed operation between 75 and 280 °C at 2 °C min^{-1}. Inject 0.2 μl of the combined test mixture and immediately start the programme and recorder chart. Mark the position of the chart

at the 275 °C point and continue the isothermal heating at 280 °C until a stable base line is obtained. Cool the oven and re-stabilise at 75 °C. Repeat the run if necessary, adjusting the attenuation and flow-rate to bring all the peaks on-scale and the elution temperature of the C_{24} hydrocarbon between 273 and 277 °C. Small alterations in the sample size may also be made. Examine the chromatogram to establish the absence of catalytic decomposition of the linalyl acetate; this is indicated by the presence of early emerging spurious peaks and a reduced peak height for the linalyl acetate. A typical tracing is shown in Fig. 1(*a*).

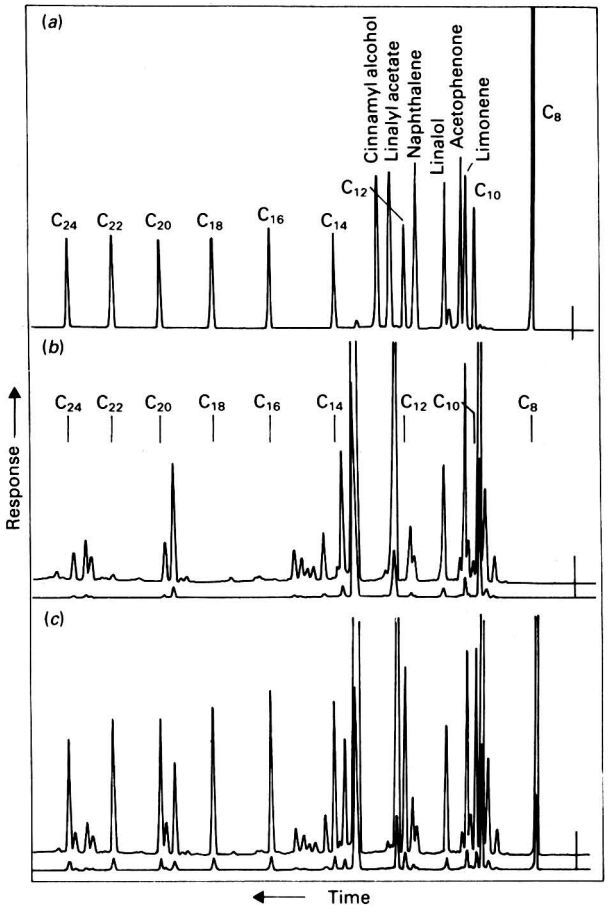

Fig. 1. Temperature-programmed gas chromatograms obtained using an OV-101 stationary phase: (*a*) typical trace with the test mixture; (*b*) bay oil; and (*c*) bay oil plus n-alkanes.

Calculation of Results

When a satisfactory tracing has been obtained, calculate the relative retention indices of the N.C. components and the R.R.I.275 point, assuming a linear span between adjacent hydrocarbon peaks. Using the values obtained for the N.C. components tabulate the results as in Table III and calculate the *g*-pack value for the column following the given worked example.

The *g*-pack value is given by $f_2\Sigma X - f_1\Sigma Y - f_3\Sigma Z$, where f_1, f_2 and f_3 are the appropriate multiplying factors.

Calculate also the elution temperatures of the N.C. components and the n-alkanes from the tracing, assuming a linear increase in the temperature from the start (75 °C) to the 275 °C point. Calculate the resolution between the limonene and the acetophenone using the peak/valley separation method (see Fig. 2, page 273 in reference 1).

The operating conditions and chromatographic system may be considered satisfactory if the results lie within the following specifications:

g-pack	1.00 ± 0.005
R.R.I.275	2375–2425
Resolution	95–100%
Time for programme	97–103 min
R.R.I. values—	
Limonene	1022–1030
Acetophenone	1036–1046
Linalol	1081–1091
Naphthalene	1167–1177
Linalyl acetate	1236–1246
Cinnamyl alcohol	1275–1285

Elution temperatures—	
C_8	87–93 °C
C_{10}	115–121 °C
C_{12}	143–149 °C
C_{14}	170–176 °C
C_{16}	196–202 °C
C_{18}	217–223 °C
C_{20}	237–243 °C
C_{22}	257–262 °C
C_{24}	273–277 °C
Limonene	117–125 °C
Acetophenone	119–127 °C
Linalol	126–134 °C
Naphthalene	136–146 °C
Linalyl acetate	148–156 °C
Cinnamyl alcohol	152–160 °C

Standardised Chromatograms of Essential Oils

As soon as the performance of a column within a chromatographic system has been satisfactorily established according to the procedure given above, it can be used under similar conditions for the analyses of essential oils. The performance of the system should be checked at least once a week.

A sample of the essential oil should be injected and run under the conditions established as above. The sample size and attenuation should be adjusted according to the sensitivity required for any subsequent examination. On completion of the run, a mixture of the essential oil with the n-alkane mixture should be chromatographed using similar conditions. Comparisons of the two chromatograms should show identical retention times and comparisons of the peak heights should be consistent with any alterations due to dilution with the n-alkane mixture or changes of the injected volume and attenuation.

These comparisons allow for a check on the reproducibility of the system and also enable the positions of the n-alkanes to be transferred from the second chromatogram [Fig. 1(*c*)] to the first [Fig. 1(*b*)] in such a manner that visual interference is avoided. In instances where a component of the essential oil overlaps or obscures an n-alkane peak, its position can be determined by comparison with the chromatogram obtained for the *g*-pack calculation [Fig. 1(*a*)]. Thus, it is possible to determine relative retention indices for any of the peaks of interest in the essential oil chromatogram.

The sensitivity of the system may be adjusted electronically to suit the purposes of the analyses, but it is recommended that for general purposes two chromatograms should be obtained such that in one a peak representing 50% of the total gives about full-scale deflection and in the other a peak representing 5% of the total gives about full-scale deflection. This does not take into account variations in response factors; sensitivity settings should be adjusted on the basis of the average peak heights found for the n-alkanes in the standardisation test run, where each n-alkane represents 5% of the total mixture. Small changes in the injection volume can also be used to adjust the peak heights obtained. Ideally a two-pen recorder should be used, allowing the chromatograms at different sensitivities to be obtained simultaneously and on the same chart, as shown in the examples [Fig. 1(*b*) and (*c*)].

Reference

1. Analytical Methods Committee, *Analyst*, 1980, **105**, 262, and references cited therein.

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

Analyst, April, 1981, Vol. 106, pp. 456–460

Analytical Methods Committee

REPORT PREPARED BY THE ESSENTIAL OILS SUB-COMMITTEE

Application of Gas - Liquid Chromatography to the Analysis of Essential Oils

Part IX.* Determination of Eugenol in Oil of Cinnamon Bark

The essential oil obtained from cinnamon leaves has a high content of eugenol whereas the genuine oil from cinnamon bark has a low eugenol content. Determination of the eugenol content of a sample reputed to be cinnamon bark oil provides a method for the detection of adulteration by addition of cinnamon leaf oil. This determination has been carried out by gas - liquid chromatography using a non-polar stationary phase.

Keywords: *Essential oils analysis*; *oil of cinnamon bark*; *eugenol determination*; *gas - liquid chromatography*

The Analytical Methods Committee has received and approved for publication the following report from its Essential Oils Sub-Committee.

Report

The constitution of the Sub-Committee responsible for the preparation of this report was: Mr. A. M. Humphrey (Chairman), Mr. C. J. Brett, Mr. E. Cummings, Dr. D. Farley, Mr. W. S. Matthews, Mr. P. Metson, Miss D. M. Michalkiewicz, Mr. M. Milchard, Mr. D. A. Moyler, Mr. A. Osbiston, Mr. R. G. Perry, Mr. J. Ridlington, with (the late) Dr. N. H. Hanson and Mr. J. J. Wilson (from September 1979) as Secretaries.

Introduction

Genuine oil of cinnamon bark is characterised by its high content of cinnamaldehyde and its accompanying characteristic fine odour. In contrast, the oil obtained from cinnamon leaves has a much lower cinnamaldehyde content and is characterised by its high content of eugenol and its harsher odour. There are several recognised grades of cinnamon bark, but the oils derived from them, whilst having a corresponding gradation of quality, are all superior in quality to the oil produced from the leaves. The quality of the bark oil reflects the market price *vis à vis* the leaf oils and it is not uncommon to find blends of oils being offered to suit particular markets. The content of total aldehydes[1] measured as cinnamaldehyde may be used as a guide to the quality, but the ready availability of synthetic cinnamaldehyde may make the result of the analysis inconclusive. In addition, the degree of polymerisation and/or oxidation of the cinnamaldehyde may give a low result. Whilst this will detract from the quality of the oil, it may give a misleading conclusion as to the degree of addition of leaf oil. It is therefore necessary to introduce an additional test for the presence of leaf oil and a measurement of the eugenol content may be used to assess the degree of addition. A gas-chromatographic method has been devised and tested by the Essential Oils Sub-Committee of the Analytical Methods Committee.

Experimental and Results

A preliminary study was made of the separation, by gas chromatography in packed columns, of eugenol from interfering compounds in cinnamon oils and it was found that only non-polar stationary phases gave a satisfactory resolution. Using OV-101 it was proposed to use vanillin as an internal standard. In the first collaborative exercise, members were asked

* For Part VIII of this series, see p. 448.

to determine the eugenol contents of two different oils using substantially the same method as given in the Appendix. The two oils were a genuine oil of cinnamon bark and the same oil to which had been added a known amount of pure eugenol. The results are given in Table I.

TABLE I

DETERMINATION OF EUGENOL IN TWO SAMPLES OF OIL OF CINNAMON

Laboratory	Eugenol in oil of cinnamon bark, % *m/m*	Eugenol in spiked oil of cinnamon bark, % *m/m*
A	3.50	13.0
B	3.37	12.9
C	3.54	13.2
D	3.32	12.6
E	4.10	13.0
F	3.47	12.8
Average	3.55	12.9
Standard deviation	0.282	0.204
Relative standard deviation (RSD), %	7.9	1.6

The spiked oil was prepared by the addition of 1 part by mass of eugenol to 10 parts by mass of the oil of cinnamon bark. Using the average figure for the eugenol content of the oil of cinnamon bark, the recovery of eugenol in the spiked sample was 96%. It was noted that the RSD for the oil of cinnamon bark was high, but this is usual for the determination of minor individual components in essential oils. In addition, several of the collaborating members reported some tailing of the peaks for both the eugenol and the vanillin internal standard. However, the results were considered satisfactory and a further collaborative trial was arranged using a wider range of commercial samples. The analytical conditions were the same as for the previous trial. The results are given in Table II.

Laboratory 3 reported difficulty in achieving adequate resolutions of the eugenol and vanillin internal standard due to tailing of the peaks and used a significantly higher operating temperature than that recommended in order to reduce the tailing. In general, the results from laboratory 3 are at variance with those from the other laboratories and Table III shows the corresponding average, etc., without the inclusion of those from laboratory 3.

Blend A was prepared by the addition of 1 part by mass of Leaf Oil Sri Lanka B to 9 parts by mass of Bark Oil English Distilled and blend B similarly from 1 part of Leaf Oil Sri Lanka B to 4 parts of Bark Oil English Distilled.

Using the average results in Table III for the eugenol contents of Bark Oil English Distilled and Leaf Oil Sri Lanka B, blend A should contain 10.0% (found 9.9%) and blend B 16.8% (found 16.6%).

Again, it was noted that the RSD increased as the level of eugenol being determined decreased.

Typical chromatograms for oils of cinnamon bark and cinnamon leaf are shown in Fig. 1.

TABLE II

DETERMINATION OF EUGENOL IN SIX SAMPLES OF OIL OF CINNAMON

	Eugenol, %					
	Bark Oil, English distilled	Leaf Oil Sri Lanka A	Leaf Oil Sri Lanka B	Blend A	Blend B	Bark Oil (so-called)
Laboratory 1	3.48	68.0	70.6	10.4	17.4	33.0
Laboratory 2	3.0	67.5	70.4	9.3	16.0	32.0
Laboratory 3	3.9	61.9	62.0	12.8	19.6	38.6
Laboratory 4	3.3	67.8	71.8	10.3	16.9	32.1
Laboratory 5	3.1	67.3	70.8	9.5	16.1	31.2
Average	3.36	66.5	69.1	10.5	17.2	33.4
Standard deviation	0.356	2.59	4.02	1.39	1.46	2.99
Relative standard deviation, %	10.6	3.9	5.8	13.3	8.5	8.9

TABLE III

AVERAGE RESULTS FROM TABLE II WITHOUT THE INCLUSION OF LABORATORY 3

	Eugenol, %					
	Bark Oil, English distilled	Leaf Oil, Sri Lanka A	Leaf Oil, Sri Lanka B	Blend A	Blend B	Bark Oil (so-called)
Average	3.22	67.7	70.9	9.86	16.6	32.1
Standard deviation ..	0.214	0.311	0.622	0.556	0.668	0.737
Relative standard deviation, %	6.61	0.46	0.88	5.61	4.0	2.3

Discussion

It was the opinion of the Committee that the results justified acceptance of the proposed procedure, but with a comment on the difficulty of obtaining sharp peaks when chromatographing phenolic compounds. This difficulty is increased when using non-polar stationary phases due to any residual activity of the support material used for the packing. It is essential that high-quality deactivated supports are used in order to minimise tailing and the choice of a phenolic compound, vanillin, as an internal standard was made in order to balance any errors due to tailing peak effects.

The noted increase of the RSD at the lower levels of eugenol determinations is probably influenced by the tailing effects of the phenols and it is particularly necessary in these analyses to match closely the peak heights of the eugenol and internal standard peaks. It is therefore desirable to have some previous knowledge of the character of the oil sample being examined or to run a semi-quantitative test chromatogram prior to the final analysis.

Because of the asymmetric nature of the peaks, it is recommended that quantitative results are calculated from peak areas, rather than peak heights, in order to preserve the accuracy of the determinations.

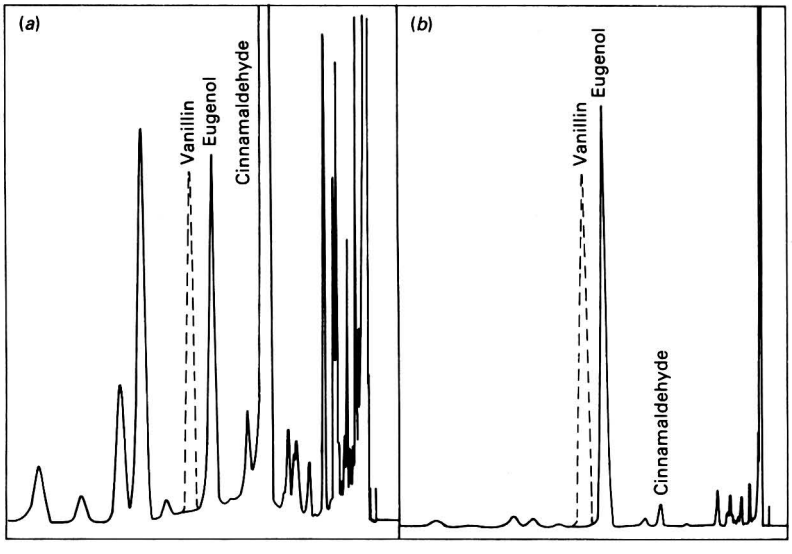

Fig. 1. Typical chromatograms of oils of cinnamon. (*a*) 10% oil of cinnamon bark in butan-1-ol, attenuation ×128; and (*b*) 10% oil of cinnamon leaf in butan-1-ol, attenuation ×2560.

The choice of solvent used is somewhat arbitrary, but any pure, medium-boiling solvent that does not interfere with the peaks being measured is satisfactory.

Conclusions

Gas - liquid chromatography can be used for the quantitative determination of eugenol in oils of cinnamon provided that suitably inert columns are chosen for use. The recommended method affords a means of determining the eugenol content in a wide variety of oils of cinnamon and the quantitative results may serve as indications of the sources of the oils.

APPENDIX

Recommended Method for the Determination of Eugenol in Oils of Cinnamon by Gas - Liquid Chromatography

Operating Conditions

It is essential that, throughout a determination, the operating conditions are maintained as constant as is practicable. It is also essential to use the detector - amplifier system within its linear range.[2]

Detector	Flame ionisation
Stationary phase	OV-101 or equivalent
Support	Chromosorb W HP, 80–100 mesh, or equivalent
Stationary phase loading	20% *m/m*
Column	Length 2 m, i.d. 4 mm, glass
Column temperature	Isothermal, 150 °C.
Injection	On-column or, if a flash heater is used, maximum temperature 200 °C
Gas flow-rates	To give satisfactory instrument performance
Chart speed	15 in h^{-1} (minimum)
Peak heights (internal standard and eugenol)	Within 40–75% of full-scale deflections
Internal standard	Vanillin, purity by gas - liquid chromatography not less than 99%
Eugenol	Purity by gas - liquid chromatography not less than 99%
Solvent	Butan-1-ol
Injection volume	Such that the internal standard and eugenol peaks fall within the linear range.

Determination of the Factor *f* for the Internal Standard (Eugenol = 1)

Make all weighings to an accuracy of 0.2 mg. Weigh about 0.45 g of eugenol and 0.70 g of vanillin and dilute the mixture with 100 ml of solvent. Inject 1.0 μl or such other volume as will ensure response within the linear range and calculate to three decimal places the factor, *f*, from the equation

$$f = \frac{am_e}{a_e m}$$

where a_e is the area of the eugenol peak, a is the area of the vanillin peak, m is the mass of the vanillin and m_e is the mass of the eugenol. Repeat twice on the same solution and use the average of the three values of f in the calculation of the eugenol content of the sample.

Determination of the Eugenol Content of the Sample

Weigh about 0.70 g vanillin and an adequate amount of the sample (0.5–10 g, according to whether more or less eugenol is present) that will give about equal heights for the two peaks and dilute with 100 ml solvent. Inject about 1.0 μl or such other volume as will

ensure a response within the linear range of the instrument and calculate the eugenol content of the sample to four significant figures from the equation

$$\text{Eugenol (\%)} = \frac{fa_e m \times 100}{am_s}$$

where f is the factor determined as described above, a_e the area of the eugenol peak, a the area of the vanillin peak, m the mass of the vanillin peak and m_s the mass of the sample.

Repeat twice on the same solution and report the average of the three results to three significant figures.

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SHORT PAPERS

Rapid Direct Complexometric Determination of Palladium(II) with EDTA*

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Keywords: *Palladium(II) determination*; *propionyl promazine phosphate indicator*; *EDTA titration*; *complexometry*

No direct complexometric method for the determination of palladium(II) has been reported. During the course of investigations on the spectrophotometric determination of palladium(II) with *N*-substituted phenothiazines,[1–3] it was observed that the intense colour of the complex of palladium and propionyl promazine phosphate (PPP) is discharged by the addition of EDTA, which indicated that PPP might be of use as a metal indicator in the complexometric determination of palladium. These studies were undertaken in order to ascertain the usefulness of PPP for this purpose.

The composition of the palladium - PPP complex has been established by the continuous variation and molar-ratio methods. The apparent stability constant of the complex has also been calculated. The proposed method has been tested for interferences due to several anions and cations.

Experimental

Reagents

De-ionised doubly distilled water was used throughout for the preparation of solutions and for dilutions. All reagents used were of analytical-reagent grade.

Palladium(II) solution. A stock solution of 0.05 M palladium(II) was prepared from palladium(II) chloride (Johnson Matthey), in 1 M hydrochloric acid and diluted to 500 ml to give a solution 0.1 M with respect to hydrochloric acid. It was standardised gravimetrically by the dimethylglyoximate method.[4]

EDTA solution. A stock 0.05 M solution of the disodium salt of EDTA was prepared and standardised against zinc chloride.[5]

Indicator solution. A solution of 0.2% propionyl promazine phosphate (Bayer, Leverkusen, W. Germany) was prepared and stored in a refrigerator.

Acetate buffer solutions. These were prepared from 0.2 M solutions of sodium acetate and acetic acid.

Apparatus

The pH measurements were made with an Elico, Model L1-10, direct reading pH meter with a glass - calomel electrode assembly. A Beckman DB spectrophotometer was employed for absorbance measurements.

Results and Discussion

PPP reacts with palladium(II) in an acetate buffer medium to form a red complex. The colour is discharged by the addition of EDTA solution leaving the yellow palladium - EDTA complex in solution.

* Presented at the 5th SAC International Conference on Analytical Chemistry, Lancaster, July 20–25th, 1980.

Effect of pH

Titrations of palladium(II) against EDTA solution were carried out at various pH values using the PPP indicator. The metal and indicator concentrations were kept constant and the pH was adjusted with acetate buffers of different pH. The results are shown in Table I.

It is evident that satisfactory results are obtained in the pH range 4.2–6.0. Below pH 4.2 and above pH 6.0, higher results are obtained and appear to be caused by the slow complexing behaviour of palladium with EDTA.

TABLE I

TITRATION OF PALLADIUM(II) AT DIFFERENT pH VALUES

Amount of palladium(II) taken, 10.64 mg (≡ 5.0 ml of 0.02 M EDTA); 1.0 ml of 0.2% indicator solution was used.

pH	3.0	3.4	3.8	4.2	4.6	5.0	5.4	5.8	6.0	6.2	6.4	6.6
Volume of 0.02 M EDTA required/ml	5.03	5.03	5.02	5.01	5.0	5.0	5.0	5.0	5.01	5.02	5.03	5.04

Concentration of Palladium

Titrations of different amounts of palladium (plus PPP solution) against EDTA were carried out at pH 5.0 (adjusted with the acetate buffer) where the colour intensity is appreciable and many bivalent metals do not interfere.

The titrations showed that when palladium is present in low concentrations (less than 0.5 mg per 100 ml) detection of the end-point becomes difficult as the intensity of the colour of the palladium - PPP complex is too low for visible detection. Up to about 40 mg of palladium in a total volume of about 100 ml can be accurately determined. With larger amounts of palladium (higher than 40 mg) erratic results are obtained as the yellow colour of the palladium - EDTA complex imparts a yellow colour to the solutions, in varying degrees.

Amount of PPP Indicator Solution

The titrimetric determination of palladium was carried out by adding different volumes of 0.2% PPP indicator solution, and it was found that 0.5–1.5 ml were sufficient at all pH values between 4.2 and 6.0. The colour change from red to yellow at the end-point is sharp and reversible.

Effect of Temperature

It was observed that the titrations could be performed within a wide range of temperatures (10–60 °C). At room temperature (27 °C) the titration can be carried out accurately by adding the EDTA dropwise near the end-point.

Composition and Stability Constant of Palladium - PPP Complex

MacNevin and Kriege[6] reported the existence of a 1:1 complex between palladium and EDTA at pH 3.7–8.9. The logarithm of the stability constant of PdY^{2-} is 18.5 ± 0.6.

Job's method of continuous variation[7,8] and a molar-ratio method[9] were employed to establish the composition of the palladium - PPP complex. Results indicated the formation of a 1:1 complex between palladium and PPP between pH 4.2 and 6.0. The apparent stability constant values of the complex were calculated by two different methods: the method of Foley and Anderson,[10] modified by Mukherji *et al.*,[11] and the molar-ratio method; the log K values at 27 °C and pH 5.0 are 5.55 ± 0.2 and 5.65 ± 0.2, respectively.

Recommended Procedure

Dilute a weakly acidic solution containing 0.5–40 mg of palladium(II) to 90 ml with water, add 10 ml of buffer solution of pH 5.0 and 1.0 ml of the 0.2% solution of the PPP indicator. Add, from a microburette, standard 0.02–0.05 M EDTA solution until the red colour completely disappears. The addition of EDTA must be dropwise near the end-point. Results for some titrations obtained using this procedure are given in Table II, which shows that the method can be used to determine amounts of palladium ranging from 0.5 to 40 mg, with an error not exceeding ±0.4 to 1.2% depending on the concentration of palladium.

The larger errors at low levels are thought to be due to the relatively low colour intensity of the palladium - PPP complex which, at high dilutions, makes detection of the end-point difficult.

TABLE II

TITRATION OF PALLADIUM(II) WITH EDTA

1.0 ml of 0.05 M EDTA ≡ 5.32 mg of palladium; pH 5.0.

Palladium(II) taken/mg	Palladium(II) found/mg	Error, %
0.106	0.108	+1.89
0.266	0.270	+1.50
0.532	0.538	+1.13
1.064	1.074	+0.94
4.256	4.280	+0.56
10.64	10.69	+0.47
15.96	16.04	+0.44
26.60	26.70	+0.38
37.24	37.10	−0.38
42.56	42.30	−0.61
53.20	52.40	−1.50

Interferences

Interferences caused by various cations and anions in the determination of palladium were investigated at pH 5.0. No interference is caused by chloride, nitrate, sulphate, oxalate, citrate and tartrate when present at a 50-fold excess. The anions iodide, thiocyanate and iodate interfere seriously. The interference from iodate is due to oxidation of the indicator with the production of a red colour, which masks the end-point. The tolerance limit of the anions acetate (in addition to the acetate in the buffer), phosphate, bromide and fluoride are given in Table III. A 20-fold excess of the cations magnesium(II), aluminium(III) and iron(II) and a ten-fold excess of calcium(II), strontium(II), barium(II) and manganese(II) do not interfere. Vanadium(V), cerium(IV), gold(III) and osmium(VIII) interfere seriously as they oxidise PPP to a red cationic radical. The tolerance limit of the cations cobalt(II) nickel(II), copper(II), ruthenium(III), rhodium(III), osmium(VI), iridium(III) and platinum(IV) are listed in Table III.

The proposed method was applied to the determination of palladium in synthetic mixtures corresponding to the palladium alloys that are used in jewellery containing 4% ruthenium and 1% rhodium. The results of these investigations are presented in Table IV.

TABLE III

TOLERANCE LIMIT OF DIVERSE IONS IN THE DETERMINATION OF PALLADIUM(II)

Amount of palladium(II) taken, 10.64 mg; pH 5.0.

Diverse ion added	Tolerance limit/mg	Diverse ion added	Tolerance limit/mg
Acetate*	150	Copper(II)	0.6
Phosphate	50	Ruthenium(III)	0.6
Bromide	20	Rhodium(III)	1.0 (2.3†)
Fluoride	10	Osmium(VI)	0.5
Cobalt	1.0	Iridium(III)	1.5 (2.5†)
Nickel(II)	0.8	Platinum(IV)	0.8 (2.0†)

* In addition to the 120 mg of acetate present in the buffer; other buffers examined did not prove satisfactory.

† In the presence of masking agent (5 ml of a 5% solution of potassium sodium tartrate).

TABLE IV

DETERMINATION OF PALLADIUM(II) IN SYNTHETIC MIXTURES CORRESPONDING TO PALLADIUM ALLOYS USED IN JEWELLERY

Palladium(II) taken/mg	Ruthenium(III) added/mg	Rhodium(III) added/mg	Palladium(II) found*/mg
4.256	0.170	0.043	4.285
10.640	0.430	0.106	10.680
15.960	0.640	0.160	16.050

* The amount of palladium(II) found is based on ten determinations.

Conclusions

The described method offers a simple and rapid procedure for the determination of palladium(II) using EDTA at room temperature. It appears to offer many advantages over the reported indirect methods.

The author is grateful to Bayer, Leverkusen, W. Germany, for the supply of pure PPP and the University Grants Commission, New Delhi, for financial assistance.

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Determination of Arsenic by Emission Spectrometry Using an Inductively Coupled Plasma Source and the Syringe Hydride Technique

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Many publications have described the use of the hydride generation method for elements such as arsenic using flames or heated silica tubes as atomisation cells. The signals measured are the absorptions at the 193.7-nm arsenic line, or occasionally emissions, using the inductively coupled plasma (ICP) as source. In the last instance, arsenic lines other than the 193.7-nm resonance line may be used, which may overcome some of the non-specific absorption effects associated with measurements in the far ultraviolet. Typically,[1,2] with the ICP, continuous flow systems are used, in which acidified sample solutions are pumped together with alkaline sodium tetrahydroborate(III) solution into a reaction chamber, and the evolved hydrides are swept into the plasma with an inert gas stream.

Smith and co-workers[3,4] have described an extremely simple hydride generation method for use in atomic absorption, which uses a disposable syringe as the hydride generation vessel. The advantages claimed for this method include rapidity (25–30 s per measurement) and simplicity, because no hydride generation apparatus or extra flow controls are required. Surprisingly, this simple hydride method does not seem to have been used in emission with an ICP source.

As part of an analytical programme to determine arsenic and other metals in a large number of vegetation samples, the author has evaluated the syringe technique, using an ICP source and the arsenic 228.8-nm emission line. Data for accuracy (from standard reference materials) and precision are included. No attempt has been made to investigate potential interference effects at this stage, although this would be necessary if the method were to be used to determine arsenic in a wider range of materials.

Experimental

Apparatus

A Plasmatherm 2500A ICP source was used, operated at 1.5 kW, with a coolant flow-rate of 13 l min^{-1}, and a sample injector flow-rate of 1 l min^{-1}. The observation height was 16 mm above the load coil.

Emission signals were measured with a Spex 1704 1-m monochromator, at a band pass of 0.3 nm. The arsenic 228.8-nm line was used throughout. Analogue output from the Spex DPC-2 photon counting unit was displayed on a chart recorder, using a 10-s photometer integration time. This displayed emission signals as one or more steps, which were summed for the total intensity.

Sample Dissolution

Samples (1 g) of NBS orchard and tomato leaves, Bowen's kale and vegetation samples, previously oven dried at 80 °C, were weighed into cleaned 50-ml calibrated flasks, and 10 ml of concentrated nitric acid and 1 ml of concentrated perchloric acid were added (both were Aristar grade reagents). After dispersion of the solid material, the flasks were heated to 50 °C over a period of 20 min until frothing had ceased, and then heated over a period of 2 h until refluxing of perchloric acid occurred. At this point, a small amount of colourless slurry remained. Dilution to 50 ml with 25% hydrochloric acid produced a clear solution in each instance, apart from NBS Orchard Leaves, which gave a slightly turbid solution.

Standards and Reagents

Known amounts of arsenic (as a 1 000 μg ml^{-1} solution) in the range 1–10 μg were added to 1-g portions of a dried cabbage sample (456-I) which, from previous measurements using instrumental neutron activation analysis (INAA) and gamma activation analysis (GAA), were known to contain insignificant amounts of arsenic. This material, with the arsenic additions, was then carried through the wet-ashing step.

A filtered 2% sodium tetrahydroborate(III) solution containing 1% of sodium hydroxide was used for the hydride generation. This was prepared daily.

Procedure

After peaking the monochromator on the chosen line, using a 100 μg ml^{-1} solution of arsenic and the conventional pneumatic nebuliser, the chart drive was activated, and samples processed at discrete time intervals.

A 4-ml sample of the acid solution was drawn into an Everett 20-ml disposable syringe, with a No. 1 needle. This type of syringe was found particularly convenient as it has a silicone sealing ring and a ridged plunger, which acts as a reproducible stop. The needle was then dipped into the alkaline sodium tetrahydroborate(III) solution, and the plunger drawn back rapidly to the stop. The syringe was then inverted, the needle tip held with a paper tissue, and gentle shaking carried out for 20 s. The needle was immediately inserted into the open end of the silicone nebuliser tube of the ICP, and the plunger depressed steadily so as to inject the 20 ml of hydrogen and arsine into the nebuliser chamber in about 5–10 s. Provided signal integration was used, the timing of this step was not critical.

The arsenic signal was then seen as successive steps on the chart recorder, which were summed for the total intensity. One sample per minute was found to be a practical sample throughout.

Results

There was apparently no difference between arsenic standards added to cabbage and carried through the wet-oxidation step and standards prepared by serial dilution of a 1000 μg ml^{-1} solution of arsenic with 25% hydrochloric acid in water. A calibration graph prepared by the addition of known amounts of arsenic to 456-I cabbage sample, and using 4 ml of digest solution per measurement, was linear up to 0.2 μg of arsenic per millilitre of digest.

Accuracy

The arsenic levels found for each material are given in Table I together with certificate or other data for these materials. The analytical data given in each instance represent the average of duplicate measurements of individual digests for the sample, measured on different occasions. There appears to be no evident bias of the results from the literature values. The results for kale are at, or only just above, the detection limit, and must therefore be regarded with caution.

Smith *et al.*[3] found a low recovery for arsenic in orchard leaves using the syringe method with atomic-absorption spectrometry, when direct signal measurement without integration was used.

TABLE I

Material	Arsenic content/μg g^{-1}	Certificate, or other value/ μg g^{-1}
Bowen's kale*	0.32, 0.10, 0.15	0.13 $\pm$ 0.02
NBS tomato leaves*	0.29, 0.25	0.27 $\pm$ 0.05
NBS orchard leaves*	10.2, 9.7, 9.5	10 $\pm$ 2
Cabbage 456–I†	<0.12, <0.10, <0.09	<1 INAA, <1 GAA
ABC–O*‡	3.2, 3.3, 3.3	<3 INAA, 5 $\pm$ 2 GAA

* Measured from a calibration graph (linear up to 10 μg g^{-1}) prepared by additions of arsenic to cabbage (456–I) and carried through the wet-oxidation step.
† Measured by a comparison with an aqueous standard calibration graph.
‡ Internal quality control material.

Blank Levels

Typical blank levels (related to the original vegetable matter dry mass) were in the range 0.12–0.17 μg g^{-1}. No attempt was made to purify the reagents used. The base line (between measurements) was extremely stable, with signal variations approximately one to two orders of magnitude lower than the blank signals.

Detection Limit

This was derived from the variation in the blank signal intensity (2δ), and was typically 0.12 μg g^{-1}. Clearly, any reduction in arsenic contamination of the reagents would be expected to lower this value, although it was felt to be adequate for the needs of the study concerned.

Precision

Using an Everett syringe, a precision of 3–5% was obtainable for orchard leaves at the 10 μg g^{-1} level. This compares favourably with the figure reported by Smith *et al.*[3] of 2–5% for the atomic-absorption technique.

Use of an alternative type of syringe that lacked the ribbed plunger worsened this figure, although no doubt a screw could be incorporated in the syringe to overcome this effect.

Discussion and Conclusion

The syringe hydride method seems to be potentially very attractive for arsenic determination with the ICP. Unlike atomic-absorption spectrometry, no hollow cathode or electrodeless discharge lamp is required, and the use of a higher wavelength arsenic line is advantageous in lessening the effects caused by light absorption. The method is also extremely easy to use, once the initial skill is acquired, which is important if large numbers of samples are to be analysed.

The accuracy of the method seems satisfactory for the materials tested, although a fuller evaluation would be needed to establish this point. The applicability of the method for other hydride-forming elements is of interest, particularly when used with a direct reader system for simultaneous multi-element analysis, and is currently under investigation.

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Simultaneous Determination of Trace Amounts of Arsenic, Antimony and Bismuth in Herbage by Hydride Generation and Inductively Coupled Plasma Atomic-emission Spectrometry

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Keywords: Arsenic, antimony and bismuth determination; herbage; hydride generation; inductively coupled plasma; atomic-emission spectrometry

In previous papers a system for the simultaneous determination of arsenic, antimony, bismuth, selenium and tellurium in aqueous solution was described, and the characteristics of this method were reported.[1,2] The elements were reduced to hydrides by sodium tetrahydroborate(III) solution in a continuous-flow system, and the hydrides injected into an inductively coupled plasma (ICP) for determination by atomic-emission spectrometry. The system was used subsequently for the determination of arsenic, antimony and bismuth in soils[3] and of selenium in soils.[4] This paper describes the use of the system for the simultaneous determination of arsenic, antimony and bismuth in herbage after the samples had been dry ashed in the presence of magnesium nitrate.

Magnesium nitrate has been used successfully as an ashing aid in the determination of arsenic[5] and antimony[6] in organic matter. Its role is the prevention of loss of analytes by volatilisation or incorporation into the walls of crucibles, and leaving the analyte residue in a readily soluble form. The method combines well with hydride generation,[7] as the ashed residue dissolves quickly in the reaction medium, dilute hydrochloric acid (1 + 1), and the reduction of the analytes to hydrides is unaffected by high concentrations of the magnesium ion.[2]

Experimental

Equipment

The equipment and experimental conditions differed from those previously reported[1] in the use of a different plasma system and an improved hydride generator.

Plasma system

The spectrometer was an Applied Research Laboratories ARL 34000C Quantovac 1-m vacuum spectrometer. The wavelengths used were: arsenic, 193.7 nm; antimony, 206.8 nm; and bismuth, 223.0 nm, all in the second order. The plasma torch was of the Fassel type, run at 1.25-kW incident power, with a viewing height 14 mm above the load coil. Argon flow-rates were: coolant gas, 12 l min^{-1}; plasma gas, 0.8 l $min;^{-1}$ and injector gas, 1.3 l min^{-1}. Under these conditions a stable plasma was obtained with a reflected power of about 15 W.

Hydride generator

The hydride generator (Fig. 1) consisted of a three-channel peristaltic pump (Watson Marlow, MHRE 200) connected via a four-port valve to the phase separator. The valve enabled the test solution to be alternated very rapidly with the blank reaction medium, effectively with no interruption of the production of hydrogen and with complete exclusion of air from the plasma. The pump tubing was of fine-bore silicone rubber (0.8 mm i.d. for the test solution and blank and 0.5 mm for the reagent) to ensure a minimum system volume and time lag. The uptake rates were 9.2 ml min^{-1} for the test solution and blank and 4.5 ml min^{-1} for the reagent. A pre-integration time of 20 s was allowed for clean-out and signal stabilisation (although this length of time was not strictly necessary for the low analyte levels found in herbage) and this was followed by two 5-s integrations, which were averaged for output. The phase separator was as previously reported and was connected with a minimum length of tubing to the plasma torch.

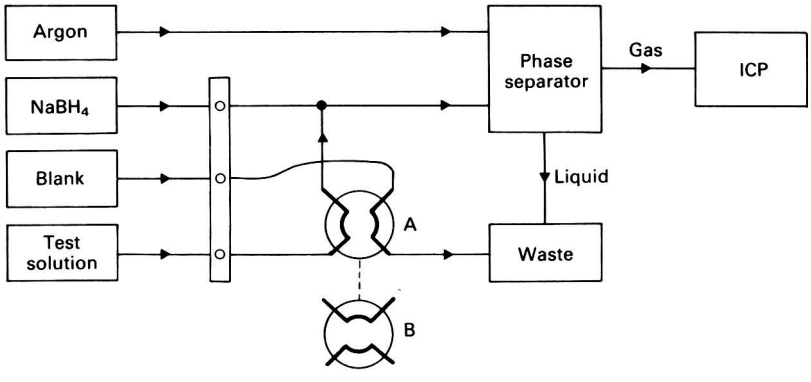

Fig. 1. Schematic diagram of the continuous-flow hydride generation system, showing the two positions of the four-port valve: (A) for analysis of the test solution and (B) for blank integrations and for changing the test solution.

Materials

Magnesium nitrate and hydrochloric acid were of AnalaR grade. De-ionised water was used throughout. Standard solutions were made by dilution of 1000 μg ml^{-1} BDH Chemicals standard solutions of arsenic, antimony and bismuth. Sodium tetrahydroborate(III) was obtained from Aldrich Chemical Co. and used as a 1% m/V solution in 0.1 M sodium hydroxide solution.

Procedure

Sample decomposition

Dry the herbage at 50 °C for 48 h and mill it to pass through a 0.5-mm screen. Weigh 1 g of sample into a 50-ml Vitreosil crucible, add magnesium nitrate solution (2 ml of saturated solution) and mix in with a glass rod. Place a batch of crucibles in a cold furnace and gradually raise the temperature (100 °C for 4 h, 200 °C for 0.5 h and 450 °C for 5–15 h). Cautiously remove the crucibles from the cooled furnace, moisten the ash with potassium iodide solution (5.0 ml, 0.2% *m*/*V*) and dissolve it in hydrochloric acid (concentrated, 5.00 ml). Transfer the solutions into test-tubes and allow any solid residue to settle.

The ashing seems to go to completion under the proposed conditions, as a pure white ash is usually obtained. It is necessary to cool the furnace before opening as otherwise the ash, which is very light, tends to be blown out of the crucibles by convection currents. Alternatively, the ashing can be undertaken in tall-form 100-ml borosilicate-glass beakers, which prevent the ash being disturbed, and are an acceptable alternative to the crucibles.

Determination of metals

Prepare a calibration blank solution of hydrochloric acid (diluted 1 + 1) and a standard solution containing analytes at the following concentrations: arsenic and antimony, 0.5 μg ml^{-1}; and bismuth, 0.2 μg ml^{-1}. Run the blank solution continuously while the plasma system is settling down (about 15 min after light-up or until a reproducible blank integration can be obtained). To run a test solution (standard or sample solution) put the uptake tube into the solution and when it can be seen running to waste (about 3 s after commencing) rapidly switch the valve and simultaneously start the integration cycle. To change test solutions, switch the valve back to "run blank," and transfer the uptake tube into a new solution. Repeat the calibration blank and standard solution measurements after every 10 sample solutions. The calibrations are linear up to twice the levels in the standard, and these levels are rarely exceeded in herbage samples. Reagent - procedure blanks should be included in every batch by applying the whole procedure to shredded ashless filter-paper or cellulose floc.

Results and Discussion

Performance of the Instrumental System

The modified hydride generator contributed markedly to the stability of the system by virtue of its uninterrupted production of hydrogen, which allowed the plasma generator to be tuned accurately to the inductive load in the plasma. In the previous system, the hydrogen flow-rate was interrupted during sample changeover, which resulted in big fluctuations in impedance matching and a constant risk of extinguishing the plasma.

The injector gas flow-rate was about double the optimum flow-rate, this being below the optimum rate for signal to noise ratio and sensitivity. However, this had two substantial advantages. Firstly, the greater dilution of the hydrogen facilitated tuning the generator to the plasma impedance. Secondly, the residence time of the hydrides in the phase separator and connecting tubing was reduced in proportion, which allowed less time for the unstable bismuth hydride to decompose. This decomposition is important if high levels of bismuth are encountered, because elemental bismuth plates out on to the connecting tubing and the injector tube of the torch. At moderate concentrations (*e.g.*, around 1 μg ml^{-1}) this causes the early onset of non-linearity of calibration, but at higher concentrations a long term memory effect due to contamination of the system can result. The sensitivity was reduced by about 30% by the use of a higher flow-rate, but the detection limits were acceptably low, regularly being below 200 pg ml^{-1} for all three analytes when determined simultaneously.

Accuracy and Precision

Validation of a new method for ultra-trace analysis is often difficult. Ideally a suite of samples should be analysed both by the proposed method and an established method and the results compared. In principle, neutron activation analysis could serve as a reference method for all three analytes, but resources were not available for its use in this work. In addition, rather variable results for arsenic were obtained by the various workers employing neutron activation methods on the Bowen's kale standard, so the method is suspect for herbage, possibly because of problems associated with the volatility of some arsenic compounds.

In this work we report the results of spiking tests and the analysis of two standard reference materials. In the spiking tests, 1-ml amounts of chloride free aqueous solution containing the three analytes were added to 1-g samples of dried pasture herbage (mostly grass species) weighed into Vitreosil crucibles. The mixtures were dried at 110 °C and then treated as normal samples by the procedure described above. The results are given in Table I, which shows the excess of analyte found (*i.e.*, above the level found in the untreated sample) compared with concentration added. The mean recovery for all three analytes was 98.7% with a standard error of 1.2%, but there seems to be a tendency for lower recoveries for bismuth (mean recovery 95.6%). These results seemed very promising despite the obvious limitations of the spiking method. Spiking experiments were also carried out on ashless cellulose floc with similar results.

Table I

Recovery of arsenic, antimony and bismuth from aqueous spikes added to pasture herbage

The spikes were added before the sample was ashed as by the proposed method.

Concentration added/ ng g^{-1} of sample	Excess of analyte recovered/ ng g^{-1} of sample		
	Arsenic	Antimony	Bismuth
20	20, 19	20, 19	16, 19
40	44, 42	41, 40	41, 39
100	95, 99	97, 101	107, 99
200	204, 202	194, 198	190, 184
400	372, 392	384, 476	392, 356

In addition, spiking experiments were carried out on both materials with selenium and tellurium solutions, which would give an indication of whether these elements could also be determined following a simple dry ashing. (The addition of potassium iodide was omitted in this instance because it prevents the formation of the hydrides.) Recoveries of both elements were low, selenium averaging about 3% recovery and tellurium about 35% recovery, and it was concluded that neither element could be determined simultaneously with the arsenic, antimony and bismuth.

The proposed method was also applied to two standard reference materials, the Bowen's kale standard and the US National Bureau of Standards' orchard leaves. The results are shown in Table II, where agreement can be seen to be good except for arsenic on the Bowen's kale standard, for which there is some doubt about the preferred value.

Blank determinations with the reagents and ashless cellulose floc were low, equivalent to less than 0.01 $\mu g\ g^{-1}$ of any of the analytes. The effect of trace amounts of analytes in the hydrochloric acid or the sodium tetrahydroborate(III) is negligible, unless they are at very high levels, because of the use of the continuous reduction system. The precisions obtained seem to be satisfactory for the levels encountered.

Table II

Results obtained by the proposed method on two standard reference materials

All results are in micrograms per gram of dried material. The ranges shown are standard deviations for complete replicate analyses.

Sample	No. of replicates	Arsenic	Antimony	Bismuth
Bowen's kale—				
Proposed method	6	0.268±0.006	0.075±0.007	<0.002
Recommended value ..	—	0.14*	0.070	—
NBS orchard leaves—				
Proposed method	3	11.98±0.08	2.77±0.02	0.004±0.001
Certified value	—	10±2	2.9±0.3	—

* Suspect result, methods disagree.

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Adsorption of Trace Metals During Filtration of Potable Water Samples with Particular Reference to the Determination of Filtrable Lead Concentration

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The separation of the dissolved and undissolved fractions of trace metals by filtration is of importance in the understanding of the distribution of such metals in water systems. Numerous authors (reference 1 and the literature cited therein) have reported adsorptive losses of trace metals on filtration. One of the principal problems in the assessment of these losses is the choice of sample type for testing. Synthetic solutions, whilst being convenient to prepare, may suffer from the lack of competition for adsorption sites from other ions and may not have the same metal speciation as natural samples. Spiked real samples (pre-filtered to remove solid material) also suffer from potentially atypical metal speciation which may affect adsorption behaviour. Unspiked real waters require a pre-filtration stage, which may disturb solution equilibrium and make the subsequent test of adsorption unrepresentative of a routine filtration.

This paper describes the assessment of adsorptive losses of lead during filtration using two commercially available filtration systems. Synthetic solutions and spiked samples were used as these allowed comparison of adsorption at one lead level.

Experimental

Filtration Apparatus

Borosilicate glass. Millipore Catalogue No. XX1054700.
Polycarbonate. Sartorius Catalogue No. SM16510.
Filter-membranes. Cellulose, 47 mm diameter, 0.45 μm pore size (Millipore HAWP0047).

Reagents and Samples

Reagents of analytical-reagent grade, or superior, were used. De-ionised water was from a mixed-bed de-ioniser (Elga C224).

Synthetic test solutions were prepared to contain 100 mg l^{-1} of sodium nitrate supporting electrolyte and to have a lead (present as the nitrate) concentration in the range 19–25 μg l^{-1} as lead. The pH was adjusted to the required value with 0.1 M sodium hydroxide solution

before spiking. Complete equilibration with atmospheric carbon dioxide was not attempted, because of the likely loss of lead to the container walls during a lengthy equilibration period. The solution was stirred for 10–15 min prior to pH determination and filtration.

Potable water samples at their natural pH were filtered through a 0.3-μm filter-membrane, spiked with a lead nitrate solution (20 mg l^{-1} of lead) to give a lead concentration in the range 19–25 μg l^{-1}, and then stirred for 10–15 min. After pH determination, the filtration test was performed. The potable waters examined were as follows.

Sample 1. pH, 7.7–7.8; total hardness, 285 mg l^{-1} calcium carbonate; alkalinity, 227 mg l^{-1} calcium carbonate; total organic carbon, 1.5 mg l^{-1}.

Sample 2. pH, 7.5–7.6; total hardness, 344 mg l^{-1} calcium carbonate; alkalinity, 210 mg l^{-1} calcium carbonate; total organic carbon, 1.6 mg l^{-1}.

Sample 3. pH, 7.0–7.1; total hardness, 53 mg l^{-1} calcium carbonate; alkalinity, 32 mg l^{-1} calcium carbonate; total organic carbon, 2.4 mg l^{-1}.

Sample 4. pH, 6.3–6.4; total hardness, 28 mg l^{-1} calcium carbonate; alkalinity, 22 mg l^{-1} calcium carbonate; total organic carbon, 0.5 mg l^{-1}.

Theoretical calculations suggest that under the conditions of these tests precipitation of lead solids should not occur.

Filtration Procedure and Determination of Adsorptive loss

The apparatus was soaked in 10% *V*/*V* nitric acid overnight, washed three times with de-ionised water, once with 0.1 M nitric acid and a further three times with de-ionised water. Between filtrations the last two stages (0.1 M acid then de-ionised water) were applied, except when the effects of rinsing with the sample were being examined. Filter-membranes were used without pre-treatment. Contamination effects (tested by filtration of a 200-ml aliquot of 0.1 M nitric acid over 13 min) amounted to 0.1 ± 0.14 μg l^{-1} of lead for polycarbonate apparatus with a filter-membrane and 0.1 ± 0.1 μg l^{-1} of lead for the glass apparatus without a filter-membrane (95% confidence limits are shown for an increase in the filtrate lead concentration).

The filtration system was assembled and 1.3 ± 0.05 ml of concentrated nitric acid (sp. gr. at 20 °C = 1.42) was pipetted into the receiving vessel (in order to acidify the filtrate to prevent a loss of lead to the vessel walls). Next, 200 ± 10 ml of test solution or spiked sample were placed in the reservoir of the system. A second aliquot of 100 ± 5 ml was immediately transferred into a 100-ml calibrated flask containing 0.65 ± 0.05 ml of nitric acid.

The suction applied to the receiving vessel was controlled to make the filtration time 13 ± 2 min. When no filter-membrane was used, the filtration time was kept at the same value by regulation of the rate of air release from the receiving vessel.

Lead loss was determined by subtracting the lead concentration of the filtrate from that of the unfiltered, acidified 100-ml aliquot.

Determination of Lead

The lead concentrations of samples were determined by atomic-absorption spectrophotometry using electrothermal atomisation and the 283.3-nm resonance line. To avoid possible matrix interferences, standards for calibration were prepared in the same matrix as the test solution or, for spiked samples, in the treated water under examination.

Results and Discussion

Fig. 1 shows the adsorptive lead losses observed when synthetic solutions, at various pH values, were passed through the two types of apparatus *without* a filter-membrane being fitted. Losses to the polycarbonate system were always below 10%, and in many tests were not statistically significant. By contrast, very large losses (greater than 80% at high pH) occurred with the glass apparatus, which consequently was not used in further tests using synthetic solutions. The fact that large losses were observed with the glass apparatus, even with no filter-membrane fitted, is consistent with loss by adsorption rather than by retention of particulate lead.

Fig. 2 shows the lead loss, at various pH values, encountered when synthetic solutions were filtered using the polycarbonate apparatus fitted with a filter-membrane.

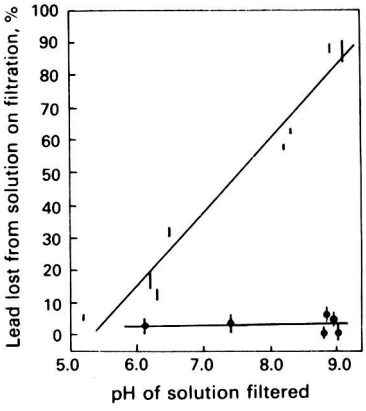

Fig. 1. Adsorptive losses of lead on filtration apparatus (without filters) *versus* pH of synthetic solution. Error bars alone = glass apparatus; error bars with ● = polycarbonate apparatus (error bars are 95% confidence limits, based on analytical error only).

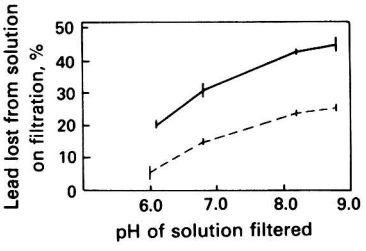

Fig. 2. Adsorptive losses of lead on polycarbonate filtration apparatus with cellulose acetate membrane filters *versus* pH of synthetic solution. Solid line, loss without "rinsing"; and broken line, loss with "rinsing" (see text for details). (Error bars are 95% confidence limits, based on analytical error only).

The effects of rinsing the polycarbonate apparatus and filter-membrane with the test solution are also shown in Fig. 2. In order to obtain the results, a second 200-ml aliquot was filtered in the same manner as the first, but the apparatus and filter-membrane were not washed in any way between the filtrations. This rinsing with the test solution secured an improved recovery of lead.

When spiked potable water samples were filtered, using both types of apparatus (with filter-membranes fitted), the observed lead losses were smaller than expected on the basis of results for synthetic solutions (Table I). Hence, these waters are partially protected from lead loss compared with the synthetic solution. The results in Table I suggest that this protection is greatest for hard waters. This is substantiated by the results in Fig. 3, which show how the presence of calcium in the synthetic solution at pH 8.8–8.9 caused a reduction in adsorptive lead loss.

It has been suggested[2] that preparatory rinsing of filtration systems with solutions containing calcium and magnesium may control lead adsorption. However, we observed that rinsing the polycarbonate apparatus and filter-membrane with a hard water (sample 1) prior to filtration of a calcium-free synthetic solution at pH 8.8. produced only a small reduction of the lead loss (42 ± 1% loss as against 48 ± 1% loss without the hard-water rinse).

Conclusions

1. Substantial adsorptive losses of dissolved lead may occur during filtration.
2. The polycarbonate apparatus gave smaller losses than the borosilicate-glass apparatus. This may be as a consequence of the high surface area of the glass equipment.
3. The presence of calcium in the filtered solution causes a reduction in adsorptive lead loss from synthetic solutions, possibly by competitive adsorption.

TABLE I

PERCENTAGE LOSS OF LEAD FROM SPIKED POTABLE WATER SAMPLES ON FILTRATION

Results have 95% confidence limits based on 6 replicate filtrations, and are the percentage loss on filtration.

Sample No.	1	2	3	4
Polycarbonate apparatus	4.6 ± 1.0	4.9 ± 0.7	11.6 ± 2.1	15.5 ± 3.3
Glass apparatus	16.7 ± 2.1	15.3 ± 0.7	25.9 ± 2.8	31.5 ± 2.0

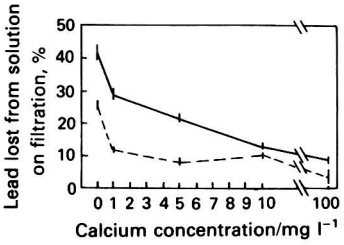

Fig. 3. Adsorptive losses of lead on filtration *versus* concentration of calcium in synthetic solution. Polycarbonate apparatus with cellulose acetate membrane filters. Solid line, loss without "rinsing"; broken line, loss with "rinsing" (see text for details). (Error bars are 95% confidence limits, based on analytical error only).

4. Pre-treatment of equipment with a solution containing calcium may reduce lead loss. It may not be particularly effective, however, and could cause desorption of lead from particulate matter in the sample. It is therefore not recommended as a routine procedure.
5. Rinsing the filtration system with the sample (*i.e.*, by pre-filtration) may produce a worthwhile reduction in adsorptive loss and is preferred as a pre-treatment.
6. It is essential that before routine application of filtration for trace metal determinations appropriate tests are conducted in order to measure the adsorptive loss likely to occur with the sample types encountered.

The advice and encouragement of Mr. A. L. Wilson, formerly Head of Analytical Division, is gratefully acknowledged. Earlier studies by Mrs. B. Orpwood and Mr. C. A. Edwards, both formerly members of Analytical Division, were helpful in guiding our work.
This paper is published by kind permission of the Director of the Medmenham Laboratory of the Water Research Centre, Dr. S. C. Warren.

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Differential-pulse Voltammetry of Sulphur Dioxide at the Parts per 10^9 Level in Air

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Keywords: *Sulphur dioxide determination*; *differential-pulse voltammetry*; *air pollution*

Electrochemical methods are used extensively in analyses for sulphur dioxide in the atmosphere. Coulometric and conductometric techniques in particular are characterised by high sensitivity, but they lack selectivity. In contrast, the possible interferences in polarography are limited to

species that react with the sulphur dioxide in the collecting medium. Notwithstanding its relatively low sensitivity, polarography has been recommended by the American Conference of Governmental Industrial Hygienists. The recommended method, based on the work of Kolthoff and Miller,[1] measures the sulphur dioxide at the dropping-mercury electrode (D.M.E.) at pH 3.9 and the sensitivity is 40 p.p.b. (parts per 10^9) with an air sample of 8401.[2]

A more sensitive method, based on the trapping of sulphur dioxide in dimethyl sulphoxide (DMSO) followed by the pulse polarographic determination of sulphur dioxide in this solvent, has been described by Garber and Wilson.[3] They quoted a sensitivity of 40 p.p.b. of sulphur dioxide and reported interferences from nitrogen oxides, water and impurities in the DMSO.

The method reported here permits the direct measurement of sulphur dioxide in aqueous solution and combines the high selectivity of differential-pulse polarography with excellent sensitivity, as it allows sulphur dioxide to be detected in air at background concentrations (0.1–1 p.p.b.) with sampling times of the order of a few minutes.

Experimental

Apparatus

Gas samples containing sulphur dioxide at concentrations of 7 p.p.b. and above were obtained by passing nitrogen - oxygen mixtures or purified air over a calibrated sulphur dioxide permeation tube (Philips P.W. 9740, 0.176 μg min^{-1} of sulphur dioxide at 25 °C).

To obtain calibration gases with sulphur dioxide contents in the parts per billion range, 7 p.p.b. gas samples were further diluted with known flow-rates of clean gas. By varying the flow-rates of the two gas streams it was possible to obtain sulphur dioxide concentrations as low as 0.1 p.p.b.

To collect sulphur dioxide from the gas mixtures a sampling apparatus similar to that described by Jaeschke[4] was employed. All tubing in contact with sulphur dioxide, the filter holder and the collecting cell were made of PTFE to minimise losses due to adsorption phenomena. A rotating vacuum pump was used to pass the gas through the filter.

The flow-rates were measured both with a flow meter and with a wet gas meter (Matheson Scientific, Cat. No. 27180–10 and 27215–10, respectively). NO_x was introduced in the carrier gas according to Hashimoto and Tanaka.[5]

Voltammetric analyses were carried out with an Amel 472 multipolarograph. The polarographic cell, having a capacity of 4 ml, was equipped with a saturated calomel electrode (S.C.E.), a platinum counter electrode and a synchronised D.M.E. The D.M.E. had a drop time of about 60 s and a mercury flow-rate of 8×10^{-2} mg s^{-1}. A delay time of 8 s was used.

Reagents

All solutions were prepared from analytical-reagent grade chemicals dissolved in triply distilled water.

High-purity nitrogen - oxygen mixtures. Supplied by SIO (Milan).

Sodium sulphite, anhydrous. Analytical-reagent grade (Merck).

Sodium sulphite solutions. A 10^{-4} M solution was prepared by dilution with deoxygenated water of 5×10^{-2} M sodium sulphite solution standardised by iodimetric titration. The solutions were kept under nitrogen and utilised within 30 min to obtain the calibration graph.

Acids. Hydrochloric and sulphuric acids were purified by addition of 10^{-5} mol l^{-1} of sodium sulphite, then the reagents were purged of sulphur dioxide with a stream of nitrogen before use. This procedure was used in measurements of sulphur dioxide concentration lower than 10^{-6} M in order to prevent losses due to reactions with trace impurities.

Procedure

Collection of sulphur dioxide from the carrier gas

Samples of sulphur dioxide were taken by passing the nitrogen - oxygen - sulphur dioxide mixture through a filter (Delbag Microsorban 98) impregnated with about 0.5 ml of 10^{-2} M sodium hydroxide solution and placed on a PTFE net in the filter holder. After sampling, the filter was rinsed in the filter holder with 10^{-2} M sodium hydroxide solution to give a final volume of 3 ml.

Analysis of sulphur dioxide solution

A 0.5-ml volume of 5 M hydrochloric or sulphuric acid, kept under nitrogen, was added to 2.5 ml of the sulphite solution previously deoxygenated for 5 min with nitrogen in the polarographic microcell. Then the D.M.E. was inserted and the voltammogram was recorded between −100 and −460 mV *versus*. S.C.E. within one drop-life.

Results and Discussion

Typical differential-pulse voltammograms that were obtained for calibration in the range of 2×10^{-8}–10^{-4} M from dilution of 10^{-4} M sodium sulphite solution are reported in Fig. 1. A logarithmic calibration graph of current *versus* sulphur dioxide concentration using hydrochloric or sulphuric acid concentrations in the range 10^{-2}–2 M as the supporting electrolyte were linear in the above range. On increasing the concentration of the acid from 10^{-2} to 2 M the sensitivity increased slightly and the maximum shifted from −420 to −352 mV *versus* S.C.E. (pulse height 50 mV). A decrease in the sensitivity at pH 2 was observed.

Bubbling nitrogen for 2 min into the polarographic cell after the voltammetric analysis led to the complete removal of sulphur dioxide (see lower curves in Fig. 1). This may be used as a further check of the presence of sulphur dioxide in the solution being analysed.

The relative standard deviation was 4% for 2×10^{-7} M sulphur dioxide solutions and the sensitivity with a signal to noise ratio of 2:1 was about 1×10^{-8} M.

The solutions to be analysed must be deoxygenated with nitrogen because oxygen interferes through its electrode reduction products. Sulphates, sulphides and nitrates at concentrations as high as 10^{-5} M do not interfere in the determination of sulphur dioxide at concentrations as low as 10^{-7} M.

Nitrites interfere by decreasing the sulphur dioxide concentration by a factor of about two on a molar basis. This effect, due to the reaction between SO_2 and NO_2^-, will occur whenever the analysis is performed in aqueous solution. The addition of diphenylamine minimises the disappearance of sulphur dioxide, as it reacts with nitrite to give the corresponding nitrosamine[6]; however, with $[NO_2^-]:[SO_2] > 1$ the nitrosamine interferes in the measurement of sulphur dioxide. Therefore, nitrogen oxides must be trapped before the sulphur dioxide collecting filter when the determination of sulphur dioxide in air is required.

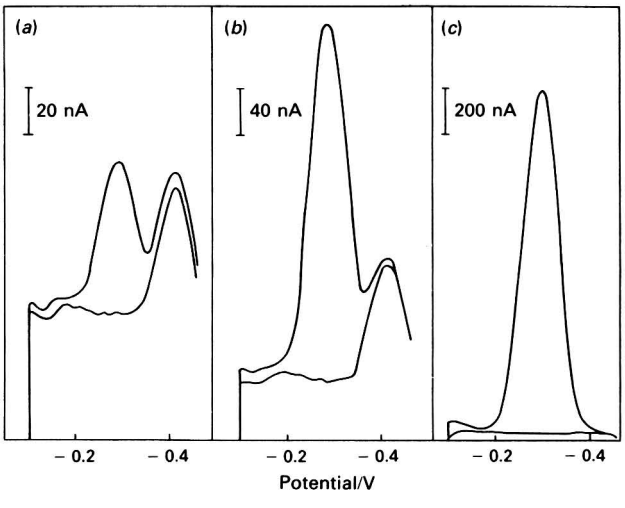

Fig. 1. Differential-pulse voltammograms of SO_2 in 1 M HCl. The concentration of the SO_2 in the solutions were: (*a*) 2×10^{-7}; (*b*) 10^{-6}; and (*c*) 5×10^{-6} M. Pulse height, 80 mV; sweep rate, 10 mV s^{-1}; temperature, 22 °C. The lower graphs are the pulse voltammograms obtained after expulsion of SO_2 from the analysing solution by purging with nitrogen.

Determination of Sulphur Dioxide in Gas Phase

Some typical results for experiments carried out using a nitrogen - oxygen mixture (3 + 1) or air as the carrier gas are reported in Table I. It is clear that sulphur dioxide concentrations at the background level can easily be measured within a few minutes of sampling and that the threshold concentration at which sulphur dioxide is not correctly measured is below 0.1 p.p.b. In some instances the collection efficiency was tested by placing two filters in series; for example, the amount of sulphur dioxide captured by the second filter was undetectable when air containing 7 p.p.b. of sulphur dioxide flowing at 10 l min^{-1} was sampled for 30 min.

TABLE I

REPRESENTATIVE EXAMPLES OF THE DETERMINATION OF SULPHUR DIOXIDE IN THE GAS PHASE

Carrier gas	Relative humidity, %	Flow-rate/ l min^{-1}	Sampling time/min	Estimated gas concentration of SO_2, p.p.b.*	Estimated solution concentration of SO_2/M × 10^7†	Measured solution concentration of SO_2/M × 10^7‡
$N_2 + O_2$	30	1	5	69.0	38.2	37.0
$N_2 + O_2$	30	3	5	22.9	38.2	39.1
$N_2 + O_2$	30	10	5	6.9	38.2	36.2
$N_2 + O_2$	30	1	10	1.38	1.52	1.58
$N_2 + O_2$	30	3	10	0.45	1.52	1.43
$N_2 + O_2$	30	10	10	0.14	1.52	1.38
$N_2 + O_2$	0	10	5	6.9	38.2	39.5
$N_2 + O_2$	0	10	30	6.9	23.0	14.5
$N_2 + O_2$	30	10	30	6.9	23.0	23.5
Air	65	10	30	7.8	26.0	26.8
$N_2 + O_2 + NO_x$§ ..	30	10	5	6.9	38.2	36.0

* Calculated from the output of the permeation tube.
† Estimated in the polarographic cell.
‡ Determined by the method presented here.
§ NO_x concentration ≈ 7 p.p.b.

For gas volumes of 100 l or below it was observed that the sulphur dioxide collection efficiency was unaffected by relative humidity in the range 0–90%, whereas for gas volumes of 300 l or above losses of sulphur dioxide were detected when the relative humidity of the carrier gas was below 20%.

Carbonation of the trapping solution, which occurs when air is sampled, has no effect on the collection efficiency. In fact, when sulphur dioxide was added to an air sample with a low sulphur dioxide content (< 1 p.p.b.), the expected value of the sulphur dioxide concentration was found irrespective of the air volumes. This result is in accord with the finding that sodium hydrogen carbonate is a good trapping agent for sulphur dioxide.[7]

The presence of NO_x (= NO + NO_2) in the gas phase, with $[NO]_x$: $[SO_2]$ = 1 and a sulphur dioxide concentration of about 20 p.p.b., leads, as previously mentioned, to a large decrease in the amount sulphur dioxide measured.

To remove this interference, a filter impregnated with a saturated solution of diphenylamine in 0.1 M perchloric acid and 0.01 M potassium thiocyanate was placed before the sodium hydroxide filter. The presence of this filter has no effect on the sulphur dioxide concentration and furthermore, when NO_x is present in the carrier gas a green colour develops on the filter, indicating the formation of the corresponding nitrosamine. In this way it was possible to measure, for example, 7 p.p.b. of sulphur dioxide in the presence of an equal amount of NO_x at a flow-rate of 10 l min^{-1} without significant loss.

The accuracy of the method was estimated to be about 7% based on the output of sulphur dioxide from the permeation tube. The sensitivity appears to be of the order of 0.1 p.p.b. and could be achieved with a sampling time of a few minutes.

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Spectrophotometric Assay of Bromhexine Hydrochloride and its Application to Binary Bromhexine - Antibiotic Mixtures

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Keywords: *Bromhexine hydrochloride assay*; *antibiotic assay*; *visible spectrophotometry*; *ultraviolet spectrophotometry*

Bromhexine hydrochloride, *N*-(2-amino-3,5 dibromobenzyl)-*N*-cyclohexylmethylammonium chloride, a mucolytic agent,[1] is commonly used in conjunction with antibiotics in the therapy of congestive respiratory disease and is included in the British Pharmacopeia.[2] Two methods for simultaneous assay of such combinations have been described.[3,4] However, of these, one[3] has poor sensitivity and is inapplicable in the common examples where the antibiotic is present in a large excess. In many instances, *e.g.*, in pharmaceutical dissolution testing, samples containing less than 80 μg of bromhexine may require measurement. The second assay,[4] which detects 1–1.5 mg of bromhexine, is of insufficient sensitivity for these examples and involves a lengthy complex extraction procedure that is impracticable in multi-sample analysis especially where binary mixtures are involved. This paper describes a rapid, automated assay of binary bromhexine - antibiotic mixtures with special reference to the antibiotic groups cephalosporins (cephalexin and cefaclor), tetracyclines (oxytetracycline) and penicillins (phenoxymethylpenicillin).

Bromhexine is measured by spectrophotometry in the visible range via chloroform extraction of the ion-pair reaction product of bromhexine and the dye bromocresol purple using the extraction method of Tsubouchi.[5] Antibiotics are measured by ultraviolet spectrophotometry.

Experimental

Reagents

Pharmaceuticals. Bromhexine Hydrochloride BP, Cephalexin BP, Oxytetracycline BP and Phenoxymethylpenicillin BP.

Cefaclor. Pharmaceutical grade.

Glacial acetic acid. Laboratory-reagent grade.

Hydrochloric acid, 0.1 N. Laboratory-reagent grade.

Bromocresol purple. Laboratory-reagent grade.

Chloroform. Analytical-reagent grade.

Methanol. Analytical-reagent grade.

Sodium sulphate. Analytical-reagent grade.

Bromocresol purple reagent. Dissolve 600 mg of bromocresol purple and 8 g of sodium sulphate in 200 ml of distilled water. Add 80 ml of glacial acetic acid and dilute to 1 000 ml with water. Store in a brown glass bottle.

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Apparatus

Autoanalysis

Liquid sampler. Technicon Instruments Sampler II.
30-*Channel proportioning pump.* Newton Instruments Co. Ltd.
Spectrophotometer. Technicon Instruments, 15-mm path length flow cell.
Ultraviolet spectrophotometer. Cecil Instruments Ltd., Model CE 272, 10-mm path length flow cell.

Dissolution testing

Dissolution apparatus. G.B. Caleva Ltd.
Microprocessor-controlled sampling system.[6]

Procedure

A schematic diagram for the parallel simultaneous autoanalysis of binary bromhexine hydrochloride - antibiotic solutions is shown in Fig. 1. Aliquots of 10 ml are sampled.

Calibration graphs

Dissolve 50 mg of bromhexine hydrochloride in 10 ml of methanol and dilute to 1 l with 0.1 N hydrochloric acid. Dissolve 500 mg of each antibiotic in separate volumes of 1000 ml of 0.1 N hydrochloric acid. Using these solutions, prepare combined standards of bromhexine hydrochloride and each antibiotic in the ratios 1 + 50, 2.5 + 125, 5.0 + 200 and 10 + 250 μg ml^{-1}.
Use these solutions to construct calibration graphs for each component.

Results and Discussion

The reaction product of bromhexine hydrochloride and bromocresol purple shows a spectrum with a single peak at 400–410 nm (Fig. 2). Equipment constraints required the visible spectrophotometric measurements to be made at 422 nm and the loss in sensitivity was estimated to be about 7%. Multiple determinations of absorbances at 422 nm were made for each of four series of bromhexine hydrochloride (plus bromocresol purple) solutions (0.5, 1.0, 2.5, 5.0, 10.0, 14.0, 15.0 and 20.0 μg ml^{-1} of bromhexine hydrochloride) in 0.1 N hydrochloric acid. The absorbance *versus* concentration graph derived from 100 determinations at 422 nm was linear over the entire range and gave the equation

$$y = 0.0351x - 0.0182$$

where y = absorbance and x = bromhexine hydrochloride concentration (in micrograms per millilitre). The correlation coefficient was 0.999. Multiple determinations showed the method to have high reproducibility. [Analysis of variance for individual data showed the regression slope was highly significant. The F-ratio (the ratio of variance $_{\text{absorbance means}}$ to variance $_{\text{absorbance residuals}}$) was insignificant.]

Assay Interference Between Bromhexine Hydrochloride and Antibiotics

Bromhexine hydrochloride shows an absorption minimum at 270 nm and zero absorbance above 330 nm[7] while the range of antibiotics tested here show absorption maxima at 260–280 nm and 340 nm and zero absorption above 400 nm. Cephalexin, cefaclor, oxytetracycline and phenoxymethylpenicillin did not give a coloured reaction product with bromocresol purple. Interference of these antibiotics at a concentration of 250 μg ml^{-1} in the bromhexine assay was less than 1%. Bromhexine, at a concentration of 20 μg ml^{-1}, showed less than 1% interference in the ultraviolet assay of cephalexin, cefaclor and oxytetracycline but did cause an unexplained increase (about 25%) in phenoxymethylpenicillin absorbance. Determination of bromhexine is precluded in the presence of erythromycin as this macrolide antibiotic reacts as a tertiary amine in the visible spectrophotometric assay.

Assay Interference by Formulation Colouring Agents

Tablets and capsule shells frequently contain colouring agents. Experience with a wide range of coloured capsule shells indicates insignificant (if any) interference of these agents in the

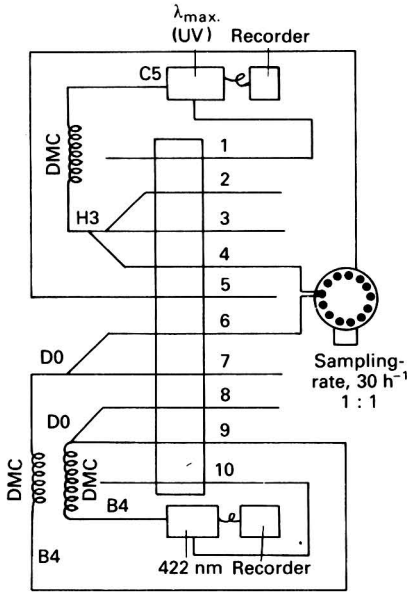

Fig. 1. Schematic diagram for the differential automated assay of bromhexine hydrochloride and antibiotics in binary mixtures. Key: A, Acidflex; S, Solvaflex; M, standard tubing; DMC, double-mixing coil; DO and H3, glass connectors, AutoAnalyzer; and B4 and C5, degassing connectors, AutoAnalyzer. (1) M, i.d. 0.065, waste; (2) M, i.d. 0.045, air; (3) M, i.d. 0.073, 0.1 N hydrochloric acid; (4) M, i.d. 0.015, sample; (5) M, i.d. 0.110, sample wash; (6) M, i.d. 0.081, sample; (7) A, i.d. 0.110, chloroform; (8) S, i.d. 0.056, bromocresol purple reagent; (9) A, i.d. 0.110, re-sample; and (10) A, i.d. 0.090, waste.

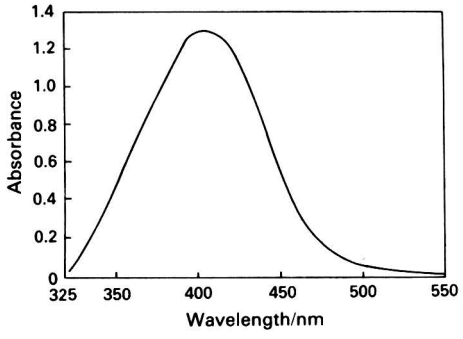

Fig. 2. Visible-region spectrum for the chloroform extract of bromhexine hydrochloride - bromocresol purple reaction product.

visible spectrophotometric assay. However, the inclusion of an equivalent capsule shell control in dissolution testing constitutes common practice to eliminate potential problems of this nature.

Reagent Concentration and Sample pH

Bromocresol purple at the 120 and 600 mg l^{-1} levels in the colour reagent gave equivalent results, but 60 mg l^{-1} did not. The five-fold excess of bromocresol purple (600 mg l^{-1}) was therefore used routinely. Dissolution testing is commonly carried out in pH conditions approximating to the acidic and neutral environments found in the gastro-intestinal tract. The colour reaction gave equivalent results with samples prepared in pH 6.8 phosphate buffer solution and in 0.1 N hydrochloric acid, confirming that the method is applicable over the range of commonly applied test conditions.

Validation of Method

Commercial and exploratory formulated bromhexine hydrochloride and bromhexine hydrochloride - antibiotic preparations were subjected to dissolution testing and their binary components analysed from 10-ml aliquots. The dissolution profiles obtained from 6 to 8 samples

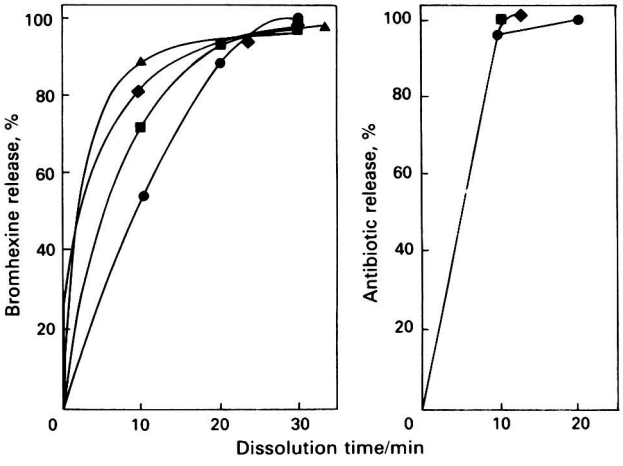

Fig. 3. Dissolution profiles for bromhexine hydrochloride and bromhexine hydrochloride/antibiotic formulated samples. ▲, Bromhexine hydrochloride 8 mg (tablet); ●, bromhexine hydrochloride 8 mg, oxytetracycline 250 mg (capsule); ◆, bromhexine hydrochloride 5 mg, cephalexin 250 mg (capsule); ■, bromhexine hydrochloride 5 mg, cefaclor 250 mg (capsule). Test conditions: dissolution testing was carried out at 37 °C in 1000 ml of 0.1 N hydrochloric acid, with a basket rotation rate of 50 rev min^{-1}. Results quoted are averages from 6–8 samples.

of each formulation are shown in Fig. 3. Variations in drug release rates from tablets and capsules due to formulation or process changes are well documented. Rapid, reproducible release rates are required for many drugs, including antibiotics, and the application of product-appropriate limits is widespread, a common standard being not less than 70% drug release in 45 min. In the samples tested here, the rate of release for each component complies easily with this limit. The drug-release profiles show recoveries of 96–99% of bromhexine and 99–102% of antibiotic from various formulations. The visible spectrophotometric method for bromhexine is therefore shown to be applicable in aqueous solution and in the analysis of binary formulated products.

Conclusion

Bromhexine hydrochloride may be rapidly determined by its reaction with bromocresol purple. The method can measure 0.5 μg ml^{-1} of bromhexine hydrochloride and is applicable to binary determinations with antibiotics in large volume dissolution determinations. Reproducibility is high and mutual assay interference in a range of binary mixtures is low. The method can be readily carried out by autoanalysis.

The authors thank Mr. G. F. Snook, Lilly Research Centre, for his comments during the preparation of this paper.

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Determination of α-Halocarbonyl Compounds by Reaction with Thiourea

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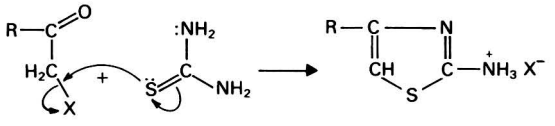
Keywords: *α-Halocarbonyl compounds*; *thiourea*; *titrimetry*

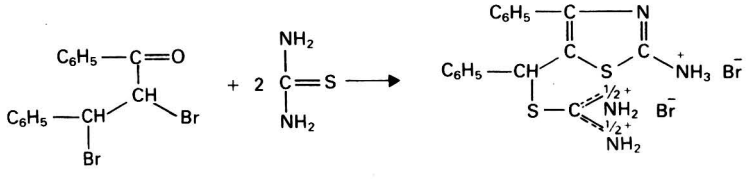
Spectrophotometry has been used for the detection and determination of α-halocarbonyl compounds by the use of asymmetric diphenylhydrazine,[1] but the procedure is subject to interference from other carbonyl compounds. Thioureas react with α-halocarbonyls to form hydrohalides of the corresponding aminothiazoles or iminodihydrothiazoles[2–4]; ω-bromoacetophenone has been employed for the determination of thioureas.[5] Thiourea reacts quantitatively with various α-halocarbonyl compounds as follows:



where R = aryl, arylamino, amino or alkoxyl group; and X = chlorine, bromine or iodine.

This reaction has been used to develop a simple alkalimetric method for the determination of α-halocarbonyls. An α-halocarbonyl compound is reacted with thiourea in ethanol and the hydrohalide of the respective aminothiazole thus formed is determined by titration against alkali, using phenolphthalein as the indicator.

Chloroacetic acid and its sodium salt react in a similar manner; with the chloroacetic acid it is difficult to establish the time for complete reaction because it requires the same amount of the alkali titrant as does the reaction product whereas the titre for the reaction product of sodium chloroacetate, as expected, is nil. The titre for 1-phenyl-2-benzoyl-1,2-dibromoethane suggests that it reacts with two molecules of thiourea to form 2-amino-4-phenylthiazole-5-benzylisothiourea dihydrobromide.



A selection of α-halocarbonyl compounds were tested (Table I). The relatively less reactive chloroacetamide[6] could be determined and the procedure seems to be applicable to many other compounds. The standard deviations recorded in the table are an indication of the precision of the method. The reaction times of the α-halocarbonyl compounds follow the order of reactivity[6,7] iodo- > bromo- > chloro-.

Experimental

A weighed amount (0.2–0.5 mmol) of an α-halocarbonyl compound, prepared and purified according to published procedures, and 1.0 mmol of thiourea are dissolved in 10 ml of ethanol. The solution is either heated on a water bath or kept at room temperature, for the time required for reaction. The solution is then titrated against 0.1 N sodium hydroxide solution,

TABLE I

DETERMINATION OF α-HALOCARBONYL COMPOUNDS

Sample*	Mass range/mg	Average recovery, %	Reaction period/h	Standard deviation, %
ω-Bromoacetophenone (4)	50–120	99.89	0.25†	0.09
ω-Bromo-*p*-chloroacetophenone (4)	50–100	99.87	0.25†	0.10
ω-Bromo-*p*-methylacetophenone (4)	40–100	99.98	0.25†	0.11
ω-Bromo-*m*-nitroacetophenone (4)	40–100	99.97	0.25†	0.10
1-Phenyl-2-benzoyl-1,2-dibromoethane (3)	30–80	100.07	0.5‡	0.06
Bromoacetamide (4)	30–100	99.97	2.0‡	0.04
Chloroacetamide (3)	20–40	100.07	3.5‡	0.08
Chloroacetanilide (4)	40–100	99.97	0.5‡	0.05
Ethyl iodoacetate (4)	40–110	99.63	0.5†	0.12
Ethyl bromoacetate (4)	40–100	99.95	1.0†	0.05
Butyl chloroacetate (3)	40–60	99.88	3.5‡	0.12

* Figures in parentheses represent number of determinations.
† At room temperature (25 °C).
‡ At reflux temperature (boiling ethanol, 78 °C).

using phenolphthalein (2 drops of 0.5% solution in ethanol) as indicator, to a light pink colour that remains for at least 1 min. However, with 1-phenyl-2-benzoyl-1,2-dibromoethane the stability is about 30 s.

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Rapid Spectrophotometric Determination of Nitrate with 4,5-Dihydroxycoumarin

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Keywords: *Nitrate determination*; *spectrophotometry*; *4,5-dihydroxycoumarin*; *chloride*; *nitrite*

Various spectrophotometric methods for the determination of nitrate have been developed. The simplest methods are based on the absorption of nitrate in the ultraviolet region.[1–3] The main disadvantages of these methods are low sensitivity and serious interference by coloured species. The direct methods widely used are based on the nitration of organic compounds, particularly phenolic compounds,[4–6] but these methods are time consuming.

Many of the indirect methods are based on the diazotisation of an aromatic amine after the reduction of nitrate to nitrite with subsequent coupling to form an azo dye.[7,8] Although indirect methods have higher sensitivity, reagents such as *N*-(1-naphthyl)ethylenediamine dihydrochloride and reductants such as cadmium are toxic.[9]

It has been reported that chloride ion catalyses the conversion of nitrate into nitrite in sulphuric acid medium,[10] and the author has previously reported a simple, rapid and selective method for the determination of nitrite in an acidic solution with 4,5-dihydroxycoumarin (DHC) dissolved in benzene.[11] Combination of these two reactions has been attempted, and in this work it was decided to optimise these reactions for the determination of nitrate. This procedure has the advantages of rapidity, simplicity and high selectivity.

Experimental

Apparatus

A Hitachi, Model 124, recording spectrophotometer was used for measuring absorption spectra and a Hitachi, Model 139, spectrophotometer was used for the determination of nitrate.

Reagents

4,5-Dihydroxycoumarin was prepared by the method described by Desai and Sethna.[12] Sulphuric acid of the highest available purity grade was obtained from Wako Pure Chemicals Industries Ltd., Osaka, Japan. All other chemicals were of analytical-reagent grade.

Nitrate standard solution. A 1×10^{-2} M solution of nitrate was prepared by dissolving sodium nitrate, which had been dried at 110 °C for 4 h, in water. Solutions of lower concentrations were prepared by dilution of this standard solution.

4,5-*Dihydroxycoumarin solution.* DHC was dissolved in benzene containing 4% *V*/*V* of methanol.

Chloride solution. A 4 M solution of chloride was prepared by dissolving sodium chloride in water.

Procedure

Transfer 5 ml of a neutral solution containing less than 7.5 p.p.m. of nitrate-nitrogen into a test-tube and add 2 ml of 4 M chloride solution and 5 ml of concentrated sulphuric acid. Stopper the tube and allow it to stand for 10–20 min, then add 5 ml of the DHC in benzene solution and 7 ml of water and shake vigorously for 30 s. Separate the benzene layer and dry it over anhydrous sodium sulphate. Measure the absorbance at 410 nm.

Caution—Benzene is highly toxic. Appropriate precautions should be taken in its use.

Results and Discussion

Effect of Acid Concentration

The concentration of sulphuric acid affects the conversion of nitrate into nitrite and the reaction for the extraction of nitrite - DHC. Fig. 1 shows the effect of sulphuric acid concentration on the absorbance when the volume of the solution before standing was kept at 12 ml and the volume of water added before shaking at 7 ml. The maximum absorbance was obtained in the range 14–15 N sulphuric acid. This acidity is achieved in the procedure by adding 5 ml of concentrated sulphuric acid.

Fig. 2 shows the effect of the final acidity when the acidity and the volume of solution before standing were kept at 15 N and 12 ml, respectively, and the volume of water added before shaking was varied between 0 and 20 ml. The maximum constant absorbance was obtained in the range 5–11 ml of water added, so that the optimum final acidity was in the range 7.8–10.6 N sulphuric acid. The water should be added after the addition of the DHC in benzene solution because, as can be seen in Table I, the addition of water before the addition of DHC solution decreased the absorbance.

Effect of Chloride Concentration

The effect of chloride concentration was examined and the results are shown in Fig. 3. A chloride concentration in the range 4×10^{-3}–12×10^{-3} M was required for the maximum

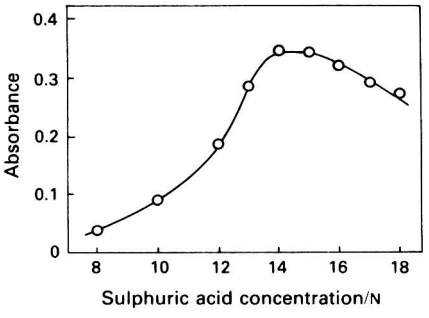

Fig. 1. Effect of sulphuric acid concentration on the conversion of nitrate to nitrite. Nitrate-nitrogen: 2.8 p.p.m.

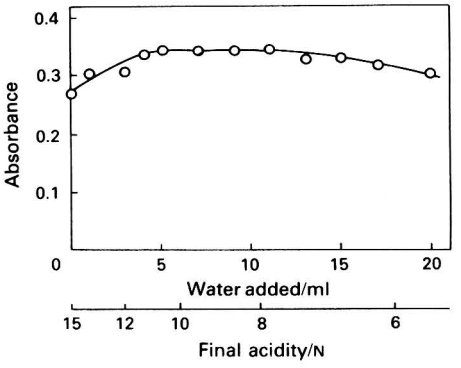

Fig. 2. Effect of final acidity. Nitrate-nitrogen: 2.8 p.p.m.

constant absorbance. Therefore, a chloride concentration of 8×10^{-3} M was chosen for the determination.

Effect of Standing Time

In order for complete conversion of nitrate into nitrite, the mixture after the addition of sulphuric acid should be allowed to stand. Fig. 4 shows the effect of standing time before the extraction. The standing time required for maximum colour development was 10–20 min; during the standing period the sample solution should not be heated and cooled because this decreases the absorbance, as shown in Table I. Afghan *et al.*[10] reported a method for the determination of chloride in which chloride ion catalysed the conversion of nitrate into nitrite and the resultant nitrite reacted with chromotropic acid to produce strongly coloured species. In their method, in order for the conversion to be successful, close control of the temperature at 75 °C was required, but in this method the conversion took place successfully only on heating a mixture of the sample solution with concentrated sulphuric acid.

TABLE I

EFFECT OF VARIATION OF THE STEPS IN THE PROCEDURE IN THE METHOD

	Absorbance*
Recommended procedure	0.345
With 7 ml of water added before the addition of the DHC in benzene solution	0.195
With cooling in an ice-bath during standing	0.197
With heating in boiling water during standing	0.302

* Absorbance measured for the determination of 2.8 p.p.m. of nitrate-nitrogen.

Effect of DHC Concentration and Shaking Time

Studies were carried out to find the optimum concentration of DHC and shaking time. The maximum constant absorbance was obtained at DHC concentrations above 2×10^{-6} M, and on shaking for longer than 10 s.

Calibration Graph

At 401 nm the nitrate - DHC system follows Beer's law over the concentration range 0.2–7.5 p.p.m. of nitrate-nitrogen. The absorbance remained unchanged for at least 24 h. A statistical study of 13 samples, each containing 2.8 p.p.m. of nitrate-nitrogen, was carried out and a relative standard deviation of 2.2% was obtained.

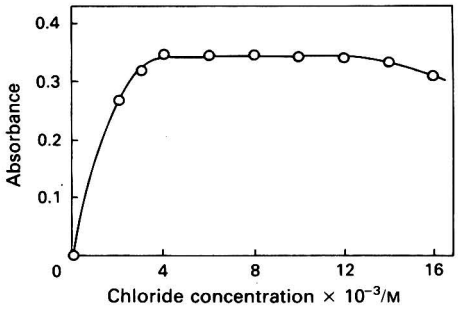

Fig. 3. Effect of chloride concentration. Nitrate-nitrogen: 2.8 p.p.m.

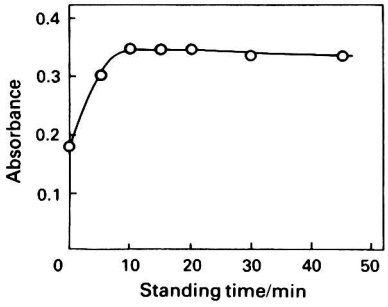

Fig. 4. Effect of standing time.

Interference Study

The effect of various potential interferents was investigated on a sample containing 2.8 p.p.m. of nitrate-nitrogen. Many of the ions examined were tolerated when present in large amounts. However, nitrite, iodate and chromium(VI) interfered at the 10-μg level. Results are shown in Table II.

Although nitrite reacts to form the same coloured species and increases the absorbance, the absorbance due to nitrite can be measured separately as follows. After the addition of the sample and the chloride solution to the tube, 12 ml of 15 N sulphuric acid are added instead of the addition of concentrated sulphuric acid in the recommended procedure; then the DHC solution is added, the mixture is shaken and the benzene layer is separated, dried and measured at the same wavelength. With this procedure, the absorbance due to nitrite can be obtained without interference by nitrate. Therefore, if the sample solution contains both nitrate and nitrite, the absorbance due to nitrite alone obtained by the procedure described above should be subtracted as the blank.

TABLE II

EFFECT OF DIVERSE IONS

Ion	Tolerance limit*
Phosphate	750 mg
Chloride	200 mg
Cadmium and sulphate	150 mg
Acetate	100 mg
Nickel, copper, iron(III) and magnesium	75 mg
Aluminium, cobalt, zinc and fluoride	50 mg
Lead	30 mg
Calcium	25 mg
Ammonium	5 mg
Chromium(III)	3 mg
Bromide	500 μg
Sulphite and iodide	100 μg
Iron(II)	25 μg
Nitrite, chromium(VI) and iodate	below 10 μg

* Amount of ion causing an error of less than 5% in the determination of 2.8 p.p.m. of nitrate-nitrogen.

Spectral Characteristics

The absorption spectrum obtained for the product was identical with that for nitrite reported previously,[11] with maximum absorption at 410 nm. The reagent at the recommended concentration shows no absorption at this wavelength.

The author thanks Mr. T. Suzuki for the preparation of 4,5-dihydroxycoumarin.

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Communication

Material for publication as a Communication must be on an urgent matter and be of obvious scientific importance. Rapidity of publication is enhanced if diagrams are omitted, but tables and formulae can be included. Communications should not be simple claims for priority: this facility for rapid publication is intended for brief descriptions of work that has progressed to a stage at which it is likely to be valuable to workers faced with similar problems. A fuller paper may be offered subsequently, if justified by later work.

Manuscripts are not subjected to the usual examination by referees and inclusion of a Communication is at the Editor's discretion.

Determination of Formaldehyde in the Atmosphere: Observations Concerning the Storage of Aqueous Samples

Keywords: Formaldehyde determination; atmosphere; water sample storage; biocides

In recent years an increasing number of laboratories have become involved in the determination of formaldehyde in the atmosphere. There are many methods of analysis available but the most widely accepted is the spectrophotometric method using chromotropic acid. Several adaptations of the method exist, but for personal sampling the NIOSH method[1] is probably the most widely known. The recommended absorbent employed in the NIOSH method is water, but when samples prepared in this manner are stored at room temperature a loss of formaldehyde is observed. This paper demonstrates that biological action is the most likely cause of the loss of formaldehyde from dilute aqueous solutions and that the addition of small amounts of biocide permits dilute formaldehyde solutions to be stored for several days without deterioration.

Experimental and Results

Procedure for the Determination of Formaldehyde

The formaldehyde concentrations were determined by the chromotropic acid method. The samples for analysis were pipetted into 50-ml borosilicate flasks, the volume was made up to 4 ml with de-ionised water, and 1 ml of chromotropic acid solution (5 g in 100 ml of de-ionised water) added followed by 45 ml of 50% *V/V* sulphuric acid. After heating in a boiling water-bath for 30 min the solutions were cooled to room temperature, diluted to the mark with 50% *V/V* sulphuric acid and measured at 570 nm in 40-mm cells using a Pye Unicam SP 800 spectrophotometer.

Stability of Dilute Aqueous Formaldehyde Solutions

The loss of formaldehyde from dilute aqueous solutions has been reported previously.[1–3] It became apparent early in our studies on the formaldehyde content of aqueous solutions that the amount of formaldehyde lost is far greater for solutions that have had air drawn through them than for those which have not. Further, the larger the volume of air passed through the solution the more rapid is the disappearance of the formaldehyde (see below). It is suggested in the NIOSH report that loss of formaldehyde is due to polymerisation, but this seems unlikely as formaldehyde polymers would rapidly depolymerise under the strongly acidic conditions of test. A more likely route would be via oxidation, or alternatively the loss of formaldehyde could be caused by biological activity.

Stabilisation of Dilute Formaldehyde Solutions

Sodium hydrogen sulphite has antioxidant and biocidal properties that would apparently make it an ideal preservative for dilute formaldehyde solutions. The NIOSH procedure recommends its use under certain circumstances and it has been adopted by some industrial analytical laboratories. However, it has the major disadvantage that in order to obtain a linear response over the region of interest the colour must be developed in the presence of concentrated sulphuric acid. The corrosive nature and high viscosity of concentrated sulphuric acid make it difficult to handle in the spectrophotometer cells. Also, there is a risk of a violent exothermic reaction should the glassware fracture during the heating and cooling processes. For these reasons, more suitable stabilising agents were sought.

To investigate the effectiveness of potential preservatives, the following experiment was conducted. Non-sterile air was drawn through 10 ml of de-ionised water, contained in a midget impinger, at a rate of 250 ml min^{-1} for 6 h using a Casella pump. Aliquots of 2 ml were pipetted into 10-ml calibrated flasks containing 5 ml of formaldehyde solution (approximately 20 $\mu g\ ml^{-1}$) in: (1) de-ionised water only; (2) de-ionised water containing 50 $\mu g\ ml^{-1}$ of mercury(II) chloride; (3) de-ionised water containing 50 $\mu g\ ml^{-1}$ of tin(II) chloride; or (4) de-ionised water containing 50 $\mu g\ ml^{-1}$ of sodium pentachlorophenate. These components were selected for investigation on the basis that mercury(II) chloride is a powerful biocide, tin(II) chloride is a biocide with reducing properties and sodium pentachlorophenate is an organic biocide that has a lower toxicity than mercury(II) compounds.

The solutions were diluted to 10 ml with de-ionised water and 2-ml samples removed by pipette and assayed for formaldehyde using the procedure described previously. The formaldehyde concentrations were measured again after a period of 4 days. The results obtained are shown in Table I.

TABLE I

EFFECT OF STABILISERS ON THE CONCENTRATION OF DILUTE FORMALDEHYDE SOLUTIONS ON STORAGE AT 21 °C

No.	Sample	Formaldehyde found/$\mu g\ ml^{-1}$	
		Initial	After 4 days at 21 °C
1	Formaldehyde in water	10.1	<0.05
2	Formaldehyde in water containing 50 $\mu g\ ml^{-1}$ of mercury(II) chloride	10.1	9.7
3	Formaldehyde in water containing 50 $\mu g\ ml^{-1}$ of tin(II) chloride	9.9	9.4
4	Formaldehyde in water containing 50 $\mu g\ ml^{-1}$ of sodium pentachlorophenate	10.1	9.6

A procedure similar to that described above was carried out, in which the effect of varying the amount of air drawn through the water before addition of formaldehyde was investigated. The changes in formaldehyde contents on storage of the unstabilised dilute solutions were measured and the results are given in Table II.

TABLE II

STORAGE OF DILUTE FORMALDEHYDE SOLUTIONS IN CONTACT WITH WATER THROUGH WHICH VARYING AMOUNTS OF AIR HAS BEEN PASSED

Volume of air passed through 10 ml de-ionised water/l	Formaldehyde found/$\mu g\ ml^{-1}$	
	Initial	After 4 days at 21 °C
0	9.5	9.0
15	10.0	7.0
105	10.1	<0.05

Considering the manner in which the samples were prepared, the results in Table II indicate that biological action is the most likely cause of the loss of formaldehyde. The effectiveness of mercury(II) and sodium pentachlorophenate as preservatives serves to strengthen this opinion, as they have little or no antioxidant properties. All of the compounds investigated in Table I appear to stabilise dilute formaldehyde solutions adequately and it is probable that many more compounds that depress biological growth would be equally effective.

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Book Reviews

THE CHROMATOGRAPHY OF HEMOGLOBIN. By WALTER A. SCHROEDER and TITUS H. J. HUISMAN. *Clinical and Biochemical Analysis, Volume* 9. Pp. xii + 255. Marcel Dekker. 1980. Price SFr68. ISBN 0 8247 6941 4.

This is the ninth volume in a series of monographs and text-books edited by Morton K. Schwartz and is a worthy successor to its predecessors. The book is a reflection of the practical experiences of the authors, gained over more than 20 years, involved in the identification, quantification and isolation of a wide variety of haemoglobins by chromatographic and ion-exchange techniques. It deals initially with the practice and concepts of chromatography showing how haemoglobin solutions may be collected and prepared for the subsequent chromatographic procedures.

Separation of the haemoglobins is discussed and detailed descriptions of the techniques together with representative separation profiles are given for a wide range of chromatographic media using both macro- and micro-techniques. Separation agents include amberlite and cellulose CM, as cationic exchangers, and sephadex and DEAE cellulose, as anionic exchangers.

Examples are given for the separation of all of the major and many of the minor haemoglobins including HbA_{1c}, which is currently being used to monitor and control patients with diabetes mellitus. While acknowledging the existence of electrophoresis it is obvious that the authors are dedicated chromatographers and put their faith in these techniques, frequently showing the ways in which their techniques are superior. They have not, however, convinced me that I should change from my electrophoresis - endosmosis techniques for routine HbA_{1c} measurements.

One unusual feature of the book is that it devotes space to the separation of non-human haemoglobins ranging from sheep to shrimps, giving details of the major haemoglobins found in them. Finally, in the days of quality control with pure haemoglobin preparations the authors explain how preparative techniques can yield isolated fractions for a variety of uses.

I found the book to be well written and easy to understand and feel that, with its comprehensive list of references, it should be readily available to the laboratory worker in both haematology and research laboratories.

J. F. STEVENS

INSTRUMENTAL HPTLC. PROCEEDINGS OF THE FIRST INTERNATIONAL SYMPOSIUM ON INSTRUMENTALISED HIGH PERFORMANCE THIN-LAYER CHROMATOGRAPHY (HPTLC), BAD DÜRKHEIM (WEST GERMANY), MAY 18–21, 1980. Edited by W. BERTSCH, S. HARA, R. E. KAISER and A. ZLATKIS. *Chromatographic Methods*. Pp. 390. Hüthig Verlag. 1980. Price DM88. ISBN 3 7785 0658 7.

The commercial manufacture of micro-particulate silicas of relatively narrow particle size distribution in the diameter range 3–10 μm has played an essential role in the rapid progress of high-performance column chromatography, but it is sometimes forgotten that these silicas have had an equally significant impact on the technique of thin-layer chromatography. This book, which is based on the proceedings of the First International Symposium on Instrumental High Performance Thin-layer Chromatography (HPTLC), provides a useful text-book for the analyst wishing to seriously consider the benefits that might be conferred by using this "improved" form of thin-layer chromatography. The theme running through the book is that by using HPTLC plates it is possible to achieve analysis more rapidly than by HPLC and with greater separating power and sensitivity than with conventional TLC. The proponents of HPTLC also claim that by using computer controlled reflectance photometers it is possible to significantly improve the quantitative accuracy of the method to such an extent that it can rival HPLC in its quantitative applications, and data are presented which certainly indicate that the former method can, under the best conditions, give standard deviations of less than 1%.

Sixteen different contributors from around the world have provided the material for this book. Six of their papers relate to practical applications ranging from the analysis of steroids in pharmaceutical preparations, antibiotics in fermentation broths, pesticides in formulations and as residues, polycyclic hydrocarbons in diesel exhaust gases and amino acids in serum to the detection of selenium in water. The remainder of the book is devoted to a variety of topics that are of considerable interest to the practising analyst, such as sample application, instrumentation (with particular

emphasis on quantitation), development techniques and the use of chemically modified stationary phases. Bearing in mind that the major limitation of HPLC at the present time is in the area of selective and sensitive monitoring, and that TLC does not suffer the same limitations, there seem to be grounds for exploring HPTLC for those applications that are proving difficult by HPLC.

A minor criticism of this book is that there is some repetition in its subject material, which can be attributed to its being based on a series of independent lectures. B. B. WHEALS

SOLUTION EQUILIBRIA. By F. R. HARTLEY, C. BURGESS and R. M. ALCOCK. Pp. 361. Ellis Horwood. 1980. Price £26 (hardback); £6.90, $17.45 (paperback). ISBN 0 85312 148 6; 0 470 26880 8.

It is a lamentable fact that all too many teachers of inorganic chemistry have but a passing interest in the quantitative aspects of complex formation between metals and ligands, frequency, in cm^{-1}, of the absorption maximum being the only numerical quantity of interest. Perhaps workers in the past have been put off by the difficulty of determining stability constants for stepwise formation of complexes reliably (there have, of course, been plenty of unreliable determinations). I feel that this book should now do for complex-formation chemistry what Albert and Serjeant's practical handbook has already done for acid - base chemistry, namely, to introduce the determination of stability constants as a routine tool in the co-ordination chemist's workshop.

The book does not give step-by-step instructions for all methods, these are available in the literature. But it does discuss in a lucid and readable manner why certain approaches should be adopted towards certain problems and when any particular method of measurement is to be preferred. Further, a number of examples are worked out in every detail to show just how potentiometry or spectrophotometry can be applied to the determination of stability constants in moderately complex examples: nickel - ethylenediamine, silver - allyl alcohol, copper - ethylenediamine - oxalate and palladium - chloride. Alternative approaches to the numerical analysis are illustrated, including the use of computer programs. Perhaps it would have been useful to include one rather simpler example such as copper - ethylene diamine, which can be studied by a combination of potentiometry and spectrophotometry in such a way as to be manageable by second year university students.

There is much more besides in this book, which readers will welcome: the attention paid to non-aqueous systems, for example (for dealing with ligands insoluble in water), and the chapters on the interpretation and application of stability constants when you have determined them. The layout of the book is pleasing and the indexing is good. I would draw attention to two errors overlooked in proof-reading: in Fig. 1.1 (axis inverted), and Eq. 7.2 (wrong sign). This book should be read by all research workers in co-ordination chemistry, and could well be useful in some advanced undergraduate courses. It is recommended and welcomed. I. L. MARR

MODERN POLAROGRAPHIC METHODS IN ANALYTICAL CHEMISTRY. By A. M. BOND. *Monographs in Electroanalytical Chemistry and Electrochemistry*. Pp. xviii + 518. Marcel Dekker. 1980. Price SFr135. ISBN 0 8247 6849 3.

With the greatly increased interest in electroanalytical methods over the last decade this book is a welcomed addition to the polarographic literature. The standard volumes on polarography (Kolthoff and Lingane, Meites, Heyrovsky and Kuta etc.) are now rather outdated or of little help concerning modern techniques and workers requiring up-to-date information have been forced in the past to obtain details from the original literature or review articles. Bond's book presents an attempt to bring together in one single volume a survey of all the important polarographic techniques (*e.g.*, sampled d.c., pulse and differential pulse, a.c. polarography and stripping voltammetry) along with many that are of only academic interest at present. The author's aim to provide a critical comparison of the various techniques at the electroanalyst's disposal has not, however, been totally successful.

A basic knowledge of conventional d.c. polarography is assumed and this is perhaps the book's weakness. The standard texts are becoming increasingly difficult to obtain and a new comer to

the field could have hoped for a "complete" up-to-date book. Extensive mathematical formulations have been avoided in the descriptions of the techniques and results presented simply without derivation. While the author considers that this facilitates the concise presentation of material of direct practical importance one is often left with the feeling of having gained little real new knowledge.

A general and useful theme throughout the book is the importance placed on understanding the electrode mechanism on which a determination is based. The polarographic response expected for reversible, quasi-reversible, irreversible processes and processes involving chemical steps are considered wherever possible for each technique so aiding the difficult problem of deciding which technique to use for a given assay. Unfortunately such a theme is not universal as, for many of the more exotic techniques, the theory is either not sufficiently advanced or, simply, experimental data is not available.

Considerable emphasis has been placed on a.c. polarography and rather less on pulse and stripping methods than would be expected from present day usage. The section on phase-sensitive fundamental harmonic a.c. polarography needs to be read with care as the discussion concerning background current is misleading and applies in many instances to the total current fundamental harmonic technique only.

There are numerous typographical errors and several odd labels on figures. Symbols are not consistently used throughout the text, for example ν and γ are used to represent kinematic viscosity. On page 245 t_p represents time for drop growth prior to pulse application while on page 351 it means pulse duration. All this is rather surprising and disappointing considering the preface suggests that the book has taken over a decade to write.

Even bearing in mind the above criticisms this is an important work bringing together for the first time a wealth of information from the literature along with much useful practical material gained from the author's own experience.

R. D. JEE

METHODEN DER ANALYTISCHEN CHEMIE. EINE EINFÜHRUNG. Band 2. NACHWEIS-UND BESTIMMUNGSMETHODEN. TEIL 1. By RUDOLF BOCK. Pp. viii + 362. Verlag Chemie. 1980. Price DM64. ISBN 3 527 25567 2.

The aim of this text is to present an introduction to the basic philosophy and techniques of modern analytical chemistry in a readily understandable form. The volumes are published in German and perhaps represent the up-to-date German language equivalents of standard English language texts, such as Fitz and Schenk, Ewing or Skoog and West; it is the opinion of this reviewer, however, that individual subject areas are not treated at the same depth as in the afore-mentioned texts so that the volume should be considered strictly introductory in nature.

Part 1 of this volume consists of two major sections. The first section is concerned with basic principles of analytical chemistry and precision and accuracy in analytical measurements. A review of elementary statistics related to such measurements is then given, and there is a clear section concerned with systematic errors. The text then goes on to define sensitivity and methods that may be used to improve accuracy. Electronic data processing methods and technology are then treated.

The second, and major chapter, of this text is concerned with analysis by measurement of electromagnetic radiation. There then follow 297 pp. concerned with absorption, emission and fluorescence techniques across the entire electromagnetic spectrum. This chapter is certainly comprehensive but does not deal in great depth with any of the techniques considered. The reader is referred to pertinent comprehensive theoretical and practical texts throughout when a more detailed understanding of an individual technique is required.

The text is clearly written (most English speaking readers should have little difficulty with its interpretation) and could be recommended for translation into English for use as an introductory text in courses designed to introduce instrumental analysis to students of chemistry, physics and biochemistry.

Part 2 of this volume will consider applications of instrumental techniques and should be most valuable for the practising analyst.

G. F. KIRKBRIGHT

FUNDAMENTALS OF FOOD CHEMISTRY. By WERNER HEIMANN. Translated by CHLOE MORTON. Pp. 344. Ellis Horwood/Avis Publishing Company. 1980. Price £22.50 (hardback); £7.50, $18 (softback). ISBN 085312 115 X/O 87055 356 9.

The book, which is in three parts, aims to serve as a basic introduction to food chemistry, and was published originally in German in 1968. This Revised Edition, which forms one of a series on food science and technology, has been translated by Chloe Morton.

Part 1, nutrition, consists of a single chapter with 1 page of text, which is little more than an expansion of the introduction. Parts 2 and 3 are more substantial although there are other short chapters; 3 pages on constituents of foods, 2 pages on minerals and trace elements, 5 pages on nutrient requirements, 2 pages on nutrient content and 3 pages on behaviour of foods during preparation and cooking. Thus 6 chapters out of 13 comprise some 16 pages of text in a book containing 320 pages (excluding reference material and an index).

Part 2 of the book brings together material from text-books on organic chemistry of proteins, fats and carbohydrates and their building blocks and puts this in the context of the complexes that are foodstuffs. Analytical matter is similarly correlated, for example, individual properties and reactions of amino acids are linked to their behaviour in foods and food digests. The properties of whole proteins are explained in the context of analytical reactions and functions in selected foods. Similar treatment is given to fats, carbohydrates, vitamins and enzymes. The fact that the book is a revision of an earlier text is perhaps most noticeable in a comparison of the contents of individual chapters, for example, the industrial extraction procedures for fats are given in some detail whilst newer technologies in protein and carbohydrates are but briefly mentioned.

Part 3 of the book contains 4 short chapters and one substantial one on food preservation. The principles of industrial processes, freezing, drying, sterilisation, irradiation and processing, are described briefly but succinctly. The Germanic origins of the book are also apparent in the sections on food additives in which pesticides and preservatives such as sulphur dioxide are classified as "incidental additives." The book is concluded with a useful list of supplementary reading, though strangely no mention is made of the commonly used journals; the analytical details are brief and citations are sketchy.

As an introductory text the book serves a useful purpose as it bridges the gap between classical text-books in chemistry and specialist texts on food technology and analysis; in this sense it can be regarded as useful to the student.

R. SAWYER

THE PARTICLE ATLAS. Second Edition. Volume V, LIGHT MICROSCOPY ATLAS AND TECHNIQUES; Volume VI, ELECTRON OPTICAL ATLAS AND TECHNIQUES. Volume V. By WALTER C. MCCRONE, JOHN GUSTAV DELLY and SAMUEL JAMES PALENIK; Volume VI edited by WALTER C. MCCRONE. Pp. vi + 1145–1456 (Volume V); and ii + 1457–1703 (Volume VI). Ann Arbor Science Publishers. 1980. Price £50 (Volume V); £50 (Volume VI). ISBN 0 250 40008 1 (Volume 5); 0 250 401961 (Volume VI).

With the rapid development of particle identification by optical and electron microscopy together with particle-analysis techniques the original Particle Atlas by McCrone covering many commonly found substances has been updated by the publication of two new volumes on particle morphology.

This collection of excellent colour photomicrographs of many particulate substances, which occur either naturally or from industrial processes as organic products or pollutants, will be of immense interest to microscopists, powder technologists and scientists who have to investigate environmental pollution, industrial effluents and forensic specimens. Volume V begins with the description of the techniques of particle characterisation by Hoffman modulation contrast and laser Raman microprobe analysis, which were not available for particle identification in the 1967 or 1973 editions of the Particle Atlas. These techniques together with dispersion staining and microchemical particle identification methods are comprehensively and lucidly described together with the advantages and limitation of each method for particle characterisation. The history, theory and instrumentation of each method are described and, in addition to a literature survey on microscopy methods, there is a comprehensive reference list for additional information if desired or required.

For fine particles that cannot be identified solely by optical morphological properties Volumes V and VI describe the specialised techniques of electron microprobe analysis (EMA), energy dispersal

X-ray analysis (EDXRA), Raman microprobe with laser excitation (MOLE) and electron microscope microprobe analysis (EMMA) and present high-quality black and white photographs at a series of increasing magnifications. The classification number of each substance is identical in both volumes to aid cross-reference.

The over-all presentation of Volumes V and VI of the Particle Atlas is of the same superb standard as seen in previous volumes. It is annoying, however, to be confronted with EDXRA figures that are askew and have not been correctly aligned in the process of publication and to see the spelling of novocaine as novacaine in Volume VI. An effort should have also been made to obtain a photoelectron micrograph of Halloysite (704) that indicated the tubular nature of this material. Likewise, the internal tubular nature of Avicel, which has more than one grade, is not readily seen in photograph 855.

The use of TEM, SEM and ion microprobe analysis (IMA), although of immense help in the analysis of sample mass in the nanogram or picogram range, has not, however, superseded the use of the optical microscope or polarised light microscopy (PLM) but has in fact complemented their use for identification of fine particles and is still capable of solving some problems that the more sophisticated instruments cannot. The advent of computer control and automation to ultra-microanalytical instrumentation, a subject discussed in the final section of Volume VI, will ensure that these two new volumes of the Particle Atlas will be readily used. The cost of the Particle Atlas, although an excellent reference book, may deter individual scientists and analysts from its purchase. However, efforts should be made to include the Particle Atlas within academic and industrial libraries.

N. G. STANLEY-WOOD

THE MEASUREMENT OF BREATH ALCOHOL. THE LABORATORY EVALUATION OF SUBSTANTIVE BREATH TEST EQUIPMENT AND THE REPORT OF AN OPERATIONAL POLICE TRIAL. By V. J. EMERSON, R. HOLLYHEAD, M. D. J. ISAACS, N. A. FULLER and D. J. HUNT. Pp. vi + 70. The Forensic Science Society and Scottish Academic Press. 1980. Price £10; $25. ISBN 0 9502425 78; ISSN 0015 7368.

This particular publication has been issued at a very opportune moment in the light of the additional drink - driving proposals in the Queen's speech in November 1980. It is the detailed report on the assessment of the substantive (evidential) breath testing equipment carried out by the Home Office in conjunction with several police forces (and published in *J., Forensic Sci. Soc.*, 1980, **20**, 3).

It covers the evaluation and comparison of the Intoxilyzer 4011A (an infrared absorption instrument), the gas chromatograph Intoximeter Mk IV, and the Breathalyzer 1000 (using visible light absorption through an acidic dichromate solution).

Descriptions of the equipment and the design of the trial are given in full. As is to be expected with such an important report, results are given in considerable detail both for laboratory studies on standard alcohol vapours and for the police station samplings. Altogether 991 valid comparisons of breath alcohol and blood alcohol were obtained by using four of each of the three instruments rotated around twelve operational sites scattered throughout Great Britain. In all instances the conversion of breath alcohol to blood alcohol on the instruments was carried out using the 1:2100 ratio.

In its comparison of the three instruments the report pulls no punches, drawing attention to design faults, failures in zero adjustments, loose control knobs, mouthpiece leakages and faulty electric cells. However, all instruments were operational for more than 95% of the time. Its conclusion is that no one type of instrument tested outshone the others and that substantive breath testing can be considered as a viable alternative to blood testing.

What is more questionable is the claim that " . . . each type of instrument produced results which were generally in good agreement with the certified blood results . . ." This statement is only valid if it is borne in mind that the instrument specifications for accuracy and precision are $\pm$10 mg per 100 ml, whilst the investigators obtained standard deviations ranging from 7.2 to 12.3 mg for two successive breath tests from the same subject. Also, insufficient emphasis is placed on the fact that figures obtained from breath alcohol values were corrected by as much as 10 mg because of delays between blood and breath sampling.

The report is addressed very clearly to the possibility of introducing substantive (evidential) breath tests in place of the present blood and urine sampling. It fails to make the point that whilst these instruments are suitable for replacing the blood sampling when suspects are well above or below the legal limit their accuracy makes it essential that a motorist should have a right to request the taking of a blood sample in more marginal cases.

This report is admirably presented and deserves to be carefully read by those who will have responsibility for changing and enforcing the future drink - drive laws.

R. C. DENNEY

TRACE CHEMISTRY OF AQUEOUS SOLUTIONS. GENERAL CHEMISTRY AND RADIOCHEMISTRY. By PETR BENEŠ and VLADIMÍR MAJER. *Topics in Inorganic and General Chemistry, Monograph* 18. Pp. 252. Elsevier. 1980. Price $56; Dfl115. ISBN 0 444 99798 9.

The subject of trace chemistry, in all its aspects, is expanding rapidly. As it is a topic that calls upon a wide array of instrumental and chemical techniques, it infiltrates almost every branch of chemical analysis. There are countless texts available on various areas of trace analysis, but the present one is completely different from any previous book in its approach, for it deals with physico - chemical properties and reactions at very low concentrations.

A good deal of emphasis is placed on radiochemical methods; hence the reactions studied are generally the many and varied phenomena of precipitation, adsorption, co-precipitation, colloidal behaviour and the influence of various factors on these phenomena. The effect of certain variables on co-crystallisation is studied and the general kinetics of co-precipitation. An attempt is made to classify the various types of co-precipitation, but this is a classification that has never been satisfactory and this attempt leaves one with some doubts. Recently, of course, IUPAC has published a recommended classification.

This is a very interesting book and contains a great deal of unusual information; it supplies over 1 000 references. All those concerned with trace analysis will find it rewarding to browse through this volume. Sometimes, when the authors wax philosophical, it is a little difficult to understand what exactly is meant, but this does not detract in any way from the value of a well conceived and authoritatively written monograph.

R. BELCHER

LIQUID SCINTILLATION COUNTING PRACTICE. By A. DYER. Pp. xiv + 100. Heyden. 1980. Price $18; £8; DM37.50. ISBN 0 85501 466 0.

This book on liquid scintillation counting technique is based on the earlier "Introduction" by the same author published in 1974. It is in a slightly larger format than the previous publication and is essentially a reprint of this earlier excellent book, but with the addition of a chapter on radioimmunoassay techniques.

The whole book gives a very good over-all survey of the field of liquid scintillation counting principles and practice, and it is a particularly useful book for those potential users of this technique who require a general knowledge of its capabilities, as well as providing a reference guide to key works for a more detailed study. The introductory chapters on the technique and fundamentals of the scintillation process itself, as well as the description of instrumentation, are particularly clearly and concisely presented. The sample preparation procedures are adequately reviewed to provide the reader with a good knowledge of the method he should employ. The scope of the technique in its possible application to other isotopes is more fully dealt with and there is a particularly informative section on the nature of quenching and its correction. Some practical hints are given in Chapter 7 with comparisons of vials, caps and problems often encountered with the different techniques described. A penultimate chapter reviews other uses of the liquid scintillation counter including flow cells, Cerenkov counting and the analytical use of chemiluminescence and bioluminescence.

This is a well presented introductory work and is a must for all those students and scientists who believe that they may have a use for this type of analytical approach in their work.

B. W. Fox

ANNUAL REPORTS ON ANALYTICAL ATOMIC SPECTROSCOPY. Volume 9. Reviewing 1979. Edited by J. B. DAWSON and B. L. SHARP. Pp. xii + 345. The Royal Society of Chemistry. 1980. Price £34; $94.25 (RSC Members £22). ISBN 0 85186 727 8; ISSN 0306 1353.

Volume 9 of this series contains over 2 000 references to publications appearing and conference papers presented during 1979. These are incorporated into a series of concise reviews and tables covering Atomisation and Excitation (Chapter 1), Instrumentation (Chapter 2), Methodology (Chapter 3) and Applications (Chapter 4). As each of these chapters is subdivided into several clearly defined sections, it is a relatively easy matter for the reader to assimilate literature relevant to any particular topic and thus to assess the state of the art in areas extending from fundamental studies on inductively coupled plasmas to dissolution methods associated with analysis of trace elements in plants. In a nutshell, this volume continues the valuable work of its predecessors and the reviewer assumes that those who have subscribed to this series in the past will need no urging to continue their subscriptions. Those unacquainted with the series are strongly encouraged to take advantage of its availability. This reviewer has always found it an invaluable asset.

The Foreword contains interesting comments, one of which, *viz.*, that concerning duplication of effort, deserves expansion. The causes of duplication appear to be several. Among these are the appearance of information in a wide variety of journals, many not remotely associated with analytical chemistry; a lack of understanding of fundamental aspects that leads to *ad hoc* solutions, which, although effective, may not themselves be understood; the fact that the concept of particular problems frequently changes, thus requiring a reassessment of so-called solutions; and the fact that there may be more than one adequate solution to a problem.

Science is dynamic indeed, and in reporting the different attempts to solve problems "Annual Reports on Analytical Atomic Spectroscopy" provides a valuable on-going commentary on the continuing development of the subject as it is evolving and in doing so fulfills an important role in the literature of chemical analysis.

JOHN AGGETT

Potentiometry of Alkoxylates

The response characteristics of barium ion-selective electrodes to various alkoxylates have been investigated in aqueous and aqueous - ethanol test solutions in the presence and absence of barium ions and in the presence of sodium and potassium salts.

The observed potentiometric response is an increase in the e.m.f. of the barium ion-selective electrode of up to about 100 mV, according to the amount of added alkoxylate in the 2×10^{-5}–10^{-3} M range. The response is linear with log[alkoxylate], but is characterised by a break in the linearity, which is attributed to the critical micelle concentration (CMC) of this class of non-ionic surfactants.

The alkoxylates studied include nonylphenoxypoly(ethyleneoxy)ethanols (NPs) of the Antarox CO series, namely, CO-430, -630, -730, -850, -880 and -890; octylphenoxypoly(ethyleneoxy)ethanols (OPs), namely, Antarox CA-620 and Triton X-100; a sorbitan-9-octadecanoate poly(ethyleneoxy)ethanol, namely, Tween 80; alkylpoly(ethyleneoxy)ethanols, namely, Dobanol 25–7, Lutensol AO7 and Synperonic 7; a fluoroalkyl poly(ethyleneoxy)ethanol, namely, Monflor 51; poly(ethylene glycol) (PEG)1540; and poly(propylene glycol) (PPG)1025. CMCs were characterised in all instances except for PEG 1540, where the increases in the e.m.f. values were smaller than for the other materials studied.

Anionic surfactants gave small decreases in e.m.f., but the cationic surfactant, benzyldimethylhexadecylammonium chloride, gave a large increase in e.m.f., characteristic of a cationic response.

Exposure to alkoxylates reduced the lifetime of the barium ion-selective electrodes.

Keywords: Non-ionic surfactants; alkoxylates; poly(alkylene glycols); ion-selective electrodes; critical micelle concentration

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Analyst, 1981, **106**, 439–447.

Application of Gas - Liquid Chromatography to the Analysis of Essential Oils

Part VIII. Fingerprinting of Essential Oils by Temperature-programmed Gas - Liquid Chromatography Using Methyl Silicone Stationary Phases

Problems of obtaining reproducible results in the "fingerprinting" of essential oils by temperature-programmed gas - liquid chromatography have been examined and reported in Part VII of this series. That report was concerned both with the general problems and with the specific use of a polar stationary phase, *i.e.*, Carbowax 20M. This report is concerned with the use of non-polar stationary phases of the methyl silicone type and the application of the method of column standardisation described in Part VII.

A collaborative study with methyl silicone stationary phases and a specification of "*g*-pack values" for the column packing has resulted in the production of a method that yields reproducible relative retention indices for the test substances limonene, acetophenone, linalol, naphthalene, linalyl acetate and cinnamyl alcohol and has been applied with satisfactory results to oils of bergamot, Jamaican ginger, Nigerian ginger, West Indian nutmeg and East Indian nutmeg. A recommended method is given for the reproducible temperature-programmed gas - liquid chromatographic fingerprinting of essential oils when methyl silicone stationary phases are used.

Keywords: Essential oils analysis; temperature-programmed gas - liquid chromatography; methyl silicone stationary phases

ANALYTICAL METHODS COMMITTEE

The Royal Society of Chemistry, Burlington House, Piccadilly, London, W1V 0BN.

Analyst, 1981, **106**, 448–455.

Application of Gas - Liquid Chromatography to the Analysis of Essential Oils
Part IX. Determination of Eugenol in Oil of Cinnamon Bark

The essential oil obtained from cinnamon leaves has a high content of eugenol whereas the genuine oil from cinnamon bark has a low eugenol content. Determination of the eugenol content of a sample reputed to be cinnamon bark oil provides a method for the detection of adulteration by addition of cinnamon leaf oil. This determination has been carried out by gas - liquid chromatography using a non-polar stationary phase.

Keywords: *Essential oils analysis*; *oil of cinnamon bark*; *eugenol determination*; *gas - liquid chromatography*

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The Royal Society of Chemistry, Burlington House, Piccadilly, London, W1V 0BN.

Analyst, 1981, **106**, 456–460.

Rapid Direct Complexometric Determination of Palladium(II) with EDTA

Short Paper

Keywords: *Palladium(II) determination*; *propionyl promazine phosphate indicator*; *EDTA titration*; *complexometry*

B. KESHAVAN
Department of Postgraduate Studies and Research in Chemistry, Manasa Gangotri, University of Mysore, Mysore 570 006, India.

Analyst, 1981, **106**, 461–464.

Determination of Arsenic by Emission Spectrometry Using an Inductively Coupled Plasma Source and the Syringe Hydride Technique

Short Paper

Keywords: *Arsenic determination*; *emission spectrometry*; *inductively coupled plasma*; *syringe hydride generation technique*

C. J. PICKFORD
Environmental and Medical Sciences Division, AERE, Harwell, Oxfordshire, OX11 0RA.

Analyst, 1981, **106**, 464–467.

Simultaneous Determination of Trace Amounts of Arsenic, Antimony and Bismuth in Herbage by Hydride Generation and Inductively Coupled Plasma Atomic-emission Spectrometry

Short Paper

Keywords: *Arsenic, antimony and bismuth determination*; *herbage*; *hydride generation*; *inductively coupled plasma*; *atomic-emission spectrometry*

BEHROOZ PAHLAVANPOUR, MICHAEL THOMPSON and LAURENCE THORNE
Applied Geochemistry Research Group, Department of Geology, Imperial College, University of London, SW7 2BP.

Analyst, 1981, **106**, 467–471.

Adsorption of Trace Metals During Filtration of Potable Water Samples with Particular Reference to the Determination of Filtrable Lead Concentration

Short Paper

Keywords: *Trace metal determination*; *potable water*; *filtrable lead*; *adsorption*; *filter-membrane*

M. J. GARDNER and D. T. E. HUNT
Analytical Division, Water Research Centre, Medmenham Laboratory, P.O. Box 16, Medmenham, Marlow, Buckinghamshire, SL7 2HD.

Analyst, 1981, **106**, 471–474.

Differential-pulse Voltammetry of Sulphur Dioxide at the Parts per 10^9 Level in Air

Short Paper

Keywords: *Sulphur dioxide determination; differential-pulse voltammetry*; *air pollution*

A. RIGO, M. CHERIDO, E. ARGESE, P. VIGLINO and C. DEJAK
Institute of Physical Chemistry, University of Venice, Venice, Italy.

Analyst, 1981, **106**, 474–478.

Spectrophotometric Assay of Bromhexine Hydrochloride and its Application to Binary Bromhexine - Antibiotic Mixtures

Short Paper

Keywords: *Bromhexine hydrochloride assay*; *antibiotic assay*; *visible spectrophotometry*; *ultraviolet spectrophotometry*

W. J. BOWTLE, AUDREY P. PRINCE and D. J. MORTIMER
The Lilly Research Centre Ltd., Erl Wood Manor, Windlesham, Surrey, GU20 6PH.

Analyst, 1981, **106**, 478–481.

Determination of α-Halocarbonyl Compounds by Reaction with Thiourea

Short Paper

Keywords: *α-Halocarbonyl compounds*; *thiourea*; *titrimetry*

SUMAN MUKHIJA and K. S. BOPARAI
School of Studies in Chemistry, Vikram University, Ujjain, 456 010, India.

Analyst, 1981, **106**, 482–483.

Rapid Spectrophotometric Determination of Nitrate with 4,5-Dihydroxycoumarin

Short Paper

Keywords: *Nitrate determination*; *spectrophotometry*; *4,5-dihydroxycoumarin*; *chloride*; *nitrite*

MOTOSHI NAKAMURA

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Analyst, 1981, **106**, 483–487.

Determination of Formaldehyde in the Atmosphere: Observations Concerning the Storage of Aqueous Samples

Communication

Keywords: *Formaldehyde determination*; *atmosphere*; *water sample storage*; *biocides*

P. R. LUDLAM and J. G. KING

Borden (UK) Limited, North Baddesley, Southampton, SO5 9ZB.

Analyst, 1981, **106**, 488–489.

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