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A237 for further information. See page xviii ⟶

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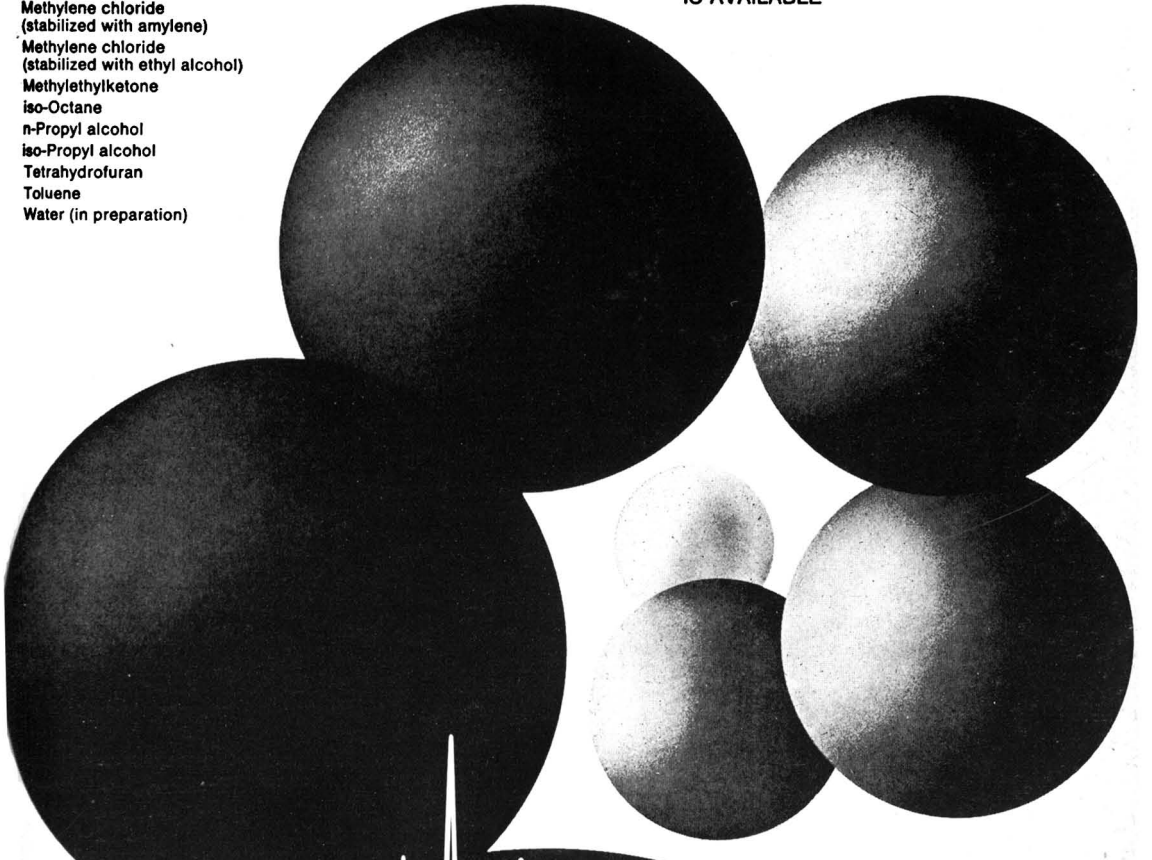

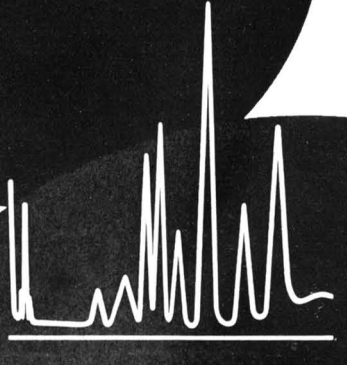

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Summaries of Papers in this Issue

Determination of Dithiocarbamates by Liquid Chromatography Using Transition-metal Salts as "Ion-pair" Reagents

A technique has been developed for the liquid-chromatographic determination on an octadecylsilyl silica reversed-phase column of *N*-alkyl- and *NN*-dialkyldithiocarbamates by adding transition-metal salts of cobalt(II) or nickel(II) to the mobile phase. The unsuccessful use of mercury(II), copper(II) and lead(II) is discussed and the mixed complexes formed when two dithiocarbamates are injected are described. The retentions of the dithiocarbamates are compared with those of related thiuram disulphides.

Keywords: *Dithiocarbamate determination*; *thiuram disulphide determination*; *liquid chromatography*; *complex formation*; *transition-metal salts*

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Analyst, 1981, **106**, 129–134.

Determination of Polynuclear Aromatic Hydrocarbons in Food, Water and Smoke Using High-performance Liquid Chromatography

A method for the separation of 17 different polynuclear aromatic hydrocarbons (PAHs) has been developed. After separation by thin-layer chromatography, the PAHs are examined by high-performance liquid chromatography using ODS and PPS stationary phases. The elution times of all 17 compounds relative to benzo[*a*]pyrene have been computed. Six representative compounds have been added to bierwurst and to water to check recovery values. Work on stubble smoke has been confined to benzo[*a*]pyrene only. The method of extraction and clean-up varies with the substrate but the method of determination of the PAH compounds in the final extract is the same in all three cases. Confirmation of identity is obtained by measuring the peak-height ratios for each PAH at two different excitation and emission wavelength combinations. The limit of detection of benzo[*a*]pyrene is 0.02 μg kg^{-1} in food, 0.3 ng l^{-1} in water and 150 pg per filter in smoke. Recoveries of the six representative compounds are generally in the range 75–100% at levels approaching the limit of detection.

Keywords: *High-performance liquid chromatography*; *polynuclear aromatic hydrocarbons*; *food*; *water*; *stubble smoke*

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Analyst, 1981, **106**, 135–145.

Voltammetric Determination of 2-, 3- and 4-Chloroaniline in Mixtures

The oxidative voltammetric behaviour of 2-, 3- and 4-chloroaniline has been investigated at the glassy carbon electrode over the pH range 1–12. The optimum pH values for the linear-sweep and differential-pulse voltammetric determination of these compounds were found to be 1.95 for 2- and 3-chloroaniline and 8.1 for 4-chloroaniline. Differentiation of the three compounds in a mixture was achieved by using a combination of high-performance liquid chromatography with voltammetric detection. This method could be used to determine concentrations of 2- and 4-chloroaniline down to 2 ng and 3-chloroaniline down to 1 ng for a 20-μl injection on to the chromatographic column.

Keywords: *2-, 3- and 4-chloroaniline determination; oxidation; high-performance liquid chromatography; voltammetric detection*

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Analyst, 1981, **106**, 146–152.

Separation of Protein-bound Copper and Zinc in Human Plasma by Means of Gel Filtration - Ion-exchange Chromatography

A procedure for the separation of protein-bound copper and zinc fractions and of potassium and magnesium in plasma, involving chromatography on columns of DEAE-Sepharose CL-6B, is described. Copper, magnesium and zinc were measured by atomic-absorption spectroscopy and potassium by flame photometry. Most plasma samples yielded one copper and two zinc fractions and these were identified by the use of protein "markers." Most of the copper appeared to be bound to caeruloplasmin and a lesser amount to albumin, whereas zinc appeared to be bound mainly to globulins with a smaller, variable, amount bound to albumin. Magnesium and potassium appeared as single peaks eluting in approximately the same fractions, ahead of the albumin peak.

Keywords: *Copper; zinc; plasma proteins; chromatography; atomic-absorption spectroscopy*

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Analyst, 1981, **106**, 153–159.

Gas - Liquid Chromatographic Determination of Major Constituents of *Piper methysticum*

A procedure is described for the quantitative determination of seven known major constituents in sun-dried roots, rhizomes and commercially powdered samples of *Piper methysticum*. A 3.0–8.0-g amount of powdered sample is extracted with chloroform in a Soxhlet apparatus for 6 h. After evaporation of solvent the extract (about 0.35 g) is dried at 100 °C for 2 h and then dissolved in chloroform to about 0.7% *m/V* concentration. The resulting solution is analysed by gas - liquid chromatography using dual 1.5 m × 4 mm i.d. glass columns, containing 3% *m/m* of OV-1 on Chromosorb W HP, and dual differential flame-ionisation detectors with nitrogen as carrier gas, the column temperature being 210 °C. There is no interference from the eight other trace constituents, non-polar low-boiling compounds or polar "tarry" material.

Keywords: Piper methysticum *analysis*; *gas - liquid chromatography*

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Analyst, 1981, **106**, 160–165.

Gas-chromatographic Determination of Trace Amounts of Lower Fatty Acids in Ambient Air Near and in Exhaust Gases of Some Odour Sources

The gas-chromatographic determination of trace amounts of the lower fatty acids (C_2–C_5) in ambient air near and in the exhaust gases of some odour sources was investigated. The sample for the gas-chromatographic determination was prepared by trapping in a pre-column packed with FFAP + orthophosphoric acid (H_3PO_4) on Carbopack C at 25 and 30 °C. The lower fatty acids were identified and quantitated from the difference between the chromatograms obtained using the FFAP + H_3PO_4 pre-column and that obtained using the FFAP + H_3PO_4 plus an alkaline pre-column. The method has been applied to the analysis of lower fatty acids in practical specimens, namely the ambient air near accumulated poultry manure, in a pig pen and a fish meal factory, and in the exhaust gases from a corn starch manufacturing factory and from a poultry manure dryer. The sample volume is as low as about 0.4 l and the method is sensitive (detection limit about 0.5 p.p.b.) and rapid (including the concentration and analysis of one sample, about 15 min are required). The coefficient of variation is less than 6%. This sensitivity and precision are adequate for use in odour pollution analysis.

Keywords: *Lower fatty acid determination*; *air analysis*; *pre-column concentration*; *odour sources*

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Analyst, 1981, **106**, 166–171.

Determination of Trace Elements in Plant Materials by a Dry-ashing Procedure. Part I. Cobalt and Molybdenum

Dry-ashing and analytical procedures for the spectrophotometric determination of cobalt and molybdenum in ashed plant extracts are described. A two-stage ashing process using nitric acid combined with either potassium hydrogen sulphate or sulphuric acid as ashing aids and oxygen enrichment during ashing facilitated the complete oxidation of plant materials.

Modified spectrophotometric procedures were devised for the quantitative determination of cobalt and molybdenum as the 2-nitrosonaphth-1-olate and toluene-3,4-dithiolate complexes in carbon tetrachloride. The extraction, chelation and phase-separation steps simplified existing published techniques, permitted more rapid sample handling, controlled interferences more effectively and provided more accurate assays. The molar absorptivities for cobalt and molybdenum were 5.1×10^4 and 2.5×10^4 l mol^{-1} cm^{-1}, respectively, and the detection limits for both elements were 4 ng g^{-1}.

Keywords: *Dry ashing*; *plant material*; *cobalt and molybdenum determination*; *spectrophotometry*

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Analyst, 1981, **106**, 172–181.

Determination of Trace Elements in Plant Materials by a Dry-ashing Procedure. Part II. Copper, Manganese and Zinc

A method is described for determining copper, manganese and zinc in ashed plant extracts by flame atomic-absorption spectrophotometry after cobalt and molybdenum have been assayed on separate aliquots of the same plant extracts. Ashing aids were necessary to maintain accuracy in the determinations. Concentrations of up to 3.5% *m/m* of silicon and calcium and 4% *m/m* of chlorine in the plants did not affect the determinations, but in some instances lower concentrations were determined in plant samples containing equal or higher levels of both added silica and calcium.

Potential interference was prevented during assays for copper by automatic background correction, for manganese and zinc by diluting the extract, and for all three analytes by selectively matching their chemical matrices with hydrochloric acid and potassium sulphate concentrations in respective working standards. By these procedures, assays for the three elements were similar to determinations by flame atomic-absorption spectrophotometry on samples digested by a standard wet-digestion method.

Keywords: *Dry ashing*; *plant material*; *copper, manganese and zinc determination*; *flame atomic-absorption spectrophotometry*

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Analyst, 1981, **106**, 182–187.

Assessment of Phosphorescence Spectroscopy for Crude Oil Identification

The application of phosphorescence techniques to crude oil identification has been studied. Conventional phosphorescence spectra are insufficiently structured to offer adequate discrimination between the crude oil samples investigated. Synchronous excitation and total contour spectra of a series of crude oils have been compiled. The latter techniques show promise as a method for differentiation between unweathered crude oils. Spectral discrimination is enhanced by the use of an external "heavy atom" quencher.

Lifetime measurements of phosphorescence decay offer limited scope, with respect to crude oil characterisation, owing to the complex nature of the observed decay rates.

Keywords: *Crude oil identification*; *phosphorescence*

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Analyst, 1981, **106**, 188–197.

Simultaneous Spectrophotometric Determination of Palladium(II) and Gold(III) with Methiomeprazine Hydrochloride: Analysis of Alloys and Minerals

Methiomeprazine hydrochloride (MMH) is proposed as a reagent for the simultaneous spectrophotometric determination of palladium(II) and gold(III). The reagent instantaneously forms an orange - red 1 : 1 complex with palladium(II) and a blue species with gold(III) in hydrochloric acid - sodium acetate buffer. The palladium - MMH complex has an absorption maximum at 480 nm with a molar absorptivity of 3.6×10^3 l mol^{-1} cm^{-1}. The blue species has an absorption maximum at 630 nm with a molar absorptivity of 1.3×10^4 l mol^{-1} cm^{-1}. Beer's law is obeyed for the range 0.4–21 p.p.m. of palladium(II) and 0.2–10 p.p.m. of gold(III). Molar excesses of 4-fold and 35-fold of the reagent are required for palladium(II) and gold(III), respectively. The concentration of two metals in an unknown mixture can be simultaneously determined in the range 0.5–15 p.p.m. of palladium(II) and 1–10 p.p.m. of gold(III) using the following equations:

$$[\mathrm{Pd}] \times 10^4 = 3.023A_{480} - 0.8403A_{630}$$

$$[\mathrm{Au}] \times 10^4 = 0.8177A_{630} - 0.1959A_{480}$$

The method has been used successfully for the determination of palladium and gold in alloys and minerals.

Keywords: *Palladium and gold determination*; *spectrophotometry*; *methiomeprazine hydrochloride reagent*

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Analyst, 1981, **106**, 198–205.

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Determination of Dithiocarbamates by Liquid Chromatography Using Transition-metal Salts as "Ion-pair" Reagents

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A technique has been developed for the liquid-chromatographic determination on an octadecylsilyl silica reversed-phase column of *N*-alkyl- and *NN*-dialkyldithiocarbamates by adding transition-metal salts of cobalt(II) or nickel(II) to the mobile phase. The unsuccessful use of mercury(II), copper(II) and lead(II) is discussed and the mixed complexes formed when two dithiocarbamates are injected are described. The retentions of the dithiocarbamates are compared with those of related thiuram disulphides.

Keywords: *Dithiocarbamate determination*; *thiuram disulphide determination*; *liquid chromatography*; *complex formation*; *transition-metal salts*

A number of methods have been developed for the determination of *N*-alkyl- and *NN*-dialkyldithiocarbamates and their salts and related thiuram disulphides,[1] because of their widespread use as fungicides, rubber vulcanisers and as pharmaceuticals.[2] However, many of these methods, including ultraviolet spectroscopy, colorimetry with copper salts and the widely used acid degradation to give carbon disulphide, are specific only for the dithiocarbamate group and cannot distinguish between compounds with different *N*-alkyl substituents.

As part of a study of the degradation of the cyclic fungicide dazomet (3,5-dimethyltetrahydro-1,3,5-thiadiazine-2-thione), we developed and briefly reported a liquid-chromatographic technique in which the individual dithiocarbamates were separated using a solvent containing nickel or cobalt salts as "ion-pair" reagents.[3] Although the sodium salts were unretained on chromatography, the non-aqueous soluble metal-ion complexes formed in the eluent were readily separated on a reversed-phase column. In this paper we describe our qualitative and quantitative studies in detail and discuss the examination of further metal ions as potential complexation reagents.

The interaction of dithiocarbamates, in particular *NN*-diethyl- and *NN*-tetramethylenedithiocarbamate, with transition-metal ions to form complexes extractable into organic solvents has been used in analytical chemistry for the separation[4] and spectrophotometric determination of a number of metals.[5] The complexes, after extraction, can be separated by thin-layer chromatography (*e.g.*, reference 6), gas chromatography (*e.g.*, reference 7) or liquid chromatography on silica gel,[8–14] bonded nitrile[15–16] or hydrocarbon (reversed-phase) columns.[17–19] In most instances the separation has been aimed at the determination of the metal ion rather than of the ligand.

Experimental

Apparatus

Liquid chromatography was carried out using a Water Associates 6000 pump connected to a Shandon Southern column (10 cm × 5 mm i.d.) packed with Hypersil-ODS and fitted with a Rheodyne 7010 valve injector with a 10-μl loop. The eluates were detected using an ALC 202 detector at 254 nm. The solvent flow-rate was 1.5 cm^3 min^{-1}.

Reagents and Standards

Metal salts. Analytical-reagent grade nickel sulphate, cobalt nitrate, copper nitrate, mercury(II) nitrate and lead nitrate were used.

Dithiocarbamates. The sodium salts of *N*-methyl-, *NN*-diethyl- and *NN*-dimethyldithiocarbamate were supplied by J. D. Cambell and Sons Ltd., Warrington, Sigma London Chemical Co. Ltd., Poole, and Aldrich Chemical Co. Ltd., Gillingham, respectively. Ammonium *NN*-tetramethylenedithiocarbamate was supplied by Sigma London Chemical Co., Ltd., Poole. Sodium *N*-ethyldithiocarbamate and ammonium dithiocarbamate were synthesised from the corresponding amine and carbon disulphide.[2]

Thiuram disulphides. Thiram (*NNN'N'*-tetramethylthiuram disulphide) was supplied by Robinson Bros. Ltd., West Bromwich, and disulfiram (*NNN'N'*-tetraethylthiuram disulphide) was supplied by Sigma London Chemical Co. Ltd., Poole.

Methanol. HPLC grade from Fisons Scientific Apparatus, Loughborough.

Procedure

Solutions of the dithiocarbamates or thiuram disulphides, approximately 500 mg dm^3 in methanol - water, were injected on to the column. Different ratios of methanol and water, containing 0.1% *m/V* of the metal salts, nickel sulphate, cobalt nitrate, copper nitrate or lead nitrate, were used as indicated for the eluent. In some runs 0.05 M phosphate buffer, pH 5, containing the metal ions was used.

As prolonged elution of methanol - water mixtures did not remove all the trace amounts of cobalt(II) from the column, a different column was used for each metal ion.

Results and Discussion

On examination of solutions of the sodium salts of *N*-methyl- or *NN*-diethyldithiocarbamic acid by liquid chromatography using an octadecylsilyl silica reversed-phase column, the solutes were eluted at the solvent front ($k' = 0$) even if water alone was used as the solvent. In some runs the sample appeared to interact with the column and no peaks were observed. Attempts to control the ionisation of the dithiocarbamates by using a phosphate buffer at pH 5 as the eluent had no effect. A lower pH was not used because the dithiocarbamates are sensitive to acid and are rapidly degraded to carbon disulphide and an amine.[5] The addition of the organic ion-pair reagents tetrabutylammonium hydroxide and cetrimide to the eluent was also studied but their use appeared to result in complete degradation of the sample. The thiuram disulphides thiram and disulfiram could be readily chromatographed without decomposition.

As a number of metal-ion complexes of *NN*-diethyl- and *NN*-tetramethylenedithiocarbamates have been successfully chromatographed on RP-8 and RP-18 reversed-phase columns,[19] it was decided to investigate the use of transition-metal ions as potential ion-pair reagents. It was hoped that on injection of the dithiocarbamate salts, the metal ions would form stable neutral complexes, suitable for chromatography. Despite the common use of organic ion-pair reagents to improve the separation or efficiency of polar compounds on liquid chromatography, there are few reports of the use of metal ions. Examples include the interaction of zinc ions with aminobenzoic acids,[20] nickel ions with aniline[21] and its metabolites and a zinc or cadmium C_{12}-dien complex with the dansyl amino acids and sulphonamides.[22,23] The more common addition of silver ions to solvents to alter the retention of olefins results not in the formation of a neutral ion-pair complex but of a polar π-complex with a shorter retention time on a reversed-phase column than the original olefin (*e.g.*, reference 24).

After initial trials using separately prepared complexes a range of solvents containing different proportions of methanol and 0.1% solutions of nickel(II) sulphate or cobalt(II) nitrate were examined. A series of sodium or ammonium salts of dithiocarbamates and related thiuram disulphides were individually injected and their capacity factors were determined (Table I). The complexes were readily detected at 254 nm, with no background interference from the metal salts in the solvent. The peak efficiencies of the complexes were comparable to those of uncomplexed eluates and suggested that mixing and complex formation occurred rapidly on injection.

A number of important fungicides, including zineb, maneb and mancozeb, are based on ethylenebisdithiocarbamic acid, $(CH_2NHCS_2)_2^{2-}$; however, its metal complexes are polymeric and attempts to examine the sodium salt using cobalt as a reagent were unsuccessful. Many dithiocarbamates form stable silver(I) salts but a trial using silver nitrate appeared to result in decomposition of the ethylenebisdithiocarbamate.

TABLE I

CAPACITY FACTORS OF DITHIOCARBAMATES AND THIURAM DISULPHIDES ON LIQUID CHROMATOGRAPHY WITH NICKEL AND COBALT REAGENTS

	Solvent					
	Methanol - water containing 0.1% Co(II) as nitrate				Methanol - water containing 0.1% Ni(II) as sulphate	
	30+70*	50+50	60+40	70+30	60+40	70+30
Dithiocarbamate—						
N-H_2-	0.25	—	—	—	—	—
N-Methyl-†	11.8	1.25	0.71	0.50	1.25	0.57
N-Ethyl-	—	7.25	2.71	1.37	3.37	1.28
NN-Dimethyl-	—	10.6	3.85	2.25	4.12	1.57
NN-Diethyl-	—	—	>30	24.7	32.1	7.14
NN-Tetramethylene-	—	—	26.5	11.8	—	—
Thiuram disulphide—						
NNN'N'-Tetramethyl (thiram)	—	2.75	1.75	0.88	1.25	0.86
NNN'N'-Tetraethyl (disulfiram)	—	—	12.7	6.37	11.8	3.71

* Methanol - 0.05 M phosphate buffer adjusted to pH 5.

† These results were obtained using methanol - water containing 0.1% Ni(II) as sulphate: 50+50, $k' = 2.86$; 40+60, $k' = 10.4$; 30+70, $k' = 25.4$.

In addition to the cobalt and nickel complexes, Schwedt also successfully chromatographed the complexes of copper, mercury and lead, although the last was unstable.[19] As each of the complexes has a different capacity factor, it appeared that it would be possible by selection of the appropriate metal ion to adjust the retention times of dithiocarbamates, when they are present in a mixture, to give the optimum resolution. The complexes of most other metal ions including iron(III), manganese, silver, chromium(III), molybdenum and vanadium were found either to decompose or to give several bands.[19]

Although the reaction with diethyldithiocarbamate followed by extraction and spectrophotometry of the complex is a widely used assay for copper(II),[2,5] when copper nitrate was used as a reagent the results were erratic and reproducible peaks could not be obtained. In addition, a strong interaction between the copper ions and thiuram disulphides appeared to take place, presumably similar to the reactions that cause colour changes on mixing aqueous solutions of disulphides and copper(II) ions,[1] whereas with nickel or cobalt ions any effect was minimal. In order to determine the product that was being formed on injection, a solution of copper(II) ions was mixed with diethyldithiocarbamate and the precipitate was dissolved by the addition of methanol. The resulting solution has a $\lambda_{max.}$ at 386 nm, whereas if the copper - dithiocarbamate complex was extracted from the aqueous solution into chloroform and then diluted with methanol - water the absorbance of the solution had a $\lambda_{max.}$ at 434 nm. The latter band corresponds to the neutral ML_2 complex, whereas the former is very similar to the ML^+ water-soluble complex formed with *NN*-diethanolaminodithiocarbamate and copper ions (ML^+, $\lambda_{max.}$ at 380 nm, and ML_2, $\lambda_{max.}$ 435 nm).[25] Thus, when used as a reagent the copper(II) ions, which are in excess, appear to form the ML^+ complex rather than the neutral ML_2 complex, which can be chromatographed. Samples of the ML_2 copper complex prepared by extraction could therefore be readily chromatographed.

Mercury(II) ions are reported to form the strongest complexes with diethyldithiocarbamate and will displace all other metal ions from complexes.[26] However, attempts to chromatograph mercury(II) diethyldithiocarbamate were unsuccessful and no peaks were obtained. Although the extracted complex is very stable, the equilibrium constant for the reaction $2HgL^+ \rightleftharpoons HgL_2 + Hg^{2+}$ is 0.[27] Therefore, the complex forms stepwise so that in the presence of an excess of mercury(II) ions only HgL^+ will be present.

In contrast to mercury and copper, lead ions form much weaker complexes[26] and it was felt that they would be a useful comparison. Schwedt found the complexes to be unstable,[19] and in two papers reported very different retentions, relative to the other complexes.[18,19] In this study an attempt to use lead nitrate as a reagent was unsuccessful, and the complex when prepared by extraction was not eluted on chromatography. A recent report has suggested that

the lead complex decomposes in solution in isobutyl methyl ketone to give the free metal after 2–3 h[28] although other papers[29] considered the complex to be stable under these conditions. Extraction of lead ions by ammonium tetramethylenedithiocarbamate was also found to fail with both high and low proportions of dithiocarbamate.[28] The disulphides chromatographed essentially unaltered in the presence of lead ions.

Two previous studies have examined in detail the separation of nickel(II) complexes on silica gel columns[8,12] and as in our degradation studies the cobalt complex gave a more suitable retention time relative to the other components in the mixture, a detailed examination of the use of cobalt(II) was carried out.

The pH of the aqueous solution was not critical and was usually not controlled except for the degradation studies.[3] In the degradation studies, as the samples were in phosphate buffers at a range of pH values it was necessary to use a pH 5 phosphate buffer in the solvent to prevent precipitation of cobalt phosphate. In extraction studies both cobalt and nickel complexes are completely extracted between pH 2 and 12.[4] Different concentrations of cobalt nitrate, 0.01, 0.5 and 0.1%, gave the same capacity factors and it is assumed that complex formation is essentially complete.

As part of the degradation study a calibration graph was prepared for *N*-methyldithiocarbamate using caffeine as an internal standard (Table II). The graph is linear from 100 to 1000 μg ml^{-1}, but a small negative intercept, also found on other calibration runs, suggested a small but constant amount of decomposition.

TABLE II

CALIBRATION OF *N*-METHYLDITHIOCARBAMATE USING METHANOL - pH 5 PHOSPHATE BUFFER [0.1% Co(II) AS NITRATE] (30+70) AS SOLVENT

Sample concentration*/ μg ml^{-1}	Peak height, scale units: *N*-Methyldithiocarbamate†	Peak height, scale units: Caffeine (internal standard)†	Ratio, sample × 10^3 to internal standard
100	6	183	32
200	14.5	183	79
300	23	183	126
400	31	177.5	175
500	40.5	177	229
600	49	178	275
700	58	179	324
800	65	178.5	364
1000	79	177	446

Slope	0.4682
Intercept	−11.5
Correlation coefficient	0.9991
Standard deviation/μg ml^{-1}	5.894

* 10-μl injection of a sample solution containing caffeine (800 μg ml^{-1}) in pH 7.0 phosphate buffer.
† For caffeine $k' = 2.0$ and for *N*-methyldithiocarbamate $k' = 18.8$.

In all the studies so far, only one dithiocarbamate was injected at a time. In view of the exchange of ligands found with nickel complexes the effect of injecting mixtures of two dithiocarbamates was determined (Table III). Four peaks were obtained in each instance corresponding to a random formation of mixed complexes of cobalt(III). The observation confirmed that, as in the test-tube reaction between cobalt and dithiocarbamates, the cobalt(II) reagent was being oxidised to yield the very stable cobalt(III) complex. This reaction is reported to occur spontaneously by oxidation with atmospheric oxygen[15,30] and explains the marked colour change from pink to green on complex formation. The mass spectrum of the extracted complex is reported to agree with the formation of a cobalt(III) - tridithiocarbamate complex.[15] The four peaks found in this work with cobalt ions can therefore be assigned to MX_3, MX_2Y, MXY_2 and MY_3 complexes.

Although a mixture of tetramethyl- and tetraethylthiuram disulphides gave two peaks on chromatography immediately after mixing, on re-examination after standing for 90 min an intermediate peak, presumably the mixed *NN*-diethyl-*N'N'*-dimethylthiuram disulphide, was also present, an effect that had also been noted earlier[31] (Table III).

TABLE III

CAPACITY FACTORS OF MIXED DITHIOCARBAMATE METAL COMPLEXES FORMED BY INJECTION OF TWO DITHIOCARBAMATES

	Dithiocarbamates		Capacity factors			
Solvent	Sample (X)	Sample (Y)	MX_3	MX_2Y	MXY_2	MY_3
30+70*	$-NH_2$	-NHMe	0.25	1.37	4.90	1.47
50+50†	-NHEt	$-NMe_2$	7.25	7.89	9.00	10.6
	-NHMe	$-NMe_2$	1.25	2.63	5.25	10.6
	-NHMe	-NHEt	1.25	2.50	4.12	7.25
70+30†	-NHMe	$-N(CH_2)_4$	0.50	1.25	4.00	11.8
	-NHEt	$-N(CH_2)_4$	1.37	2.87	5.87	11.8
	$-NMe_2$	$-N(CH_2)_4$	2.00	3.62	6.75	11.8
	$-N(CH_2)_4$	$-NEt_2$	11.8	15.0	19.2	24.7
	-NHMe	-NHEt	0.50	0.75	1.00	1.37
	-NHEt	$-NEt_2$	1.37	2.75	10.0	24.7

* 30+70 methanol - 0.05 M phosphate buffer (pH 5) containing 0.1% Co(II) as nitrate.
† Methanol - water containing 0.1% Co(II) as nitrate.

If a disulphide and dithiocarbamate were mixed and injected (Table IV), interchange apparently also occurred, leading to all possible combinations of disulphides, mixed disulphides and mixed complexes, although some of the assignments are tentative as not all the possible standards were available. Whether this exchange occurs via oxidation, reduction and cleavage of the S—S bond or by amine exchange[32] is not known.

TABLE IV

CAPACITY FACTORS FOR MIXTURES OF THIURAM DISULPHIDES AND DITHIOCARBAMATES

	Disulphide		Dithiocarbamate	Capacity factors of complexes							
Solvent*	A–A	B–B	X	A–A	A–B	B–B	MX_3	MX_2A	MXA_2	MA_3	Other
50+50	$-NMe_2$	—	$-NMe_2$	2.75			10.6				
	$-NMe_2$	—	-NHMe	2.75						10.5	
70+30	$-NMe_2$	$-NEt_2$	—	0.75		6.25					
	(after 90 min)	(after 90 min)		0.75	2.25	6.25					
	$-NMe_2$		$-N(CH_2)_4$	—	1.5†	3.0†	1.75	3.5	6.5	11.8	
	$-NEt_2$		$-NMe_2$	6.25	—	—	2.12	2.62	4.75	—	1.12
	$-NEt_2$		$-N(CH_2)_4$	6.37	4.37†	3.0†	11.8	14.4	19.1	24.7	8.4

* Methanol - water containing 0.1% Co(II) as nitrate.
† A tentative assignment assuming that tetramethylenedithiocarbamate is yielding the corresponding disulphide (B—B) and a mixed disulphide (A—B).

Hence, the use of nickel or cobalt ions as complexation reagents is a useful technique for the determination of individual dithiocarbamates but the interpretation of the results can be complicated if dithiocarbamates or thiuram disulphides with different amino functions are present in the sample.

The method has been successfully applied to the determinations of *N*-methyldithiocarbamate formed during the degradation of dazomet *in vitro*, in cell cultures and in bacterial cell cultures under the same chromatographic conditions used to monitor dazomet and methyl isothiocyanate.[33]

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Determination of Polynuclear Aromatic Hydrocarbons in Food, Water and Smoke Using High-performance Liquid Chromatography

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A method for the separation of 17 different polynuclear aromatic hydrocarbons (PAHs) has been developed. After separation by thin-layer chromatography, the PAHs are examined by high-performance liquid chromatography using ODS and PPS stationary phases. The elution times of all 17 compounds relative to benzo[*a*]pyrene have been computed. Six representative compounds have been added to bierwurst and to water to check recovery values. Work on stubble smoke has been confined to benzo[*a*]pyrene only. The method of extraction and clean-up varies with the substrate but the method of determination of the PAH compounds in the final extract is the same in all three cases. Confirmation of identity is obtained by measuring the peak-height ratios for each PAH at two different excitation and emission wavelength combinations. The limit of detection of benzo[*a*]pyrene is 0.02 $\mu g\ kg^{-1}$ in food, 0.3 $ng\ l^{-1}$ in water and 150 pg per filter in smoke. Recoveries of the six representative compounds are generally in the range 75–100% at levels approaching the limit of detection.

Keywords: *High-performance liquid chromatography*; *polynuclear aromatic hydrocarbons*; *food*; *water*; *stubble smoke*

Polynuclear aromatic hydrocarbons (PAHs) occur in crude coal tar products and they are also formed during the pyrolysis of coal, oil and other forms of organic matter. Contamination of the environment with PAHs may occur through the discharge of industrial wastes, accidental spillages, combustion processes of industry, motor vehicle emissions and in food during preparation and cooking. Lo and Sandi[1] have reviewed the occurrence of PAH residues in food, and Harrison *et al.*[2] have paid particular attention to their distribution in water supplies. The identification and determination of these compounds in various parts of the environment, air, food, water, etc., is of interest as some of them are known to be strongly carcinogenic,[3] whilst others are non- or only weakly carcinogenic.

A number of analytical techniques have been employed for the extraction, separation and identification of PAHs in various different substrates. Chromatographic techniques have been widely used, especially thin-layer chromatography (TLC)[4] and gas - liquid chromatography.[5] In recent years, however, high-performance liquid chromatography (HPLC) has been developed and this technique possesses certain advantages with its fast analysis times and ability to utilise directly certain molecular properties of the compounds of interest, such as ultraviolet absorption and fluorescence. Most workers using high-performance liquid chromatography in the field of PAH analysis have preferred to use reversed-phase columns, particularly octadecylsilane (ODS).[6–9] There is also an increasing tendency to use fluorescence detectors.[8–11] Generally a number of PAH compounds, not all of which are carcinogenic, will be present concomitantly. Hence the analytical method must be capable of separating and identifying minute amounts of individual compounds in a complex mixture.

Methods are proposed for the separation and determination of PAHs in foodstuffs, water and smoke. The food method is capable of separating 17 different PAH compounds. For water samples attention has been confined to the separation and measurement of the six compounds listed by the World Health Organization (WHO) as indicators of pollution.[12] Additionally, some samples of smoke produced during controlled straw stubble burning were analysed for benzo[*a*]pyrene only. Although the sample preparation and clean-up necessarily require

different approaches for the three different types of sample, the eventual separation and measurement of the PAHs in the final extract utilises the same HPLC system under identical conditions. Fig. 1 shows a flow diagram of the different procedures used for the three types of sample. The methods as presented in this paper will detect 0.02 μg kg^{-1} in food, 0.3 ng l^{-1} in water and 150 pg of benzo[*a*]pyrene when deposited on a filter.

Experimental

Foods

The extraction procedure is essentially that described by Hanus *et al.*,[9] followed by clean-up using a silica gel Sep Pak cartridge. The PAHs are then separated into two groups by TLC on acetylated cellulose. After recovery from the TLC plate, each group is subjected to HPLC. Detection is by fluorescence at two different wavelength combinations.

Reagents

Unless stated otherwise, all solvents are re-distilled over charcoal.

Acetonitrile.

Acetylated cellulose for TLC (21% *acetate*).

Celite 545.

Diethyl ether.

Ethanol.

Lead acetate reagent. Dissolve 200 g of lead(II) acetate trihydrate in warm water, then add 3 ml of acetic acid and dilute to 1 l with water.

Methanol. Analytical-reagent grade.

Potassium hydroxide. Analytical-reagent grade.

Sodium sulphate (*granular, anhydrous*). Heat for 5 h at 300 °C.

Silica gel. Sep Pak from Waters Associates Ltd.

Toluene. Analytical-reagent grade.

2,2,4-*Trimethylpentane.*

Procedure

Weigh 100 g of homogeneous sample into a 500-ml beaker, add 275 ml of acetonitrile and blend for 3 min with a top-drive macerator. Add 25 ml of the lead acetate reagent and blend for a further 3 min at high speed.

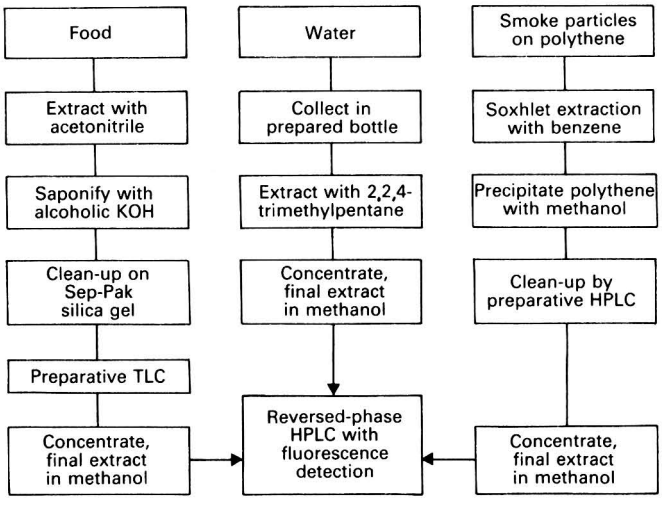

Fig. 1. Flow diagram for analysis of different samples for PAHs.

Filter the mixture with suction through a Whatman No. 541 filter-paper into a Büchner flask. Collect 150 ml of filtrate plus the equivalent volume of the calculated water content of 50 g (based on a previously dried sample) in a measuring cylinder. The total volume of extract will now be equivalent to 50 g of the original sample. Transfer the collected filtrate to a 500-ml separating funnel and shake vigorously with 50 ml of 2,2,4-trimethylpentane for 30 s. Add 200 ml of distilled water and 30 ml of saturated sodium sulphate solution and shake for 2 min. Allow the layers to separate then transfer the lower aqueous phase into a second 500-ml separating funnel. Pass the organic extract through a 3-cm layer of anhydrous granular sodium sulphate, contained in a filter-paper and funnel, into a 250-ml round-bottomed flask. Partition the aqueous phase in the second separating funnel with 50 ml of 2,2,4-trimethylpentane. Allow to separate then discard the aqueous layer, and pass the organic phase into the first separating funnel to rinse it, then pass through the same sodium sulphate layer into the 250-ml flask. Reduce the combined solvent in the flask nearly to dryness using a rotary evaporator at 60 °C, and remove the last traces of solvent with a stream of nitrogen.

Add 25 ml of ethanol, 1.5 g of potassium hydroxide and a few boiling chips to the residue. Fit a reflux condenser and boil under reflux for 40 min. Transfer the solution whilst still hot into a 250-ml separating funnel and rinse the flask successively with 30 ml of hot ethanol followed by two rinses with 50 ml of distilled water, adding each in turn to the separating funnel. Add 25 ml of saturated sodium sulphate solution, mix thoroughly and allow the contents to cool. Rinse the flask finally with 50 ml of 2,2,4-trimethylpentane, add this to the separating funnel and shake the mixture for 2 min. Allow the layers to separate and transfer the lower aqueous layer to a second 250-ml separating funnel. Wash the organic phase in the first separating funnel by swirling with two 100-ml portions of distilled water. Discard the aqueous portions in turn and pass the organic portion through a 3-cm layer of anhydrous granular sodium sulphate contained in a filter-paper and funnel, into a 250-ml flask. Partition the reserved aqueous phase contained in the second separating funnel with 50 ml of 2,2,4-trimethylpentane. Discard the aqueous phase, transfer the organic phase to the first separating funnel, rinse and then pass the solvent through the same sodium sulphate layer into the 250-ml flask. Rinse the two separating funnels successively with 30 ml of 2,2,4-trimethylpentane, finally passing the solvent through the sodium sulphate layer into the 250-ml flask. Concentrate the combined extracts to 2 ml on the rotary evaporator. Connect a silica gel Sep Pak to a suitable syringe, then wash it with 10 ml of 2,2,4-trimethylpentane. Add the sample extract to the Sep Pak, rinse the flask with two 2-ml portions of 2,2,4-trimethylpentane and add each of the washings to the Sep Pak. Discard all the eluate from the Sep Pak up to this point.

Elute the PAHs with 20 ml of 2,2,4-trimethylpentane followed by 20 ml of acetonitrile - 2,2,4-trimethylpentane (2 + 98). Collect the eluent in a 100-ml pear-shaped flask, concentrate to about 200 μl and apply the extract as a 10-cm streak to a TLC plate of acetylated cellulose prepared as described below. Rinse the flask with 1 ml of 2,2,4-trimethylpentane, concentrate and transfer to the TLC plate.

Thin-layer chromatography

Thin-layer chromatography plates (20 × 20 cm) are prepared by thoroughly mixing 50 g of 21% acetylated cellulose for TLC (Schleicher and Schüll) with 100 ml of 95% ethanol. Spread the plates to a thickness of 0.5 mm. Allow the plates to dry in air for 1 h and then store in a desiccator cabinet until required.

Place the development solvent of ethanol - toluene - water (17 + 4 + 4) in a lined tank to equilibrate for 30 min.

At the start line on the TLC plate at a distance of 2.5 and 3 cm from both ends of the sample streak, spot standards of fluoranthene and anthanthrene.

When the TLC plate has fully developed examine it under ultraviolet light to establish the position of the PAH standards.

Using the edge of a spatula, scribe straight lines on the plate as follows:

(*i*) Horizontal line at 0.5 cm above the start line.

(*ii*) Horizontal line just above anthanthrene.

(*iii*) Horizontal line just below fluoranthene.

(*iv*) Horizontal line at R_F 0.6.

(*v*) Vertical lines between the sample and standards.

Fig. 2 illustrates the scribing of the developed plate. Scrape off the side portions of the plate containing the standards and discard. Similarly, remove and discard the lower and upper portions of the plate. Designate the three remaining portions of the plate as A, B and C in ascending order then remove and bulk portions A and C into a 10-ml stoppered test-tube. Similarly transfer portion B into a second stoppered test-tube. To the combined A and C portions add 5 ml of diethyl ether and shake for 1 min, or use an ultrasonic bath. Set the tube aside for the powder to settle. Prepare a small filter column by plugging the first 1 cm of the shoulder of a long-form Pasteur pipette with glass-wool, followed by a 1-cm layer of Celite. Wash the column with 10 ml of diethyl ether before use.

Transfer the supernatant solvent in the test-tube to the small column and collect the filtered extract in a 25-ml pear-shaped flask. Repeat the extraction and filtering procedure a further three times to use a total volume of 20 ml. Extract the TLC powder from portion B separately, in a similar manner. Concentrate each portion to about 1 ml and transfer quantitatively to two small vials and evaporate off the diethyl ether using a stream of nitrogen. Re-dissolve each residue in 500 μl of methanol ready for the injection of 20 μl on to the HPLC system (see below).

Carry out a blank determination omitting the sample, and subtract the result from the sample results.

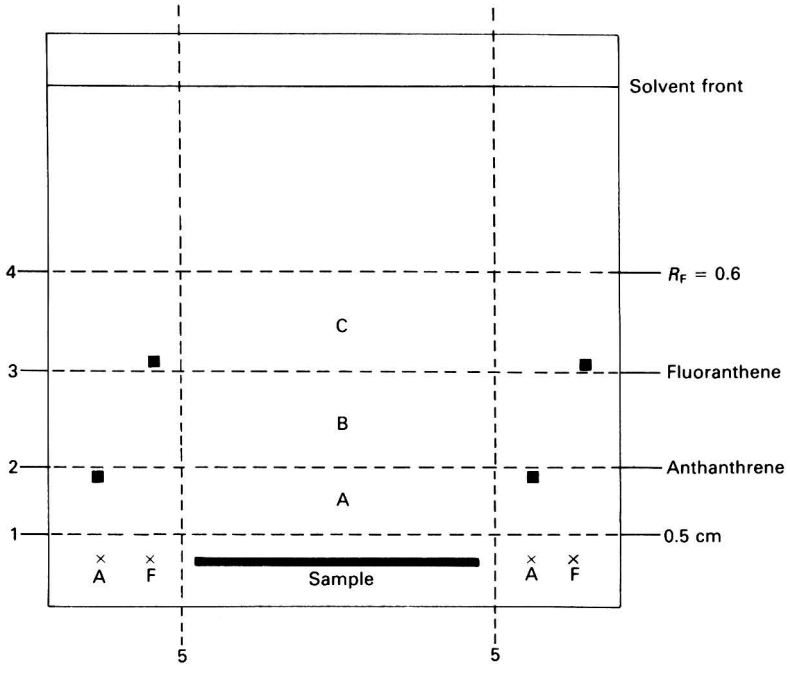

Fig. 2. Scribing of the acetylated cellulose TLC plate.

Results

Table I shows the R_F values of 17 PAHs separated on acetylated cellulose under the conditions of the method. Table II gives the recovery of six PAHs from a sample of spiked bierwurst, whilst Table III gives three results from a survey of 46 samples of a wide range of smoked foods purchased in the UK. For spiking the bierwurst the PAH compounds were dissolved

TABLE I

THIN-LAYER CHROMATOGRAPHY OF PAH COMPOUNDS ON 21% ACETYLATED CELLULOSE

PAH	R_F	Position	PAH	R_F	Position
Benzo[*b*]fluoranthene ..	0.16	A	7-Methylbenz[*a*]anthracene	0.28	B
Benzo[*a*]pyrene	0.16	A	12-Methylbenz[*a*]anthracene	0.29	B
Indeno[1,2,3-*cd*]pyrene ..	0.20	A	Benzo[*k*]fluoranthene ..	0.29	B
Anthanthrene	0.21	A	Dibenz[*a,h*]anthracene ..	0.30	B
Fluoranthene	0.41	C	3-Methylcholanthrene ..	0.30	B
Benzo[*ghi*]fluoranthene ..	0.41	C	Perylene	0.33	B
9,10-Dimethylanthracene	0.42	C	Benzo[*ghi*]perylene ..	0.33	B
Pyrene	0.44	C	Benzo[*e*]pyrene	0.36	B
			Benz[*a*]anthracene ..	0.36	B

in methanol and the required volume was added from a syringe in droplets to the minced bierwurst. It was then left for at least 1 h in a stream of air to remove methanol. Levels of fluoranthene found were significantly higher in most foods than the levels of the other PAH compounds detected. However, smoking of foods by modern controlled processes appears to add only very low levels of carcinogenic PAH compounds to the diet.

Water

This method has been designed for water samples which need to be transported from the site of sampling to the analytical laboratory. The extraction procedure consists of shaking the sample with 2,2,4-trimethylpentane. After concentration, the extract is injected on to the HPLC system consisting of a reversed-phase column and fluorescence detector.

Reagents

2,2,4-*Trimethylpentane.* Re-distilled over charcoal.
Sodium sulphate (*anhydrous, granular*). Heat at 300 °C for 5 h.
Methanol. Analytical-reagent grade.

TABLE II

RECOVERY OF PAH COMPOUNDS FROM SPIKED BIERWURST

PAH	Spiking level/μg kg^{-1}	Recovery, %
Fluoranthene	2.4	70
Benzo[*k*]fluoranthene	0.8	110
Benzo[*b*]fluoranthene	2.0	110
Benzo[*a*]pyrene	2.0	100
Indeno[1,2,3-*cd*]pyrene	40	95
Benzo[*ghi*]perylene	10	95

TABLE III

LEVELS OF PAH COMPOUNDS FOUND IN THREE DIFFERENT SMOKED FOODS

	PAH/μg kg^{-1}					
Food	Fluoranthene	Benzo[*k*]-fluoranthene	Benzo[*b*]-fluoranthene	Benzo[*a*]-pyrene	Indeno[1,2,3-*cd*]pyrene	Benzo[*ghi*]-perylene
Bacon	7.8	0.05	0.30	0.05	2.5	3.75
Kippers	2.4	0.10	0.35	0.10	2.7	4.3
Cheese	4.2	0.15	0.30	0.20	ND*	0.60

* ND = less than 0.02 μg kg^{-1}.

Procedure

Prepare the bottle for collecting the water sample as described below. To a 2.5-l brown-glass Winchester bottle (such as an empty methanol reagent bottle) fitted with a screw-cap and a PTFE insert, add 2 l of tap water and mark the bottle at the meniscus. Discard the water, rinse the bottle with distilled water, then methanol, then finally with 2,2,4-trimethylpentane and invert the bottle to dry. Add exactly 100 ml of 2,2,4-trimethylpentane to the bottle and screw the cap on firmly. The bottle is now ready for collecting the water sample. The water sample is poured into the bottle until the lower meniscus reaches the 2-l mark, then the bottle is tightly sealed. The sample can now be transported or left for a number of days before the analysis takes place with little loss of PAH compounds.

Extraction

If the sample appears cloudy add 10 g of sodium sulphate; if the water is clear this is not necessary. Shake the bottle on a laboratory shaker for 10 min, then transfer the contents into a 2-l separating funnel. Allow the two layers to separate and run off the lower, water layer to waste. Rinse the sample bottle with 20 ml of 2,2,4-trimethylpentane, add the rinsings to the solvent in the separating funnel and mix. Filter the bulk of the solvent extract through a 10-g layer of anhydrous sodium sulphate contained in a Whatman No. 1 paper in a filter-funnel. Collect 60 ml of the filtered extract (≡ 1 l of water sample), concentrate it nearly to dryness on a rotary evaporator at 60 °C, then transfer it quantitatively into a small vial and evaporate to dryness using a stream of nitrogen. Re-dissolve the residue in 200 μl of methanol ready for the injection of 20 μl on to the HPLC system (see below).

Development of the method

The method was tested by the addition of a solution in methanol, of the six PAH compounds listed in Table II, to water taken from the River Thames at Waterloo, where the river is tidal and well below the last point where it is abstracted for use as drinking water. Immediate extraction of filtered and spiked samples gave satisfactory recovery figures. After storage for 3 d in glass bottles, recoveries were only 59–81%; if 2,2,4-trimethylpentane was present at the time of sampling, however, the recoveries after 3 d were 74–96%. These results are shown in Table IV and indicate that PAHs can be adsorbed on to a glass surface. Further confirmation of this was obtained when spiked tap water was examined using a membrane filter concentration step when recoveries of 60–85% were obtained, dropping to 45–60% after 24 h in a glass bottle. The decision whether to examine a filtered or unfiltered sample of water should be made before the bottle is filled, as the removal of particulate matter can considerably reduce the PAH level. Table V shows the effect on PAH recoveries of both filtering and adding 2,2,4-trimethylpentane at the sampling stage. Work carried out on sampling procedures indicates

TABLE IV

RECOVERIES OF PAHs FROM FILTERED WATER AFTER 3 DAYS IN GLASS

PAH	Solvent treatment	Original determination/ ng l^{-1}	PAH added/ ng l^{-1}	Final determination/ ng l^{-1}	Recovery, %
Fluoranthene	Without solvent	10.2	50	41.5	69
Benzo[*k*]fluoranthene		0.7	4	3.1	66
Benzo[*b*]fluoranthene		0.2	1	0.9	75
Benzo[*a*]pyrene		1.8	10	9.6	81
Indeno[1,2,3-*cd*]pyrene ..		Not detected	20	12.0	60
Benzo[*ghi*]perylene		2.9	15	10.6	59
Fluoranthene	With solvent	15.3	50	48.3	74
Benzo[*k*]fluoranthene		1.3	4	4.4	83
Benzo[*b*]fluoranthene		0.3	1	1.1	81
Benzo[*a*]pyrene		4.2	10	13.6	96
Indeno[1,2,3-*cd*]pyrene ..		Not detected	20	17.6	88
Benzo[*ghi*]perylene		4.4	15	15.9	82

TABLE V

EFFECTS OF FILTRATION AND SAMPLING PROCEDURE ON RIVER THAMES WATER

PAH	PAH concentration/ng l^{-1}			
	Filtered		Unfiltered	
	No solvent	With solvent	No solvent	With solvent
Fluoranthene	10.2	15.3	540	667
Benzo[*k*]fluoranthene	0.7	1.3	85	99
Benzo[*b*]fluoranthene	0.2	0.3	17	20
Benzo[*a*]pyrene	1.8	4.2	294	430
Indeno[1,2,3-*cd*]pyrene	Not detected	Not detected	Not detected	Not detected
Benzo[*ghi*]perylene	2.9	4.4	500	541

that those which are based on concentration steps involving a degree of filtration, such as the on-column concentration methods and the membrane methods, may not be appropriate for unfiltered samples or for those samples requiring transportation from the sampling site, as the PAHs will be partially retained by particulate matter and by the glass container. With polluted water emulsions may form when the sample is shaken with the solvent. If sodium sulphate is added after the emulsion has formed the emulsion can still be difficult to break, whereas the addition of the salt before the extraction starts will prevent the emulsion from forming.

Peaks were obtained corresponding to indenopyrene but the ratio was 1.0 instead of 0.1 as found for standards. Other PAHs detected possessed ratios very close to standard values. Hence, the presence of indenopyrene was not confirmed. This observation was supported by further measurements at 355 nm excitation and 500 nm emission.

Benzo[*a*]pyrene in Smoke

Interest has been expressed in the possible formation of benzo[*a*]pyrene during the burning of straw and stubble. Experiments were designed by AERE, Harwell, in which straw was burned under controlled conditions in a wind tunnel. Samples were taken in a high-volume sampler with a glass-fibre filter backed by two bubblers filled with hexane. Approximately 0.5–1 m^3 of air was sampled at each burn. In later experiments, the concentration of benzo[*a*]-pyrene was determined as a function of particle size using an Anderson cascade impactor by which samples are collected at several stages on thin polythene sheets. The results of the stubble burning study have now been published.[13]

Analysis of samples

The hexane extracts presented no problems; they were evaporated to dryness and the residue was re-dissolved in methanol before examination by HPLC (see below).

As charcoal is a very powerful adsorbent for PAHs, difficulties were envisaged for the recovery of benzo[*a*]pyrene from filters ingrained with soot from the smoke. Recovery experiments were carried out to test the efficiency of various solvents for extracting benzo[*a*]-pyrene from activated charcoal, as this material should represent the most difficult case encountered with the samples. Glass-microfibre filter-papers were used for a series of experiments in which activated charcoal was spread over the surface, then ground in by rolling with a glass rod. Excess of charcoal was removed by shaking the papers. A 2 $\mu g\ \mu l^{-1}$ standard solution of benzo[*a*]pyrene (50 μl) was then spotted evenly over the surface using a syringe and the paper was then allowed to dry in air. Soxhlet extraction was carried out on the impregnated filters by placing them in pre-extracted thimbles and using 150–200 ml of solvent for 7 h. The solvents selected for trial were benzene, cyclohexane, acetone and dichloromethane, these solvents having been used in the past for the extraction of PAHs from air samples.[14–17] After 7 h, the extracts were evaporated and re-dissolved in methanol for quantitation by HPLC. Table VI shows the recoveries obtained for each solvent and the superiority of benzene (with a mean recovery of 88%) over the other solvents for this particular work.

TABLE VI

RECOVERY OF BENZO[*a*]PYRENE FROM CHARCOAL-IMPREGNATED FILTERS

Solvent	Recovery, %	Mean recovery, %
Benzene	100,82,80,88	88
Cyclohexane	5, 5, 6, 6	6
Acetone	17,17, 8,15	14
Dichloromethane	4, 5, 6,20	9

Reagents

All solvents should be re-distilled over charcoal.

Benzene.

Caution—Benzene is highly toxic and appropriate precautions should be taken.

Acetonitrile.

Methanol.

2,2,4-*Trimethylpentane.*

Procedure

Extract the sample of smoke-impregnated glass-fibre filter-paper in a Soxhlet apparatus for 7 h with benzene. Concentrate the extract to a low volume using a rotary evaporator at 60 °C, quantitatively transfer it into a small vial and take just to dryness with a stream of nitrogen. Re-dissolve the residue in 200 μl of acetonitrile and inject 100 μl into a semi-preparative HPLC column composed of a 300 × 7 mm i.d. stainless-steel tube packed with Spherisorb ODS, 5 μm, using methanol - water (9 + 1) as the mobile phase at a flow-rate of 2 ml min^{-1}. Then collect the portion of the eluate containing benzo[*a*]pyrene, previously determined by the injection of a standard, in a stoppered test-tube. Add an equal volume of distilled water and mix the contents. Extract the benzo[*a*]pyrene by shaking with half the original volume of 2,2,4-trimethylpentane. Repeat the extraction and concentrate the combined extracts just to dryness, re-dissolve in 100 μl of methanol and inject 20 μl on to the HPLC system (see below).

If samples of smoke are collected on an Anderson impactor using polythene discs, then the same extraction procedure is used with a minor modification. At the end of the Soxhlet extraction period the polythene will have partially dissolved in the benzene and is removed by precipitation by the addition of 100 ml of methanol, followed by filtration through a Whatman No. 40 paper prior to the concentration stage.

Fig. 3 shows an extract of smoke subjected to semi-preparative HPLC on an ODS column together with the final determination of benzo[*a*]pyrene on a PPS analytical column.

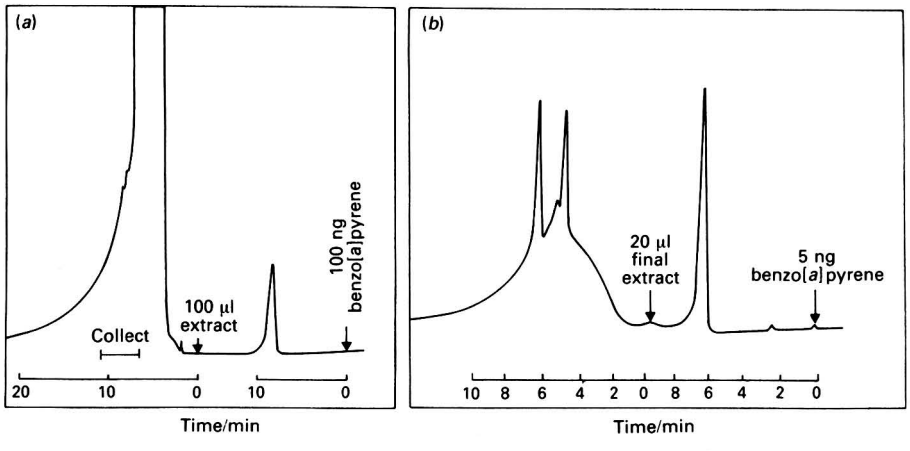

Fig. 3. High-performance liquid chromatogram of smoke extract on (*a*) preparative column of ODS, and (*b*) benzo[*a*]pyrene portion from (*a*) on an analytical column of PPS.

High-performance Liquid Chromatography

In recent years the separation and detection of PAHs by HPLC has been effected primarily with bonded phases using a fluorescence detector, which imparts both selectivity and high sensitivity to the system. In this work two different bonded phases, octadecylsilane (ODS) and phthalimidopropylsilane (PPS), have been examined. HPLC columns containing these two packings give different orders of elution for many PAHs and their preparation and performance have been compared in a previous paper[18] using methanol - water (9 + 1) as the mobile phase. Additionally, PPS has been used with a non-polar solvent in a normal-phase system to give a separation of the six PAHs designated by the WHO, found in water,[19] which is superior to that obtained using ODS or other similar systems.

Two types of fluorescence detector have been examined during this work. A wide-range filter instrument has proved useful for general surveillance work, whilst a more expensive double monochromator scanning instrument, which can be programmed to give a number of pre-set wavelength combinations, has been used as a further aid to PAH identification.

Apparatus

HPLC pumps. Waters Associates M6000 reciprocating pump and Applied Chromatography Services Ltd., Model 750/03, reciprocating pump.

Detectors. Aminco FluoroMonitor and Perkin-Elmer 3000 Fluoresence Spectrometer.

The HPLC system now in routine use consists of a dual-piston reciprocating pump supplying the mobile phase of methanol - tetrahydrofuran - water (20 + 2 + 3) at 1.5 ml min^{-1} through a Rheodyne valve fitted with a 20-μl loop, to a 15 cm × 4.6 mm i.d. stainless-steel column of either ODS or PPS bonded to LiChrosorb and Partisil 5, respectively. The eluate is monitored at two wavelength combinations and the ratio of the peak heights of each PAH at the two wavelength combinations lends a further aid to identification. The settings used for the six PAH compounds listed by the WHO as indicators for water pollution are excitation at 290 nm and emission at 430 nm (λ_1), and excitation at 282 nm and emission at 457 nm (λ_2). Compounds are identified both by their elution times and by comparison of the values of the ratios of peak heights λ_1/λ_2 with standards. If necessary further confirmation can be obtained by using the alternative column packing and by scanning the peak. Fig. 4 shows the chromatograms obtained for the six WHO polynuclear compounds at the two different wavelength combinations stated in the above method and the ratio of peak heights obtained. Table VII lists the elution times of 17 PAHs relative to benzo[*a*]pyrene on the two columns, and their

TABLE VII

ELUTION TIMES RELATIVE TO BENZO[*a*]PYRENE OF PAHS ON TWO REVERSED-PHASE COLUMNS AND THEIR PEAK-HEIGHT RATIOS AT TWO WAVELENGTH COMBINATIONS

PAH	Relative elution times* (benzo[*a*]pyrene = 1.00)		Peak-height λ_1: λ_2 ratio†
	ODS on LiChrosorb	PPS on Partisil	
Benzo[*b*]fluoranthene	0.86	0.84	1.3
Benzo[*a*]pyrene	1.00	1.00	3.8
Indeno[1,2,3-*cd*]pyrene	1.42	1.32	0.1
Anthanthrene	1.76	1.63	3.4
Fluoranthene	0.42	0.52	0.5
Benzo[*ghi*]fluoranthene	0.60	0.83	0.9
9,10-Dimethylanthracene	0.52	0.75	1.5
Pyrene	0.45	0.61	6.4
7-Methylbenz[*a*]anthracene	0.81	0.82	3.6
12-Methylbenz[*a*]anthracene	0.80	0.72	2.3
Benzo[*k*]fluoranthene	0.90	0.78	3.4
Dibenz[*a*,*h*]anthracene	1.24	1.00	4.8
3-Methylcholanthrene	1.85	1.11	2.7
Perylene	0.84	1.21	0.8
Benzo[*ghi*]perylene	1.34	1.68	3.3
Benzo[*e*]pyrene	0.81	1.07	3.7
Benz[*a*]anthracene	0.60	0.64	1.8

* Mobile phase = methanol - tetrahydrofuran - water (20 + 2 + 3).
† For values of λ_1 and λ_2 see text.

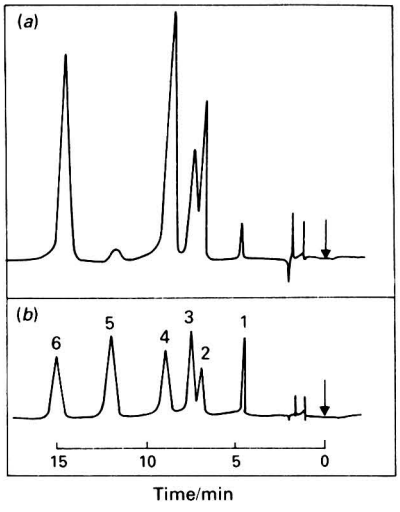

Fig. 4. High-performance liquid chromatogram of the six WHO polynuclear compounds separated on PPS, at two different sets of wavelengths. (*a*) Excitation at 290 nm and emission at 430 nm and (*b*) excitation at 282 nm and emission at 457 nm. 1, Fluoranthene; 2, benzo[*k*]fluoranthene; 3, benzo[*b*]fluoranthene; 4, benzo[*a*]pyrene; 5, indeno[1,2,3-*cd*]pyrene; 6 benzo[*ghi*]perylene.

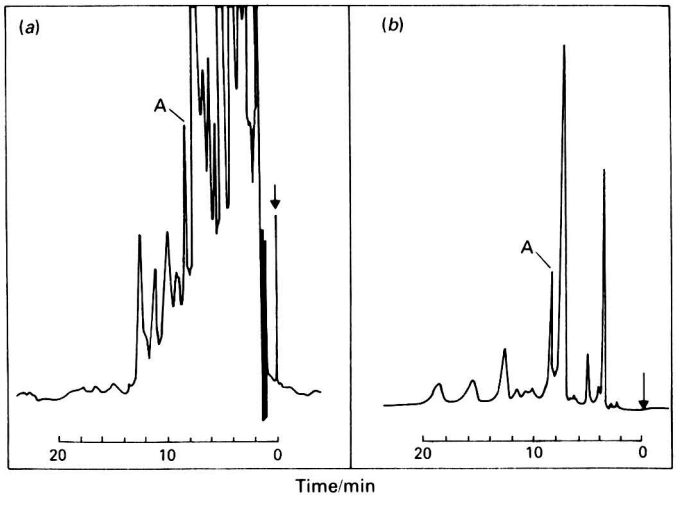

Fig. 5. Comparison of high-performance liquid chromatograms of mussels with different detection systems: (*a*) ultraviolet at 280 nm and 0.01 a.u.f.s. and (*b*) fluorescence at 10% of full sensitivity. A, Benzo[*a*]-pyrene peak.

peak-height ratios at the two wavelength combinations. Fig. 5 illustrates the advantages of sensitivity and selectivity of fluorescence detection over ultraviolet detection for the determination of PAHs in mussels.

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Voltammetric Determination of 2-, 3- and 4-Chloroaniline in Mixtures

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The oxidative voltammetric behaviour of 2-, 3- and 4-chloroaniline has been investigated at the glassy carbon electrode over the pH range 1–12. The optimum pH values for the linear-sweep and differential-pulse voltammetric determination of these compounds were found to be 1.95 for 2- and 3-chloroaniline and 8.1 for 4-chloroaniline. Differentiation of the three compounds in a mixture was achieved by using a combination of high-performance liquid chromatography with voltammetric detection. This method could be used to determine concentrations of 2- and 4-chloroaniline down to 2 ng and 3-chloroaniline down to 1 ng for a 20-μl injection on to the chromatographic column.

Keywords: 2-, 3- *and* 4-*chloroaniline determination*; *oxidation*; *high-performance liquid chromatography*; *voltammetric detection*

Chlorine-substituted phenylamides are widely used as herbicides and have been found to metabolise *in vivo* to monochloroanilines.[1–6] For example, isopropyl *N*-(3-chlorophenyl)-carbamate is converted into 3-chloroaniline through microbial action in the soil.[2–6] *N*-Demethylation of 4-chloro-*N*-methylaniline has also been reported.[7]

A variety of methods exist for the determination of these monochloroanilines. For example, gas chromatography has been used to separate mixtures of mono- and dichloroanilines[8–10] and thin-layer chromatography with fluorimetric detection has been used to determine the hydrolysis products of carbamate- and urea-containing herbicides.[11] However, both methods are time consuming. Colorimetric methods have also been used in this regard, but these usually involve derivatisation (by diazotisation) and are generally non-specific.

Monochloroanilines can be oxidised at a variety of solid electrode surfaces and the potential of oxidation has been shown to be dependent on the nature of the substitution in the ring system.[12] It was the purpose of this investigation to study the inherent voltammetric behaviour of 2-, 3- and 4-chloroaniline at the glassy carbon electrode with a view to developing a method for their determination using differential-pulse voltammetry. However, this technique was found not to have the required selectivity, and a method was therefore developed for their determination using a combination of high-performance liquid chromatography (HPLC) with voltammetric detection. This method has recently been applied to the determination of many amino- and phenolic-containing compounds of biological importance.[13,14]

Since acceptance of this paper another paper dealing with the determination of 2-chloraniline by HPLC with voltammetric detection has been published.[15] This paper demonstrates the advantageous possibilities of using this technique in environmental analysis.

Experimental

Apparatus

Linear-sweep, differential-pulse and cyclic voltammograms were recorded using a PAR, Model 174A, Polarographic Analyser in conjunction with a Servoscribe, Model 1S 541.20, potentiometric recorder. A three-electrode cell system was employed that incorporated a glassy carbon indicator electrode (Tokai GC-305; calculated area 0.39 cm^2), a saturated calomel

reference electrode and a platinum counter electrode. A Spectra-Physics, Model 3500, liquid chromatograph was operated in conjunction with a Metrohm, Model EA 1096, electrochemical detector, the potential of which was controlled by the PAR 174A. The detector was operated in the amperometric mode with glassy carbon as the material of both the indicator and counter electrodes and silver - silver chloride as the reference electrode.

Procedures

Solutions of the monochloroanilines were prepared by dissolving the compounds in either 0.1 N sulphuric acid or in Britton - Robinson buffers of pH 1.95–12.0 containing 50% V/V of ethanol, so that the final concentration was approximately 5×10^{-4} M. Linear-sweep voltammetry was carried out on these solutions using the following operating conditions: initial potential, 0.0 V; scan rate, 10 mV s^{-1}; and current range, 0.02 mA. Between successive runs, the indicator electrode was cleaned by washing it with distilled water and drying it with a tissue. In the cyclic voltammetric experiments a scan rate of 50 mV s^{-1} and a chart speed of 60 cm min^{-1} were employed.

Following selection of the optimum pH for the determination of the individual monochloroaniline, calibration graphs were constructed using the linear-sweep and differential-pulse modes. A scan rate of 50 mV s^{-1} was employed in the former instance and 5 mV s^{-1} in the latter. The pulse height was 100 mV, the "drop time" 1 s and the time constant 0.3 s for differential-pulse voltammetry.

For the construction of chromatovoltammetric curves, the current arising from the oxidation of 2-, 3- or 4-chloroaniline following HPLC separation was plotted as a function of the applied potential (in the range 0.6–1.2 V), which was increased stepwise (50 mV) over a series of injections. The separation was carried out on a 5-μm LiChrosorb RP-8 column (250 $\times$ 4.6 mm i.d.) with a water - methanol - acetonitrile mixture (75 + 20 + 5) containing sodium dihydrogen orthophosphate (6 g l^{-1}) and 85% orthophosphoric acid (2.5 ml l^{-1}) as eluting agent. For analytical determinations, the column was operated at a flow-rate of 2 ml min^{-1} and the compounds were detected amperometrically at an applied potential of +1.2 V *vs.* silver - silver chloride.

Results and Discussion

Linear-sweep Voltammetry

Linear-sweep voltammograms of 2-, 3- and 4-chloroaniline were recorded over the pH range 1–12. The variation of E_p and i_p with pH for the single peak (peak A) exhibited by these chloroanilines using linear-sweep voltammetry is shown in Fig. 1. The first break in

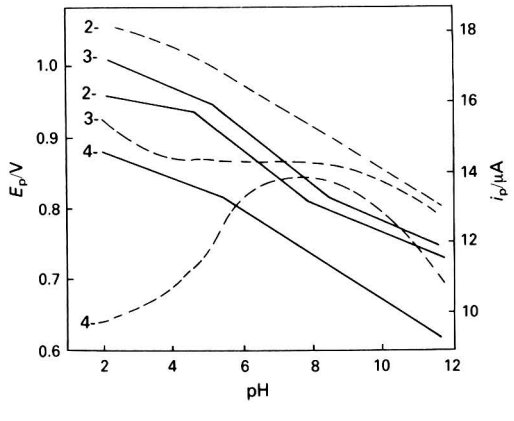


Fig. 1. Variation of E_p (solid lines) and i_p (broken lines) with pH for 2-, 3- and 4-chloroaniline.

each of the respective E_p *versus* pH curves corresponds to the pK_a value relating to deprotonation of the anilinium species, and although the values obtained using this method are higher than those obtained using spectrophotometry (Table I), the trend of increasing pK_a values in the series 2-chloroaniline < 3-chloroaniline < 4-chloroaniline is maintained. It is interesting that at pH < pK_a, 4-chloroaniline behaves differently from the other two isomers in that the i_p value is small in strongly acidic media and increases as the pH is increased, whereas the reverse occurs for the other two compounds. In addition, 4-chloroaniline does not exhibit a pK_a value in the pH region 8–9.

Values of αn_a, obtained from the equation[16]

$$\alpha n_a = \frac{0.048}{E_p - E_{p/2}}$$

where $E_{p/2}$ is the potential corresponding to $i_{p/2}$, were calculated at various pH values (Table II), and were shown to decrease with increasing pH, indicating an increasing irreversibility of the electrode process with increasing deprotonation of the compounds in the bulk of solution.

TABLE I

pK VALUES FOR 2-, 3- AND 4-CHLOROANILINE

Compound	pK_a (voltammetry)	pK_a' (voltammetry)	pK_a (spectrophotometry)
2-Chloroaniline	4.8	8.0	2.65
3-Chloroaniline	5.4	8.6	3.46
4-Chloroaniline	5.5	—	4.15

Cyclic Voltammetry

The cyclic voltammetric behaviour of 2- and 3-chloroaniline was found to be similar over the pH range 1–12. At pH < pK_a, the cyclic voltammetric pattern was as shown in Fig. 2 (for 2-chloroaniline). From this it can be seen that the process giving rise to peak A is irreversible, but that some reduction of the product of oxidation occurs on the reverse scan. In 0.1 N sulphuric acid - 50% ethanol, two peaks appear for 2-chloroaniline on the first reverse scan at +0.47 V and +0.33 V, the more negative of which appears to be quasi-reversible. On increasing the pH these two cathodic peaks decrease in size until at pH >8.0 they disappear, leaving only peak A.

The cyclic voltammetric behaviour of 4-chloroaniline was found to be similar to that of the other two isomers except that this compound exhibited only one cathodic process on the reverse scan at +0.37 V. This peak, which was reversible, was also found to disappear in solutions of pH > 8.0.

Chromatovoltammetric Behaviour

The hydrodynamic chromatovoltammograms obtained for 2-, 3- and 4-chloroaniline are shown in Fig. 3. From this it can be seen that 4-chloroaniline ($E_{1/2}$ = +0.91 V) is more easily oxidised than either 3-chloroaniline ($E_{1/2}$ = + 1.00 V) or 2-chloroaniline ($E_{1/2}$ = +0.96 V, +1.13 V) in water - methanol - acetonitrile (75 + 20 + 5) containing phosphate ions as supporting electrolyte (pH ≈ 3). It is interesting that for 2-chloroaniline (and to a

TABLE II

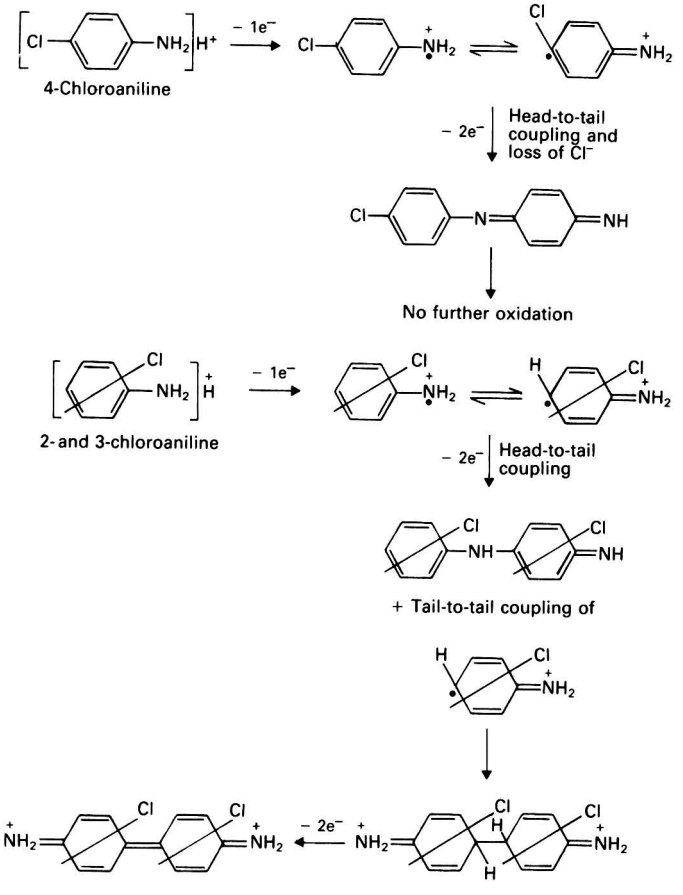
αn_a VALUES FOR 2-, 3- AND 4-CHLOROANILINE

pH	2-Chloroaniline	3-Chloroaniline	4-Chloroaniline
1.95	0.9	0.6	0.9
4.15	0.7	0.5	0.6
6.10	0.7	0.6	0.7
8.10	0.6	0.5	0.7
10.40	0.4	0.5	0.6
11.80	0.4	0.4	0.5

lesser extent 3-chloroaniline) there are two processes involved in the over-all oxidation reaction at the glassy carbon electrode in the hydrodynamic mode, a feature that was not observed in either the linear-sweep voltammetric or the cyclic voltammetric experiments in quiescent solution.

Mechanism of Oxidation

Based on these experimental findings, and bearing in mind the work carried out by previous workers,[17] the following mechanisms can be postulated to explain the oxidation of 2-, 3- and 4-chloroaniline with pH $< pK_a$:



Similar mechanisms are likely to be in operation at $pK_a < pH < pK_a'$, but at pH $> pK_a'$ there is likely to be a change in the nature of the coupling process with more head-to-head coupling. This has been shown to be the case for aniline in the pH range 7–14, where increasing amounts of azobenzene were formed following oxidation at a variety of solid indicator electrodes.[18]

Analytical Applications

Optimum pH values for the determination of these compounds by linear-sweep voltammetry and differential-pulse voltammetry were found to be 1.95 for 2- and 3-chloroaniline and 8.1 for 4-chloroaniline. Calibration graphs of i_p (nA) *versus* concentration (ng ml^{-1}) were constructed and the response factors calculated. These data, together with the concentration range studied and coefficients of variation obtained using both voltammetric techniques, are given in

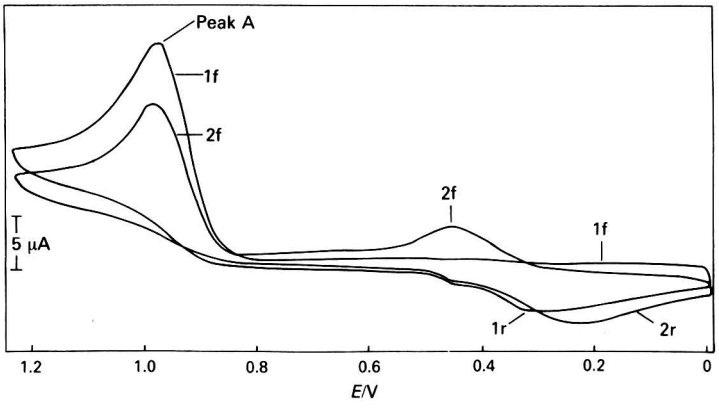


Fig. 2. Cyclic voltammogram of 2-chloroaniline in 0.1 N H_2SO_4 - 50% EtOH (f = forward; r = reverse). Scan rate, 50 mV s^{-1}; starting potential, 0 V.

Table III. As expected, greater sensitivity was achieved using the differential-pulse mode. The limit of detection of this technique was found to be 0.2 μg ml^{-1} for all three compounds.

Differential-pulse voltammetry was also investigated as a means of differentiating between 3- and 4-chloroaniline in mixtures. A supporting electrolyte of Britton - Robinson buffer (pH 3.0) - 50% ethanol gave rise to the best resolution (Fig. 4), but it is obvious that this selectivity is not sufficient for trace analytical studies. It was decided, therefore, to employ the combination of HPLC with voltammetric detection for this purpose. Using the conditions

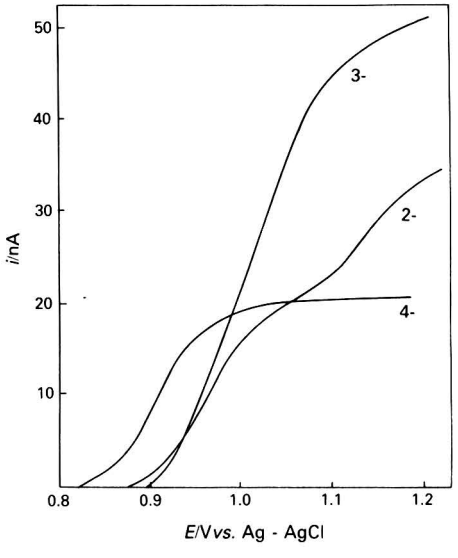


Fig. 3. Hydrodynamic chromatovoltammograms of 2-, 3- and 4-chloroaniline. Concentration, 100 ng per 20 μl; flow-rate, 1.2 ml min^{-1}.

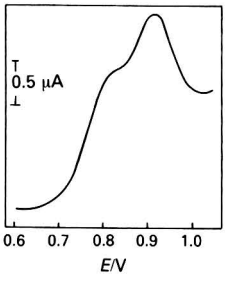


Fig. 4. Differential pulse voltammogram of a mixture of 3-chloroaniline (1.17×10^{-4} M) and 4-chloroaniline (1.04×10^{-4} M) in Britton - Robinson buffer, pH 3 - 50% EtOH. Scan rate, 5 mV s^{-1}; pulse height, 100 mV.

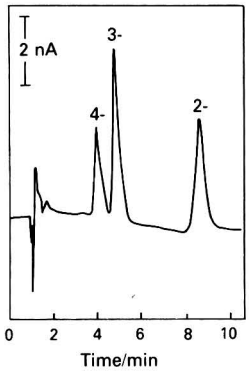


Fig. 5. Separation of a 10-ng mixture of 2-, 3- and 4-chloroaniline by HPLC with voltammetric detection. Flow-rate, 2 ml min^{-1}; applied potential, +1.2 V.

described previously under Experimental, a good separation of the three isomers was effected (Fig. 5) and the method could determine concentrations of 2- and 4-chloroaniline down to 2 ng and 3-chloroaniline down to 1 ng for a 20-μl injection on to the column. This method should prove to be useful for the trace analysis of these compounds in environmental samples, whereas the differential-pulse voltammetric method might be of application in formulation studies.

TABLE III

LINEAR-SWEEP (LSV) AND DIFFERENTIAL-PULSE VOLTAMMETRIC (DPV) DATA FOR 2-, 3- AND 4-CHLOROANILINE

Polarographic mode	Compound	Supporting electrolyte	E_p/V	Response factor/ nA ng^{-1} ml	Coefficient of variation, % (a)*	(b)†	Concentration range studied/μg ml^{-1}
DPV	2-Chloroaniline	0.1 N H_2SO_4 - 50% EtOH	0.940 ±0.010	0.93	6.8	2.2	0.64–12.70
DPV	3-Chloroaniline	0.1 N H_2SO_4 - 50% EtOH	0.990 ±0.010	0.88	7.1	2.4	0.64–12.70
DPV	4-Chloroaniline	Britton - Robinson buffer (pH 7) - 50% EtOH	0.665 ±0.005	0.82	6.2	2.0	0.64–12.70
LSV	2-Chloroaniline	0.1 N H_2SO_4 - 50% EtOH	1.015 ±0.010	0.63	8.2	3.3	0.64–12.70
LSV	3-Chloroaniline	0.1 N H_2SO_4 - 50% EtOH	1.060 ±0.010	0.60	8.4	3.4	0.64–12.70
LSV	4-Chloroaniline	Britton - Robinson buffer (pH 7) - 50% EtOH	0.730 ±0.005	0.55	8.0	2.9	0.64–12.70

* (a) Based on 10 determinations at 0.64 μg ml^{-1}.
† (b) Based on 10 determinations at 12.70 μg ml^{-1}.

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Separation of Protein-bound Copper and Zinc in Human Plasma by Means of Gel Filtration - Ion-exchange Chromatography

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A procedure for the separation of protein-bound copper and zinc fractions and of potassium and magnesium in plasma, involving chromatography on columns of DEAE-Sepharose CL-6B, is described. Copper, magnesium and zinc were measured by atomic-absorption spectroscopy and potassium by flame photometry. Most plasma samples yielded one copper and two zinc fractions and these were identified by the use of protein "markers." Most of the copper appeared to be bound to caeruloplasmin and a lesser amount to albumin, whereas zinc appeared to be bound mainly to globulins with a smaller, variable, amount bound to albumin. Magnesium and potassium appeared as single peaks eluting in approximately the same fractions, ahead of the albumin peak.

Keywords: *Copper*; *zinc*; *plasma proteins*; *chromatography*; *atomic-absorption spectroscopy*

Over 90% of the copper in human plasma is firmly bound to an α_2-globulin, caeruloplasmin. The remainder, constituting the labile pool, is less firmly bound, in large part to albumin and in smaller part to amino acids, especially to histidine, threonine and glutamine.[1,2] Zinc in plasma is likewise mainly bound to albumin and to an α_2-globulin, although there are conflicting reports as to the relative distribution between these two proteins.[3] Some zinc may also be bound to amino acids.[4]

Various techniques have been used to study the binding of copper and zinc to specific proteins in plasma, including salt fractionation,[5] electrophoresis,[5–8] gel filtration[9] and sucrose density gradient centrifugation.[3] Some of these techniques are time consuming, subject to contamination or require specialised apparatus that is not always available in a clinical chemistry laboratory. A suitable method for clinical investigations should provide a rapid and reproducible separation of large and small molecules, with minimum dilution, contamination or loss of trace metals, and, moreover, should be suitable for automated analysis. In this study we examined several types of gel filtration and ion-exchange chromatography, in combination with atomic-absorption and -emission spectroscopy; a modified ion-exchange procedure is described.

Experimental

Apparatus

A schematic diagram of the apparatus is shown in Fig. 1. Acrylic plastic columns with an internal diameter of 0.9 cm and 30 or 60 cm long were used (Pharmacia Fine Chemicals, Uppsala, Sweden, Type K9/30 or K9/60). Solutions were fed to the tops of the columns by a variable-speed peristaltic pump (Stalprodukter, Uppsala, Sweden) and a constant-pressure head was maintained by means of an over-flow pipe. Ionic strength gradient elution was used in some experiments, the gradients being produced by using a second channel of the peristaltic pump to inject, with stirring, a concentrated solution of the eluent into a large reservoir of starting buffer. This system produced a nearly linear gradient according to the equation

$$\frac{dc}{dt} = \frac{f(C_c - C)}{V}$$

provided $C_c \gg C$, where f = flow-rate into and out of the reservoir, C_c = concentration of concentrated eluent, C = concentration of dilute eluent and V = volume of liquid in mixing vessel.

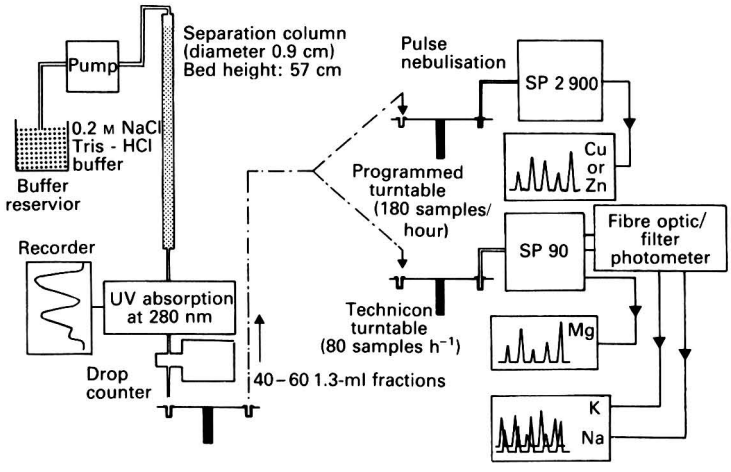

Fig. 1. Schematic diagram of apparatus used for separation of plasma proteins and the determination of Cu, Zn, Mg, Na and K.

The absorbance of the effluent at 280 nm was monitored by a Uvicord II with chart recorder (LKB Produkter, Bromma, Sweden). A laboratory-made electronic unit, based on a logarithmic circuit (Module 757P, Analog Devices Ltd.), was inserted between the detector and chart recorder to produce a signal that was proportional to absorbance instead of absorption, as was the case with the original system. Fractions of effluent were collected in a Radi Rac collector fitted with a drop counter (LKB Produkter).

The concentrations of copper and zinc in the fractions were determined by flame atomic-absorption spectroscopy (Pye Unicam, Model SP2900). A programmable laboratory-made turntable with continuously variable sampling and wash times and with facilities for replicate determinations was used to supply samples to the instrument. The sampling and wash times in the present application were 3 and 7 s, respectively, and this cycle was performed twice on each fraction, the total consumption of sample being less than 0.2 ml. The output of the instrument was presented on a chart recorder (Philips), an extra capacitor being incorporated to give a response time of about 1 s. Sodium, potassium and magnesium were measured simultaneously, using a modified atomic-absorption spectrophotometer (Pye Unicam, Model SP90). Magnesium was determined by atomic-absorption spectroscopy and sodium and potassium by flame-emission spectroscopy, the light emitted from the flame being collected and transmitted to external filter photometers by means of a fibre-optic system.

Reagents

Reagents of the highest available purity, usually AnalaR or Aristar grade, were used in order to minimise the trace-element background signal. Chelex 100 (Bio-Rad Laboratories, Watford) was used in some experiments to reduce contamination still further. All gels were manufactured by Pharmacia Fine Chemicals. Solutions were prepared with singly glass-distilled water. Eluting solutions contained tris(hydroxymethyl)methylamine (Tris), hydrochloric acid and sodium chloride. Commercial standard solutions for atomic-absorption spectroscopy (BDH Chemicals) were diluted with eluting solutions to minimise errors arising from contamination or interference. Albumin, caeruloplasmin and α-, β- and γ-globulins were purchased from Sigma London Chemical Co.

Procedure

Powdered gels were prepared in Tris - hydrochloric acid buffer, pH 7.4 or 8.6, ionic strength 0.05 mol l^{-1}. After loading on to the column, all gels were washed with three bed volumes of the eluting fluid (initial eluting fluid in the case of gradient elution). Heparinised plasma from

normal adults (0.5 ml) was added to the column and the flow-rate of the eluting fluid was adjusted to about 15.0 ml h^{-1}. Between 30 and 40 fractions (1.5 ml) were collected in plastic sample cups, which were then transferred manually to the turntables of the SP2900 and SP90 instruments and analysed without further treatment, using the instrument settings recommended in the manufacturer's handbooks. Total concentrations of copper and zinc in plasma were determined after 10-fold dilution with 0.1 mol l^{-1} hydrochloric acid.[10,11] The wavelengths used were sodium 589.0, potassium 766.5, magnesium 285.2, copper 324.8 and zinc 213.9 nm.

Results

Gel Filtration

Chromatography on Sephadex G-150 gave only partial separation of protein-bound copper or zinc under the experimental conditions used [Fig. 2(*a*)]. The use of Sepharose CL-6B and a longer column gave improved separation of both proteins and metals [Fig. 2(*b*)]. There appeared to be at least two zinc fractions but only one copper fraction.

Some improvement in the fractionation of both copper and zinc was achieved with Sephacryl S-200 [Fig. 3(*a*)], with both copper and zinc exhibiting at least two fractions. Similar results were obtained with Sephacryl S-300, for both copper and zinc [Fig. 3(*b*)], but the separation of plasma proteins was still poor.

Ion-exchange Chromatography

The use of DEAE-Sephadex A-50 with gradient elution gave a better separation of plasma proteins than could be achieved with gel filtration. There were also several copper and zinc peaks [Fig. 4(*a*)]. However, the reproducibility of results on repeated runs with the same

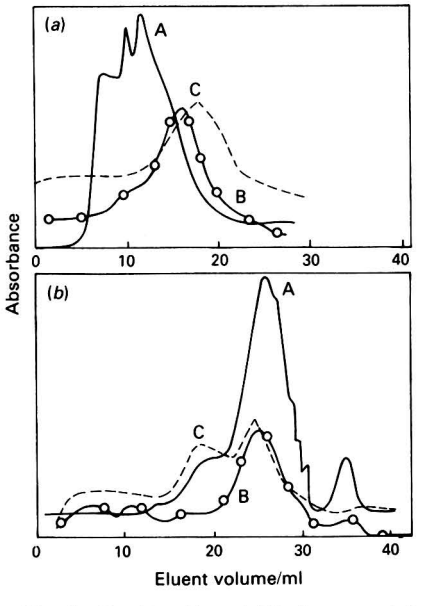

Fig. 2. Fractionation of (A) plasma proteins, (B) copper and (C) zinc. (*a*) Sephadex G-150; bed height, 30 cm; flow-rate, 12 ml h^{-1}; eluent, 0.1 mol l^{-1} Tris - HCl buffer, pH 8.0, and 1.0 mol l^{-1} NaCl. (*b*) Sepharose CL-6B; bed height, 57 cm; flow-rate, 14 ml h^{-1}; eluent, 0.1 mol l^{-1} Tris - HCl buffer, pH 7.4, and 0.2 mol l^{-1} NaCl.

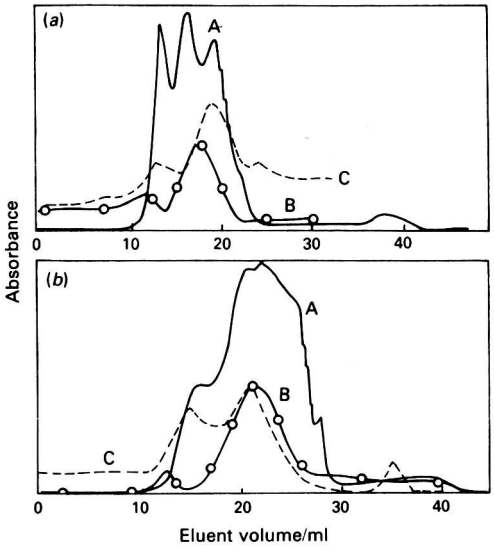

Fig. 3. Separation of (A) plasma proteins, (B) copper and (C) zinc. (*a*) Sephacryl S-200; bed height, 57 cm; flow-rate, 7 ml h^{-1}; eluent, 0.1 mol l^{-1} Tris - HCl buffer, pH 7.4, and 0.2 mol l^{-1} NaCl. (*b*) Sephacryl S-300; bed height, 57 cm; flow-rate, 14 ml h^{-1}; eluent, 0.1 mol l^{-1} Tris - HCl buffer, pH 8.0, and 0.5 mol l^{-1} NaCl.

sample was poor, owing to changes in bed volume and flow-rates as the gel settled. A comparable degree of separation of proteins and associated metals was achieved by using DEAE-Sepharose CL-6B in place of DEAE-Sephadex [Fig. 4(*b*)]. The use of the more rigid agarose gel gave better reproducibility than was the case with DEAE-Sephadex but both ion-exchange procedures suffered from the disadvantage that a heterogeneous mixture of proteins and metals was eluted in the first fractions owing to the salts already present in the plasma samples. Preliminary de-salting on a small column of Sephadex G-25 was unsatisfactory because it resulted in unwanted dilution of the sample and loss of copper and zinc. The possibility of using an eluting buffer with a fixed ionic strength, comparable to that of plasma, was therefore investigated.

Chromatography on DEAE-Sepharose CL-6B with an Eluent of Constant Ionic Strength

The protein and metal elution patterns for a system using DEAE-Sepharose CL-6B and an eluent of constant ionic strength are shown in Fig. 5. With this procedure the early "self-elution" of protein, copper and zinc was eliminated and an ultraviolet absorbance pattern with four peaks was obtained. Copper and zinc were each partially separated into two fractions. In Figs. 2–4, the curves for copper and zinc have an elevated base line attributable to contamination of the eluting solution by about 0.01 p.p.m. of those elements. The contamination arose from the presence of copper and zinc in the Tris and sodium chloride used to prepare the eluting solution. In the final procedure this contamination was reduced by passing the eluting fluid through a column of Chelex 100 (30 × 0.9 cm). This treatment removed at least 98% of the copper and 80% of the zinc, as shown by the near zero base lines in Fig. 5.

Identification of Ultraviolet Absorption Peaks

An attempt was made to identify the ultraviolet absorption peaks in Fig. 5 by eluting α-, β- and γ-globulins, albumin, caeruloplasmin and lithium urate under the same experimental conditions and using copper as a marker for caeruloplasmin. This procedure was followed

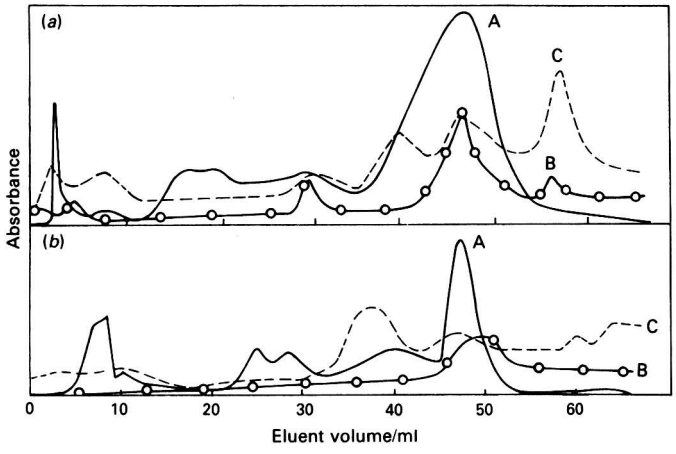

Fig. 4. Separation of (A) plasma proteins, (B) copper and (C) zinc. (*a*) DEAE-Sephadex A-50; Tris - HCl buffer, pH 8.3, with a linear salt gradient of 0.0–0.4 mol l^{-1} NaCl; bed height, 57 cm; flow-rate, 18 ml h^{-1}. (*b*) DEAE-Sepharose CL-6B; Tris - HCl buffer, pH 8.6, with a linear salt gradient of 0.0–0.25 mol l^{-1} NaCl; bed height, 57 cm; flow-rate 11 ml h^{-1}.

because the ultraviolet absorbance peak for caeruloplasmin would be masked by the higher concentrations of other proteins present in the sample. Fig. 6(*a*) shows the pattern obtained with a mixture of albumin, caeruloplasmin and lithium urate. Albumin and caeruloplasmin both gave peaks at 24 ml whereas lithium urate emerged much later at 56 ml. A mixture of α- and β-globulins, albumin, caeruloplasmin and lithium urate, on the other hand, gave peaks at 10, 19, 27 and 31 ml and a late peak at 50 ml [Fig. 6(*b*)], while a mixture of α-, β- and γ-

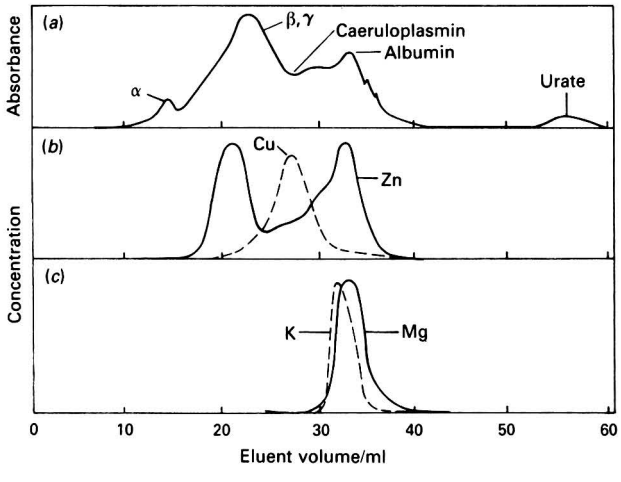

Fig. 5. Separation patterns of (*a*) plasma proteins, (*b*) copper and zinc and (*c*) magnesium and potassium on DEAE-Sepharose CL-6B, using an eluent of constant ionic strength (Tris - HCl buffer, pH 8.6, containing NaCl, 0.2 mol l^{-1}; total ionic strength = 0.25 mol l^{-1}). Bed height, 54 cm; flow-rate, 14 ml h^{-1}.

globulins, caeruloplasmin and lithium urate gave major peaks at 11 and 20 ml, small peaks at 30 and 40 ml and a late peak at 57 ml [Fig. 6(*c*)]. Finally, a mixture of α-, β- and γ-globulin with additional albumin, caeruloplasmin and lithium urate gave four peaks at 10, 18, 25 and 52 ml [Fig. 6(*d*)].

Lithium urate eluted at a relatively constant volume, whatever the composition of the mixture, but the proteins showed some variability, particularly caeruloplasmin, which tended to elute with albumin at about 25 ml if albumin was present and at a higher value if albumin was absent. The identity of the absorbance peaks in Fig. 5 therefore cannot be deduced with absolute certainty from Fig. 6(*a*)–(*d*). It may be noted that the albumin peak in Fig. 5 is abnormally low; this is because the sample was obtained from a patient suffering from malabsorption and mild renal failure.

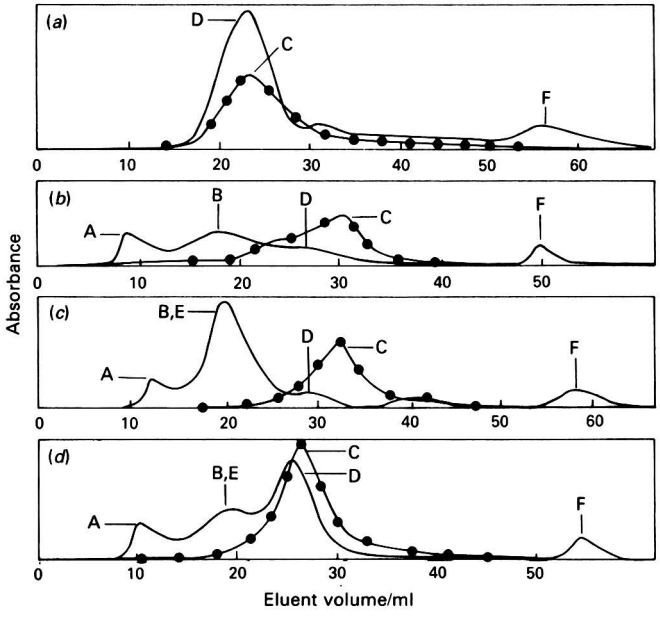

Fig. 6. Separation of mixtures of proteins (——), copper (●——●) and lithium urate on DEAE-Sepharose CL-6B. The sample volume was 0.5 ml in all instances; other conditions were as in Fig. 5. The test solutions had the following composition: (*a*) albumin 20 g l^{-1}, caeruloplasmin 20 g l^{-1}, lithium urate 0.3 g l^{-1}; (*b*) α-globulins 27.2 g l^{-1}, β-globulins 9.2 g l^{-1}, albumin 5.6 g l^{-1}, caeruloplasmin 0.8 g l^{-1}, lithium urate 0.05 g l^{-1}; (*c*) α-globulins 27.2 g l^{-1}, β-globulins 9.2 g l^{-1}, γ-globulins 20.0 g l^{-1}, albumin 5.6 g l^{-1}, caeruloplasmin 6.0 g l^{-1}, lithium urate 0.05 g l^{-1}; (*d*) α-globulins 27.2 g l^{-1}, β-globulins 9.2 g l^{-1}, albumin 25.6 g l^{-1}, caeruloplasmin 0.8 g l^{-1}, lithium urate 0.05 g l^{-1}. Peaks: A, α-globulins; B, β-globulins; C, caeruloplasmin; D, albumin; E, γ-globulins; and F, lithium urate.

Discussion and Conclusions

Recent reports indicate that both copper and zinc concentrations are higher in serum than in plasma, possibly owing to liberation of these metals from platelets or other cells during the process of clotting.[12,13] Plasma is therefore to be preferred to serum and was used throughout this study. Moreover, the protein elution pattern of plasma showed fewer signs of change with ageing than was the case with serum.

The ion-exchange procedure described above departs from usual practice in that an eluting buffer of fixed ionic strength is used instead of one with a steadily increasing concentration. The use of a single-strength buffer is less effective in separating fractions but it avoids the need

to de-salt the specimen, with the attendant risk of loss of metals. The procedure finally adopted gave good reproducibility of both protein and metal patterns on repeated runs on the same sample, a feature of some importance in comparing one patient with another and in deriving quantitative data.

The identity of the protein peaks has been partly established but more detailed examination of the degree of homogeneity of each peak is needed, using more refined techniques such as electrophoresis or ultracentrifugation. The distribution of copper and zinc between albumin and the various globulins is broadly in agreement with published data,[1,3,4] most of the copper being bound to caeruloplasmin and to a lesser extent to albumin, whereas zinc appeared to be bound mainly to globulins, although the relative distribution of zinc between albumin and globulins appeared to differ from one individual to another; this aspect is being examined in greater detail.

So far, it has not been possible to detect copper or zinc bound to small molecules such as amino acids because of the low signal to noise ratio in this area of the chromatogram, but this limitation can probably be overcome by the use of atomic-absorprion spectroscopy with electrothermal atomisation and work on this problem is in progress.

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Gas - Liquid Chromatographic Determination of Major Constituents of *Piper methysticum*

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A procedure is described for the quantitative determination of seven known major constituents in sun-dried roots, rhizomes and commercially powdered samples of *Piper methysticum*. A 3.0–8.0-g amount of powdered sample is extracted with chloroform in a Soxhlet apparatus for 6 h. After evaporation of solvent the extract (about 0.35 g) is dried at 100 °C for 2 h and then dissolved in chloroform to about 0.7% *m/V* concentration. The resulting solution is analysed by gas - liquid chromatography using dual 1.5 m × 4 mm i.d. glass columns, containing 3% *m/m* of OV-1 on Chromosorb W HP, and dual differential flame-ionisation detectors with nitrogen as carrier gas, the column temperature being 210 °C. There is no interference from the eight other trace constituents, non-polar low-boiling compounds or polar "tarry" material.

Keywords: Piper methysticum *analysis; gas - liquid chromatography*

Chemical and pharmacological investigations of constituents of the sun-dried roots and rhizomes (known locally as "waka" and "lawena," respectively) of *Piper methysticum*, which began in 1860,[1–3] led to the publication of numerous papers and reviews.[4–6] To date at least seven major and eight trace constituents have been isolated from the roots of the shrub[4–7] (see Table I). A number of these constituents have been shown to possess interesting physiological properties such as sleep-producing,[8,9] antimycotic,[10] local anaesthetic,[11] anti-convulsive[12–14] and smooth muscle contraction[15] effects.

The beverage prepared by straining the powdered roots or rhizomes of *Piper methysticum* with water is a national drink, and is held in high esteem in all traditional Fijian ceremonies. It is also a common traditional drink in other Pacific islands such as Samoa, Tonga and Hawaii. During an investigation by the author of the quality of commercially powdered samples of *Piper methysticum* (known locally as "yaqona" or "kava"), it became necessary to determine the amounts of major constituents. Numerous reports[5–8,16] exist on the column and thin-layer chromatographic separation of the active constituents, but these methods are unsatisfactory and there does not appear to be a simple and rapid method for the quantitation of these constituents. This paper, which deals with the gas - liquid chromatographic analysis of the seven major constituents of *Piper methysticum*, aims to bridge this gap.

Experimental

Structures of the compounds concerned are shown in Fig. 1.

Apparatus

An electrothermal melting-point apparatus, a Hewlett Packard, Model 5730A, gas - liquid chromatograph, a Model 3380A integrator and a Model 7123A recorder were used.

All glassware was cleaned with chromic acid, washed with distilled water and oven dried at 100 °C.

Reagents

All reagents and solvents were of analytical-reagent grade unless otherwise stated. The solvents were chloroform, methanol and benzene. Aluminium oxide of Brockmann activity II (Merck) was used.

Standard Solutions

Standard solution A of major constituents

Prepare the following standard solution in chloroform (*m/V*): 0.12% 7,8-dihydrokawain (1), 0.12% kawain (2), 0.03% 5,6-dehydrokawain (6), 0.02% 5,6,7,8-tetrahydroyangonin (7), 0.075% 7,8-dihydromethysticin (5), 0.075% yangonin (4) and 0.12% methysticin (3).

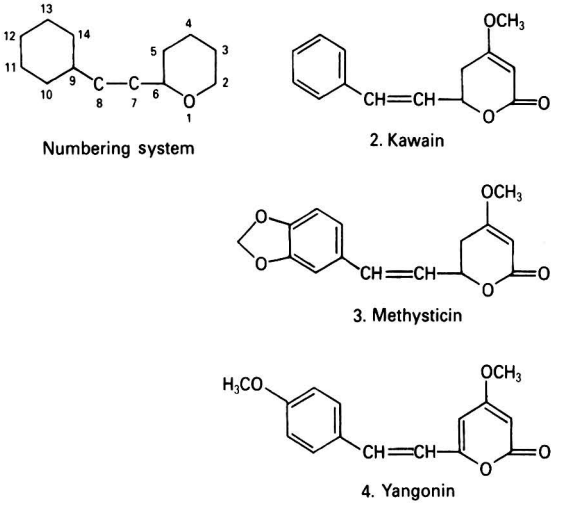

Fig. 1. Structures of the three parent compounds of *Piper methysticum*. The structural formulae for all other kava pyrones, 1 and 5–15, can be derived by using the numbering system above and diagrams 2, 3 and 4.

Standard solution B of trace constituents

The trace constituents were provided by Professor R. Hansel of Frieie Universität, Germany. Prepare a 0.003% *m/V* solution of each of *cis*-5-hydroxykawain (8), 7,8-dihydroyangonin (9), 5,6-dihydroyangonin (10), 5,6-dehydromethysticin (11), 11-methoxyyangonin (12), 11-hydroxyyangonin (13), 11-methoxy-12-hydroxy-5,6-dehydrokawain (14) and 10-methoxyyangonin (15).

Standard solution C of all active constituents

Prepare this solution by combining solutions A and B. It has concentrations of constituents as in the original solutions.

Standard solutions A, B and C have similar constituent ratios and concentrations to those in the samples and sample solutions. The standard and sample solutions are stable for at least 6 months if stored in a refrigerator.

Isolation of Major Constituents

Yangonin (4), methysticin (3), 7,8-dihydromethysticin (5), kawain (2), 5,6-dehydrokawain (6) and 7,8-dihydrokawain (1) were separated by column chromatography on aluminium oxide and purified by repetitive crystallisation as described elsewhere.[8] 5,6,7,8-Tetrahydroyangonin (7) was isolated by a combination of column chromatography[8] and the proposed gas - liquid chromatography and purified by crystallisation from methanol. 7,8-Dihydromethysticin (5), 7,8-dihydrokawain (1) and 5,6,7,8-tetrahydroyangonin (7) were obtained by catalytic hydrogenation of the parent compounds methysticin (3), kawain (2) and yangonin (4), respectively. Hydrogenation was carried out at room temperature on a 2-g sample using 0.1 g of platinum dioxide and 75 ml of ethyl acetate and was complete in 4 h. After filtration and distillation of the solvent *in vacuo*, the hydrogenated products were recrystallised.[8] The hydrogenated products had similar melting-points and mixed melting-points to the naturally occurring compounds.

Preparation of Samples

The dried roots and rhizomes were obtained from local markets, cut into thin slices and ground to pass a No. 25 sieve. Commercial powders were further ground to pass a No. 25 sieve.

Moisture Determination

Dry 5 g of prepared sample at 100 °C for 5 h.

Extraction of Constituents

Extract 3.0 g of prepared roots or 5.5 g of rhizomes or 8.0 g of commercial powders with chloroform in a Soxhlet apparatus for 6 h. Evaporate the solvent on a water-bath and dry the extract at 100 °C for 2 h. Dissolve the dried extract (about 0.35 g) in chloroform and make up to 50 ml with chloroform in a calibrated flask to give a concentration of about 0.7% *m/V* (sample solution D).

Gas - Liquid Chromatography

Inject 3 μl each of standard solution A and sample solution D into the gas chromatograph using the following conditions: column, dual 1.5 m × 4 mm i.d. glass containing 3% *m/m* of OV-1 on Chromosorb W HP; detector, dual differential flame-ionisation; carrier gas, nitrogen at 300 kPa and 60 ml min^{-1}; fuel gases, hydrogen at 150 kPa and 60 ml min^{-1} and air at 180 kPa and 240 ml min^{-1}; column temperature, 210 °C; detector temperature, 300 °C; injection port temperature, 250 °C; and integrator, sensitivity 0.3–1.0 mV min^{-1}, attenuation 64, chart speed 5 mm min^{-1}.

Determination of Polar "Tarry" Material in Extract by Clean-up on an Aluminium Oxide Column

Wash 4–5 g of aluminium oxide contained in a glass column (12 mm diameter) with 20 ml of chloroform. Apply a suitable amount (generally about 0.35 g) of extract in 5 ml of chloroform to the washed column, elute with 50 ml of chloroform and collect the eluate in an already dried and weighed 100–150-ml conical flask. Evaporate the solvent on a water-bath and dry the extract at 100 °C for 2 h to give a cleaned-up extract. The difference in mass represents polar "tarry" material.

Thin-layer Chromatography

Thin-layer chromatography was carried out on 0.5-mm silica gel G plates with benzene - methanol (98.5 + 1.5) as developing solvents and the spots were revealed with iodine vapour.

Results and Discussion

The seven major and eight trace constituents of the roots and rhizomes of *Piper methysticum* are as presented in Table I and Fig. 1. The constituents are all derivatives of the three variant compounds, namely kawain (2), methysticin (3) and yangonin (4). The major constituents have traditionally been isolated by column chromatography[8,16] and the trace constituents by preparative thin-layer chromatography.[7] We have found that neither column nor thin-layer chromatography could be used for the quantitative determination of the constituents, as in the former the separation is incomplete and in the latter all of the major constituents have similar R_F values (Table I). The proposed gas - liquid chromatographic analysis of the major constituents overcomes this problem and offers a simple and rapid technique for their determination in *Piper methysticum*.

Extraction of Constituents

Chloroform,[8] diethyl ether[16] and ethanol[16] have been used for the extraction of constituents of *Piper methysticum*. Some of the constituents, such as methysticin (3) and yangonin (4), are insoluble in diethyl ether but all constituents are soluble in chloroform. We therefore used chloroform for the extraction work. It was found that 95% and 99% of the constituents are extracted in the first 2 h and 4 h, respectively, and we therefore used 6 h as the extraction time. The difference in the amounts of roots, rhizomes and commercial powders of *Piper methysticum* taken for extraction is to ensure that uniform amounts of extract (*ca.* 0.35 g) are obtained.

Quantification and Linear Range

A typical chromatogram is shown in Fig. 2, and the tested linear range limits and retention times of the various constituents are reported in Table I.

TABLE I

CHROMATOGRAPHIC CHARACTERISTICS AND CONCENTRATIONS OF CONSTITUENTS OF *Piper Methysticum*

Compound No.	Constituents	Melting-point/°C	Retention time (GLC) at 210 °C/min	Tested limit of linear range/μg	Retention time at 240 °C/min	R_F (TLC)	Average content of constituents on dry mass basis, % *m/m*		
							Roots (6 samples)	Rhizomes (6 samples)	Commercial powder (12 samples)
	Major constituents—								
1	7,8-Dihydrokawain	55–57[17]	5.88	4.8	2.4	0.41	2.37	1.20	0.70
2	Kawain	107[17]	8.01	4.8	2.8	0.43	1.90	1.17	0.84
3	Methysticin	139–140.5[18]	27.0	—	8.0	0.39	2.12	1.00	0.69
4	Yangonin	155–157[19]	25.63	3.0	8.0	0.48	1.73	0.70	0.47
5	7,8-Dihydromethysticin	117–118[18]	18.33	3.0	6.4	0.39	1.12	0.69	0.61
6	5,6-Dehydrokawain	138–139[8]	10.27	1.2	3.6	0.52	0.81	0.32	0.18
7	5,6,7,8-Tetrahydroyangonin	99–100[7]	13.12	0.75	4.4	0.43	0.39	0.20	0.14
	Total of 1–7	—	—	—	—	—	10.44	5.28	3.63
7a	Tarry material	—	—	—	—	0.00	0.71	0.55	0.41
	Total of 1–7a	—	—	—	—	—	11.15	5.83	4.04
	Chloroform extract	—	—	—	—	—	12.21	6.61	4.69
	Other constituents—	—	—	—	—	—	1.06	0.78	0.65
	I Non-polar compounds		<5.0		<2.0	>0.65			
	II *Trace constituents*—								
8	*cis*-5-Hydroxykawain	120–122[7]	9.58	—	4.0	0.10			
9	7,8-Dihydroyangonin	104–106[7]	11.80	—	3.2	0.48			
10	5,6-Dihydroyangonin	122–124[7]	Decomposes	—	Decomposes	0.37			
11	5,6-Dehydromethysticin	230–231[7]	37.54	—	10.8	0.50			
12	11-Methoxyyangonin	155–157[7]	50.18	—	13.6	0.40			
13	11-Hydroxyyangonin	196–200[7]	50.18	..	13.6	0.18			
14	11-Methoxy-12-hydroxy-dehydrokawain	119–220[7]	50.18	—	13.6	0.19			
15	10-Methoxyyangonin	191–192[7]	56.12	—	14.8	0.42			

For 7,8-dihydrokawain (1), kawain (2), 5,6-dehydrokawain (6), 5,6,7,8-tetrahydroyangonin (7) and 7,8-dihydromethysticin (5), quantification was effected by comparisons of either standard and sample peak heights or integrated peak areas. However, the peak of yangonin (4) could not be completely separated from that of methysticin (3), which appears as a shoulder on the former. However, the peak height of yangonin (4) is linearly related to amount, irrespective of the amount of methysticin (3) present, and therefore can be determined. However, as the peak height of methysticin (3) is not linearly related to amount, the amount of methysticin (3) is determined by matching its peak heights in a standard and a sample. This is made simple by the fact that the ratio of yangonin (4) to methysticin (3) in roots, rhizomes and commercial powders of *Piper methysticum* is reasonably constant (1.7:2.1, 1.54:2.1 and 1.5:2.1, respectively).

Clean-up of Extract

The total amount of the major constituents 1–7 (see Table I) was found to be lower than that in the total chloroform extract. Thin-layer chromatography of standard C and a chloroform extract of *Piper methysticum* root sample showed that in addition to the major and trace constituents, the root extract contained some non-polar compounds and polar "tarry" material. The polar "tarry" material had an R_F value of 0. The amount of "tarry" material could be determined as described under Experimental. Between 10 and 60 ml of standard solution A, representing 0.056–0.336 g of major constituents, and between 10 and 40 ml of standard solution B, representing 0.0021–0.0084 g of trace constituents were subjected to the clean-up procedure, and subsequent analysis showed no significant adsorption of active constituents on the column at these concentrations. However, clean-up of 0.25–40 g of a chloroform extract of *Piper methysticum* roots on aluminium oxide showed an average adsorption of 0.7% *m/m* (based on the dry mass of the sample) of polar "tarry" material; thin-layer chromatography of the cleaned-up extract showed the absence of polar "tarry" material. However, the amount of the major constituents in the cleaned-up extract was the same as that in the unpurified extract. Hence, although the clean-up procedure provides a method for the determination of polar "tarry" material, it is not necessary for the determination of active constituents.

Interferences

Using the concentration ranges of standards and samples mentioned under Clean-up of Extract, it was established that heating for 6 h under reflux during extraction and drying for 2 h at 100 °C had no significant effects on the analysis. There was no interference by

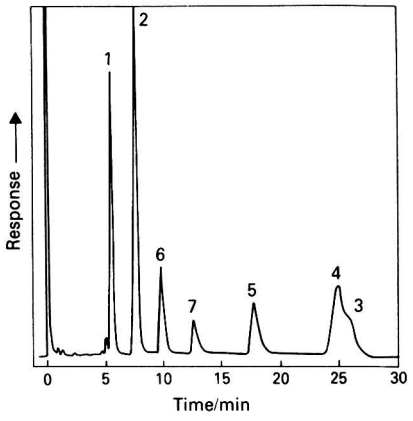

Fig. 2. Gas - liquid chromatogram obtained from a 3-μl injection of a 0.7% m/V solution of chloroform extract of *Piper methysticum*. Other conditions are as stated in text. Peaks: 1 = 7,8-dihydrokawain; 2 = kawain; 3 = methysticin; 4 = yangonin; 5 = 7,8-dihydromethysticin: 6 = 5,6-dehydrokawain; and 7 = 5,6,7,8-tetrahydroyangonin.

the trace constituents, as these have different retention times (see Table I). In addition, the comparatively low concentration of trace constituents in the samples did not give rise to any peaks under the given conditions of analysis. The analysis was complete within 0.5 h. A column temperature of 210 °C was used to distinguish the methysticin (3) peak from the yangonin (4) peak within a reasonable time. An increase in temperature resulted in the collapse of these two peaks, whereas a decrease in temperature only increased retention times without any improvement in the separation of the two peaks. It is clear from Table I that the proposed method is not satisfactory for the determination of trace constituents, owing mainly to the similar and relatively high retention times of some of the constituents. We are currently studying methods of analysis of trace constituents. There is also no interference by polar "tarry" material or non-polar low-boiling compounds, as the latter elute before any of the active constituents.

Precision

Satisfactory reproducibilities were obtained by the proposed method (Table II).

TABLE II

CONTENTS OF MAJOR CONSTITUENTS, CHLOROFORM EXTRACT AND POLAR "TARRY" MATERIAL FROM 10 RUNS ON A RHIZOME SAMPLE OF *Piper methysticum*

Constituent	Content, % m/m										Average	Standard deviation
7,8-Dihydrokawain	1.05	1.14	1.11	1.06	1.11	1.07	1.08	1.08	1.08	1.09	1.08	2.78
Kawain	1.22	1.22	1.25	1.25	1.28	1.31	1.21	1.27	1.29	1.28	1.26	2.38
Methysticin	1.15	1.10	1.12	1.21	1.17	1.17	1.13	1.17	1.19	1.17	1.16	2.59
Yangonin	0.82	0.82	0.87	0.84	0.87	0.79	0.81	0.83	0.84	0.86	4.84	3.57
7,8-Dihydromethysticin	0.68	0.66	0.66	0.65	0.71	0.62	0.66	0.68	0.68	0.69	0.67	2.99
5,6-Dehydrokawain	0.28	0.30	0.30	0.30	0.31	0.29	0.29	0.31	0.30	0.30	0.30	3.33
5,6,7,8-Tetrahydroyangonin	0.128	0.127	0.127	0.129	0.130	0.121	0.121	0.128	0.125	0.126	0.127	1.57
Polar "tarry" material	0.71	0.73	0.66	0.61	0.67	0.64	0.72	0.65	0.71	0.63	0.67	5.97
Chloroform extract	6.56	7.02	6.78	6.92	6.56	6.54	6.79	6.66	6.96	7.00	6.78	2.80

Conclusion

A simple and rapid gas - liquid chromatographic technique has been developed for the determination of the major constituents of *Piper methysticum*. This initial study has also shown considerable variations in the amounts of major constituents in roots, rhizomes and commercial powders. It is expected that the proposed method of analysis will find use in the column chromatographic separation and purification of the major constituents of *Piper methysticum*. The procedure described here is also being used for a study of variations in the amounts of constituents in roots, rhizomes and commercial powders of local *Piper methysticum* as part of a general survey of the quality of the local product.

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Gas-chromatographic Determination of Trace Amounts of Lower Fatty Acids in Ambient Air Near and in Exhaust Gases of Some Odour Sources

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The gas-chromatographic determination of trace amounts of the lower fatty acids (C_2–C_5) in ambient air near and in the exhaust gases of some odour sources was investigated. The sample for the gas-chromatographic determination was prepared by trapping in a pre-column packed with FFAP + orthophosphoric acid (H_3PO_4) on Carbopack C at 25 and 30 °C. The lower fatty acids were identified and quantitated from the difference between the chromatograms obtained using the FFAP + H_3PO_4 pre-column and that obtained using the FFAP + H_3PO_4 plus an alkaline pre-column. The method has been applied to the analysis of lower fatty acids in practical specimens, namely the ambient air near accumulated poultry manure, in a pig pen and a fish meal factory, and in the exhaust gases from a corn starch manufacturing factory and from a poultry manure dryer. The sample volume is as low as about 0.4 l and the method is sensitive (detection limit about 0.5 p.p.b.) and rapid (including the concentration and analysis of one sample, about 15 min are required). The coefficient of variation is less than 6%. This sensitivity and precision are adequate for use in odour pollution analysis.

Keywords: Lower fatty acid determination; air analysis; pre-column concentration; odour sources

Organic acids and their derivatives are very important in industry; however, several of these compounds are primary irritants[1] and the lower fatty acids are frequently implicated in odour pollution. In this context, their identification and quantitation are extremely important, as is their analysis in foods, in cigarettes and cigarette smoke and in drugs. Because of their low odour threshold (below 1 part per 10^9 in air), the determination of trace concentrations of these compounds in air presents a formidable task.

The direct gas-chromatographic determination of these free lower fatty acids at low concentrations has been limited by adsorption and decomposition in the analytical column, "ghosting" phenomena and tailing of eluting peaks. Kuksis[2] reviewed recent developments in the methodology and application of gas - liquid chromatography to the analysis of free fatty acids in the underivatised form. Two special issues of the *Journal of Chromatographic Science* were devoted to a comparison of techniques for the analysis of fatty acids and fatty acid esters.[3] DiCorcia and co-workers[4–7] reported the complete separation of C_2–C_5 lower fatty acids at the nanogram level using gas - liquid - solid chromatography (GLSC) without adsorption and tailing. The peaks obtained were generally sharp.

In this study, a simple and rapid procedure for the accurate determination of trace amounts of lower fatty acids (C_2–C_5) in ambient air near and in the exhaust gases of some odour sources is described. The acids were collected in a pre-column using FFAP (0.1%) + orthophosphoric acid (H_3PO_4) (0.1%) on Carbopack C (80–100 mesh) at 25 and 30 °C. For desorption the pre-column was heated from room temperature to 200 °C in 30 s, then held at that temperature for 30 s. The sample was subsequently introduced into the GLSC system with FFAP (0.3%) + H_3PO_4 (0.3%) on Carbopack B (60–80 mesh) as the packing in the analytical column. The packing of the main analytical column and the trapping pre-column produced peaks free from tailing. The identification of the peaks of the acids was carried out by the disappearance method using an alkaline pre-column.

Experimental

Reagents and Materials

The lower fatty acids (C_2–C_5) and other reagents used were obtained from PolyScience (Niles, Ill., USA), Wako Pure Chemical Industries (Osaka, Japan), Katayama Chemical

Industries (Osaka, Japan) and Tokyo Kasei Kogyo (Tokyo, Japan). All reagents were of guaranteed or analytical-reagent grade.

A standard solution of lower fatty acids was prepared by dissolving the acids in distilled water to give a concentration of each acid of 0.1 mmol per 10 ml. Calibration graphs were obtained using the standard solution diluted 1 + 10 or 1 + 100.

The column packings were purchased from Wako.

Apparatus

The gas chromatograph used was a Shimadzu, Model GC5AP$_5$F, equipped with on-column injection, a flame-ionisation detector (FID) and a digital integrator (Shimadzu, Model ITG-2A), for the determination of retention times and quantitative analysis. The detector signal was recorded at 10 mV full scale simultaneously on a Shimadzu, Model R-201, double-pen recorder.

GLSC Conditions

The main analytical column was glass (1.5 m × 3 mm i.d.), packed with 0.3% FFAP + 0.3% H_3PO_4 on Carbopack B (60–80 mesh). This column was pre-conditioned at 250 °C for 10 h with a constant flow of nitrogen (60 ml min^{-1}) before being connected to the FID. The chromatographic conditions for the FID were as follows: main analytical column temperature, 200 or 220 °C; injection port and detector temperature, 250 °C; carrier gas (nitrogen) flow-rate, 50 ml min^{-1}; hydrogen and air flow-rates, 50 ml min^{-1} and 1.0 l min^{-1}, respectively.

Pre-column Collection and Injection Methods for Sample Gases

The pre-column consisted of a 16 cm × 4 mm i.d. glass tube packed with 0.1% FFAP + 0.1% H_3PO_4 on Carbopack C (80–100 mesh). The pre-column was pre-conditioned at 250 °C for 2 h with a constant flow of nitrogen (60 ml min^{-1}) before being used for the collection of the sample gases. The sample gases were collected directly in the pre-column at 25 and 30 °C using a vacuum pump (Mini-Vac, Model PS-05, Yamato, Japan, maximum rate 5 l min^{-1}) and a gas meter (T-3 Dry Test gas meter, Chubushinagawa Seisakusho, Japan, 1 l rev^{-1}). Pre-concentration of the lower fatty acids (C_2–C_5) in sample gases was carried out by using the FFAP + H_3PO_4 adsorption pre-column and the FFAP + H_3PO_4 pre-column plus an alkaline pre-column. The FFAP + H_3PO_4 pre-column and the alkaline pre-column were connected with a PTFE tube (2 cm × 1 mm i.d.) and the sampling rate was about 1 l per 4 min. Then the FFAP + H_3PO_4 pre-column was connected to the carrier gas line of the gas chromatograph.

After about 5 min, a constant flow-rate of the carrier gas was maintained, and the FFAP + H_3PO_4 pre-column was heated from room temperature (21 °C) to 200 °C in 24 s, this temperature was maintained for 30 s, then the column was cooled to room temperature.

For the preparation of the calibration graphs, the volume of the standard solution and the diluted solution of the lower fatty acids (C_2–C_5) injected into the FFAP + H_3PO_4 pre-column was usually 1–5 μl.

Alkaline Pre-column

The alkaline pre-column consisted of 2% sodium hydroxide on glass beads (30–60 mesh) (Wako), packed into a glass tube (2 cm × 5 mm i.d.). This column was pre-conditioned at 150 °C for 2 h with a constant flow of nitrogen (60 ml min^{-1}) before being used for the collection of the sample gases. This column was placed in the front port of the FFAP + H_3PO_4 pre-column, and used for the identification of the lower fatty acid peaks by means of the disappearance method.

Results and Discussion

Separability of the Lower Fatty Acids and Other Compounds on the Main Analytical Column (FFAP + H_3PO_4) by the Direct Injection Method

The retention times of about 130 compounds, including eight lower fatty acids, on the main analytical column (FFAP + H_3PO_4) were determined by the direct injection method at a column temperature of 200 °C.[8] According to previous work,[8] the following peaks overlapped: acetic

acid with *cis*-but-2-ene, *trans*-but-2-ene, buta-1,3-diene and isopropyl chloride; propionic acid with 2,2-dimethylbutane, cyclohexane, *sec*-butyl chloride and isobutyl chloride; isobutyric acid with isobutyl formate; butyric acid with ethyl acrylate; isovaleric acid with hept-1-ene; and valeric acid with amyl formate; however, *cis*-but-2-ene, *trans*-but-2-ene, buta-1,3-diene and 2,2-dimethylbutane passed through the FFAP + H_3PO_4 pre-column during the pre-concentration of the sample gases at 25 or 30 °C, because these compounds have low boiling-points, and the other compounds also passed through the alkaline pre-column.

Calibration Graphs

The FID response produced a straight line in the approximate range 10–1 000 ng of the six lower fatty acids (Fig. 1), and the detection limit at twice the noise level was about 0.5 ng. Therefore, when the pre-concentration volume is 1 l, the minimum detectable concentration is about 0.5 parts per 10^9 (p.p.b.). These sensitivities are adequate for use in odour pollution analysis.

Repeatability of the FFAP + H_3PO_4 Pre-column Injection Method

The repeatability and uniformity of the retention times and peak areas (as counts on the digital integrator) of the six lower fatty acids were evaluated by the FFAP + H_3PO_4 pre-column injection method. As can be seen from Table I, they showed good uniformity and repeatability. The amounts of the lower fatty acids tested were 60–100 ng.

TABLE I

REPEATABILITY OF RETENTION TIMES AND PEAK AREAS (AS COUNTS ON DIGITAL INTEGRATOR) OF SIX LOWER FATTY ACIDS USING THE FFAP + H_3PO_4 PRE-COLUMN INJECTION METHOD ($n = 6$)

	Retention time		Peak area	
Acid	Value ± standard deviation/min	Coefficient of variation, %	Value ± standard deviation, counts	Coefficient of variation, %
Acetic acid	1.24 ± 0.03	2.4	6034 ± 337	5.6
Propionic acid	1.71 ± 0.05	2.9	8992 ± 413	4.6
Butyric acid	2.88 ± 0.09	3.1	13602 ± 277	2.0
Isobutyric acid	2.47 ± 0.08	3.2	10899 ± 540	5.0
Valeric acid	5.75 ± 0.09	1.6	14897 ± 548	3.7
Isovaleric acid	4.92 ± 0.10	2.0	14190 ± 455	3.2

Relationship Between Recovery of the Lower Fatty Acids on the FFAP + H_3PO_4 Pre-column and the Nitrogen Carrier Gas Volume Passed Through the Pre-column

This test was carried out at 40 °C and a constant flow-rate of nitrogen of 0.25 l min^{-1}. The amounts of each lower fatty acid used were the same as in Table I. Quantitative recovery was obtained up to a sampling gas volume of about 5 l, except that the recovery of acetic and propionic acids decreased slightly (Table II).

Typical Gas Chromatograms of Lower Fatty Acids (C_2–C_5) in Practical Specimens

Fig. 2(A) shows a typical gas chromatogram of acetic (peak 1), propionic (2), isobutyric (3), butyric (4) and isovaleric acid (5) in ambient air near accumulated poultry manure. The volume concentrated was 0.4 l, and the sample air was trapped directly in the FFAP + H_3PO_4 pre-column. The pre-concentration procedure was as described under Experimental. Fig. 2(B) shows a typical gas chromatogram of sample air collected on the FFAP + H_3PO_4 pre-column, but passed through the alkaline pre-column. In this chromatogram, the peaks of isobutyric, butyric and isovaleric acids have disappeared completely. The lower fatty acids were identified and quantitated from the difference between chromatograms (A) and (B) in Fig. 2.

Fig. 2(C) shows a typical gas chromatogram of small amounts of the six lower fatty acids.

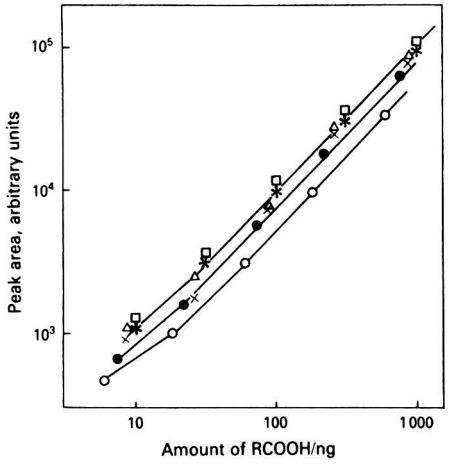

Fig. 1. Calibration graphs for six standard lower fatty acids using FFAP + H_3PO_4 pre-column: ○, acetic acid; ●, propionic acid; ×, isobutyric acid; Δ, butyric acid; □, isovaleric acid; and *, valeric acid. Main analytical column conditions: 0.3% FFAP + 0.3% H_3PO_4 on Carbopack B (60–80 mesh); 1.5 m × 3 mm i.d., glass; 220 °C; N_2, 50 ml min^{-1}; FID. Pre-column conditions: 0.1% FFAP + 0.1% H_3PO_4 on Carbopack C (80–100 mesh); 16 cm × 4 mm i.d., glass; temperature programmed from room temperature (21 °C) to 200 °C in 24 s and maintained at 200 °C for 30 s.

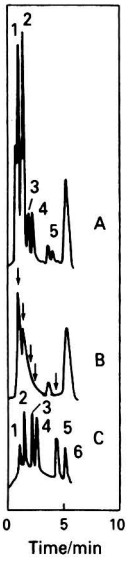

Fig. 2. Typical gas chromatograms of lower fatty acids (A), (B) in ambient air near an accumulated poultry manure and six standard lower fatty acids (C). (A) Sample volume, 0.4 l; FFAP + H_3PO_4 pre-column. Peaks: 1, acetic acid; 2, propionic acid; 3, isobutyric acid; 4, butyric acid; and 5, isovaleric acid. (B) Sample volume, 0.4 l; FFAP + H_3PO_4 pre-column, but passed through an alkaline pre-column. (C) Six standard lower fatty acids; FFAP + H_3PO_4 pre-column. Peaks: 1, acetic acid (6 ng); 2, propionic acid (7.4 ng); 3, isobutyric acid (8.8 ng); 4, butyric acid (8.8 ng); 5, isovaleric acid (10.2 ng); 6, valeric acid (10.2 ng). Main analytical column temperature, 220 °C. FID sensitivity, 2×10^2. Other conditions as described under Experimental.

The concentrations of the six lower fatty acids detected in practical specimens are listed in Table III, which also gives the odor characteristics (quality and intensity) of the sample gases.

Deibel[9] found butyric acid, ethanol and acetoin to be the chief volatile compounds in accumulated poultry manure. Burnett[10] used a combination of gas chromatography and organoleptic techniques to determine the compounds responsible for the offensive odour. The volatile components of liquid manure supernatant were identified, using the cold trapping method with acetone - dry-ice and gas chromatography, as mercaptans, organic acids, indole and skatole. Sulphur compounds, organic acids and skatole were implicated as major malodorous components. The acidic volatile compounds reported in poultry liquid manure were acetic, propionic, isobutyric, butyric, isovaleric and valeric acids. The acidic compounds in swine manure have been reported to be formic, acetic, propionic and butyric acids.[11,12]

Yasuhara and Fuwa[13] isolated seventeen carboxylic acids and four phenols from liquid swine manure by steam distillation and identified them by gas chromatography - mass spectrometry. The major components were butyric, isovaleric, benzoic and phenylacetic acids and *p*-cresol. Only the carboxylic acid fraction has the same odour as liquid swine manure. Butyric, isovaleric and phenylacetic acids have a very strong odour of manure.

TABLE II

RELATIONSHIP BETWEEN RECOVERY OF SIX LOWER FATTY ACIDS ON THE FFAP + H_3PO_4 PRE-COLUMN AND NITROGEN CARRIER GAS VOLUME PASSED THROUGH THE PRE-COLUMN

Values given are percentage recoveries at 40 °C.

Acid	Gas volume/l		
	0.5	1.5	5.0
Acetic acid	100	98.9	86.5
Propionic acid	100	99.4	94.9
Butyric acid	100	100	100
Isobutyric acid	100	100	100
Valeric acid	100	100	100
Isovaleric acid	100	100	100

Okabayashi *et al.*[14] analysed trace concentrations (1–15 p.p.b.) of lower fatty acids (C_2–C_5) in air from a poultry manure farm and exhaust gases from heated domestic animal faeces such as pig, cow and hen, using chemical trapping with 50 ml of a 1% aqueous solution of sodium hydroxide in two impingers, followed by an acidic diethyl ether extraction method.

Hoshika and co-workers[15,16] reported analyses of sulphur compounds, lower aliphatic amines, ammonia and lower fatty acids in the exhaust gas from a poultry manure dryer. At least four lower fatty acids in the exhaust gas were identified (isovaleric, isobutyric, butyric and valeric acids, 200–15900 p.p.b.) by the use of chemical trapping with 10% sodium hydroxide solution and decomposition with concentrated sulphuric acid and extraction with diethyl ether.

Tsuji and co-workers,[17,18] reported the detection of lower fatty acids (C_2–C_6) in the exhaust gas from a poultry manure dryer (18–770 p.p.b.), in the air of a pig house (2.2–260 p.p.b.) and in ambient air near accumulated cow manure (2.4–190 p.p.b.) using an alkaline filter-paper collection method followed by acidic decomposition and diethyl ether extraction.

TABLE III

DETERMINATION OF CONCENTRATIONS OF LOWER FATTY ACIDS (C_2–C_5) IN PRACTICAL SPECIMENS

Sample gas	Volume/l	Fatty acid concentration, p.p.b.						Odour characteristics	
		Acetic acid	Propionic acid	Butyric acid	Isobutyric acid	Valeric acid	Isovaleric acid	Quality	Intensity
Ambient air near accumulated poultry manure	0.4	304	82	6	6	n.d.*	3	Rancid, putrid, faecal, rotten eggs, ammoniacal	Strong
Ambient air near a fish meal factory	0.4	30	n.d.*	4	3	n.d.*	n.d.*	Fishy	Moderate
Air in a pig pen	0.2	1540	990	247	164	20	41	Faecal, putrid, pungent	Strong
Exhaust gas from a corn starch manufacturing factory	0.3	2000	32	57	34	182	22	Sulphury, pungent, rancid, sour	Very strong
Exhaust gas from a poultry manure dryer	0.2	1696	1190	546	399	37	250	Ammoniacal, rancid, rotten eggs, faecal, putrid	Very strong
Odour recognition threshold concentration[14]	—	1000	34	1	1.3	0.62	2.7	—	—

* n.d. = not detected (concentration less than 0.5 p.p.b.).

Horiba *et al.*[19] reported analytical results for lower fatty acids (C_4–C_5) in ambient air of a fish meal factory (2.4–15.9 p.p.b.) and a pig house (1.6–11.3 p.p.b.) using an alkaline filter-paper collection method followed by acidic decomposition and chloroform extraction.

However, these methods[14–19] are not suitable for the routine analysis of large numbers of samples, because the trace analysis of lower fatty acids (C_2–C_5) in ambient air and exhaust gas samples requires large sample volumes (about 10–1 000 l).

In the method described here, the sample volume was as small as about 0.4 l and the time required for the whole procedure, including the concentration and analysis of one sample, was about 15 min. The coefficient of variation was less than 6%. As shown in Table III, the concentrations of the lower fatty acids measured, especially butyric and isobutyric acids, were well above the odour thresholds. Therefore, these compounds may be responsible for the unpleasant odours frequently associated with poultry, fish and pig waste.

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Determination of Trace Elements in Plant Materials by a Dry-ashing Procedure

Part I.* Cobalt and Molybdenum

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Dry-ashing and analytical procedures for the spectrophotometric determination of cobalt and molybdenum in ashed plant extracts are described. A two-stage ashing process using nitric acid combined with either potassium hydrogen sulphate or sulphuric acid as ashing aids and oxygen enrichment during ashing facilitated the complete oxidation of plant materials.

Modified spectrophotometric procedures were devised for the quantitative determination of cobalt and molybdenum as the 2-nitrosonaphth-1-olate and toluene-3,4-dithiolate complexes in carbon tetrachloride. The extraction, chelation and phase-separation steps simplified existing published techniques, permitted more rapid sample handling, controlled interferences more effectively and provided more accurate assays. The molar absorptivities for cobalt and molybdenum were 5.1×10^4 and 2.5×10^4 l mol^{-1} cm^{-1}, respectively, and the detection limits for both elements were 4 ng g^{-1}.

Keywords: *Dry ashing*; *plant material*; *cobalt and molybdenum determination*; *spectrophotometry*

The discovery that in plants there are critical concentrations of trace elements[1–3] below which both plant and animal production may be affected has led to the increased use of plant analyses for revealing nutritional inadequacies.[2–4] In plant analysis, either wet-digestion or dry-ashing methods[5–7] have been used to oxidise and dissolve the plant material. An advantage of dry-ashing procedures is that large amounts of plant material (up to 10 g) can be digested and consequently low levels of cobalt and molybdenum can be determined by spectrophotometric procedures.

In our laboratory, determinations of cobalt, molybdenum, copper, manganese and zinc were required on plant samples of low mass and low nutrient content. A method in which all determinations could be made on a single digested extract by standard spectrophotometric techniques was required. This paper describes the development and testing of such a procedure.

In the dry-ashing procedure reported, the ashing aids, potassium hydrogen sulphate,[8–10] nitric acid and sulphuric acid,[5,6] increase the rate of oxidation and improve the extraction of trace elements as non-basic, less fusable sulphates in the plant ash,[7] and oxygen supplied during ashing increases the rate of oxidation and decreases the residual carbon content of the final ash.[5,6] The effect of these ashing aids was assessed on cobalt and molybdenum determinations for a variety of plant materials. In addition, standard spectrophotometric procedures for determining cobalt[11] and molybdenum[12] in the ashed plant materials were modified and tested. Aliquots of the ashed plant extracts were chelated with 2-nitrosonaphth-1-ol (2NN) or toluene-3,4-dithiol, for cobalt and molybdenum determinations, respectively, and were then concentrated in carbon tetrachloride. The methods for determining copper, manganese and zinc concentrations on the same extract are detailed elsewhere.[13]

Recommended Dry-ashing Procedure

Reagents

Analytical-reagent grade chemicals and glass-distilled water are used for all determinations.

Nitric acid, 25% *V*/*V*, + *potassium hydrogen sulphate solution*, 12.5% *m*/*V* (*acidified potassium hydrogen sulphate solution*).

* For Part II of this series, see p. 182.

Nitric acid, 25% *V/V*, + *sulphuric acid*, 25% *V/V*, *ashing solution* (*nitric - sulphuric acid ashing solution*). Add the sulphuric acid last.

Hydrochloric acid, 32% *m/m*.

Hydrochloric acid, 5.5 M.

Apparatus

Glassware is washed in 1% *V/V* Teepol detergent once, de-mineralised water three times, 1 M hydrochloric acid once and distilled water five times.

The samples are ashed in a thermostatically controlled Townson muffle furnace (Model 202CH), with doors at the front and back of the furnace chamber to facilitate the removal of gaseous products and rapid cooling. Stainless-steel oxygen inlets in the front and back doors are connected via silicone-rubber tubing to a flow gauge (0–100 ml min^{-1}) and an oxygen cylinder. A simmerstat controls the rate of heating. The muffle furnace has acid-resistant stainless-steel fittings near gaseous exits.

Procedure

Dispense 3 ml of acidified potassium hydrogen sulphate solution into a Pyrex ashing vessel (*e.g.*, a Jobling Cat. No. 0362–033 Pyrex bottle) graduated at 55 ml and fitted with a PTFE-lined screw-cap. Add 4.0 g of oven-dried ground plant material and uniformly apply a further 3 ml of acidified potassium hydrogen sulphate solution. Place the uncapped Pyrex bottle in the muffle furnace pre-heated to 120 °C and increase the furnace temperature in four thermostatically controlled stages:

(*a*) 120–175 °C with the doors ajar to allow for the removal of water and nitric acid (usually 1 h);

(*b*) 175–350 °C until charring has moderated (usually 1 h);

(*c*) 350–550 °C for removal of sulphuric acid and organic by-products (usually 1½ h); and

(*d*) 550 °C with both doors closed for 10 h, in the presence of ducted oxygen at 100 ml min^{-1}.

Allow the furnace to cool to below 300 °C before fully opening the doors. Remove the sample when the temperature is below 150 °C, allow to cool to room temperature and add 3 ml of nitric - sulphuric acid ashing solution.

Re-heat the sample in the muffle furnace in increasing temperature steps to 150 °C (45 min), 350 °C (1 h) and 550 °C (2 h) and with oxygen for about 10 h at 550 °C or until a white to greyish white ash is obtained. Repeat the cooling procedure described above.

Add 10 ml of 5.5 M hydrochloric acid, two small glass beads and 20–30 ml of distilled water and swirl the contents after each addition. Either disperse the silica in an ultrasonic bath, if available, or on a hot-plate at 80–90 °C for 30 min with periodic swirling. Dilute to nearly 55 ml with distilled water, cap tightly and agitate the contents vigorously for 15 min on a shaker. Allow to cool to room temperature, dilute to 55 ml, re-seal and again mix well. Allow the silica to settle (*e.g.*, overnight standing) and decant the aqueous phase into graduated culture tubes (*e.g.*, Corning 9826 Pyrex tubes fitted with PTFE-lined screw-caps) ready for analysis.

Recommended Method for Cobalt Determination

Reagents

Sodium citrate solution, 40% *m/V*. Filter through a Whatman No. 1 filter-paper.

Bromothymol blue, 0.04% *m/V* - *methyl red*, 0.125% *m/V*, *mixed indicator*. Dissolve 0.08 g of bromothymol blue and 0.25 g of methyl red in 100 ml of distilled water and ethanol, respectively. Mix 1 + 1 by volume.

Sodium hydroxide solutions. Dissolve 400 g of sodium hydroxide in distilled water and dilute to 1 l (10 M). Dilute 1 + 9 *V/V* and 1 + 4 *V/V* with distilled water to prepare 1 and 2 M sodium hydroxide solutions, respectively.

Hydrochloric acid, 12 M.

Sodium L(+)*-tartrate solution*, 3.5 N, *pH* 5.6. Add about 33 ml of 10 M sodium hydroxide solution to 50–55 ml of chilled solution containing 26 g of tartaric acid and mix rapidly.

Add 3 drops of mixed indicator, adjust the pH to approximately 5.6 (golden orange) with sodium hydroxide solution or hydrochloric acid and dilute to 100 ml with distilled water.

Potassium gluconate solution, 2 N. Dissolve 47 g of potassium gluconate in distilled water and dilute to 100 ml.

Combined sodium tartrate - potassium gluconate solution. Mix 100 ml of 3.5 N sodium tartrate solution with 100 ml of 2 N potassium gluconate solution. Adjust the pH of the solution to 5.6 (golden orange) with sodium hydroxide solution or hydrochloric acid. Dilute to 250 ml with distilled water. The solution can be stored in a refrigerator for 4–5 d.

Hydrogen peroxide, 30% *m/m* (100 *volume*).

Purified 2-nitrosonaphth-1-ol, 0.5% *m/V solution in ethanol* (2*NN*). BDH Chemicals laboratory-reagent grade 2NN was used in this study. 2NN is purified initially by Needleman's procedure,[14] except that 0.5 M sodium hydroxide extracts containing dissolved 2NN are combined and washed twice with 50-ml aliquots of carbon tetrachloride before the 2NN is precipitated with 0.15 M hydrochloric acid. Purified 2NN, after storage for 2–3 months, produces unacceptably high blank readings with the method outlined here. By shaking 0.55% *m/V* purified 2NN, which has been stored for up to 12 months, in ethanol with 0.4% *m/V* activated charcoal for 15 min, filtering it through Whatman GF/A filter-paper and using it for cobalt analysis within 2 d, blank readings approaching those for freshly purified 2NN can be obtained.

Carbon tetrachloride. AnalaR grade.

Standards

A 10 mg l^{-1} cobalt stock solution in 0.1 M nitric acid is prepared as described by Rooney[15] and diluted 1 + 99 *V/V* with distilled water to obtain a 0.1 mg l^{-1} cobalt solution. Cobalt working standards (0–0.5 μg of cobalt) are prepared by adding appropriate aliquots of the 0.1 mg l^{-1} cobalt solution to culture tubes, diluting to 15 ml with distilled water and analysing as for 15-ml ashed plant extracts. The working range can be extended to 0–2 mg of cobalt without affecting the linearity of the calibration graph.

Apparatus

A Varian 634 ultraviolet - visible spectrophotometer, fitted with a Hellma 178 quartz-glass micro flow cell (80 μl capacity, 10 mm light path) is used. Carbon tetrachloride containing the cobalt - 2NN complex is drawn into the flow cell through 1.4 mm i.d. polyethylene tubing by suction, and the sample volume is controlled by a vacuum control valve (Fig. 1) and a small vacuum pump. Waste solution is collected in a glass vacuum flask containing a 20-mm layer of water. A vortex mixer is also used.

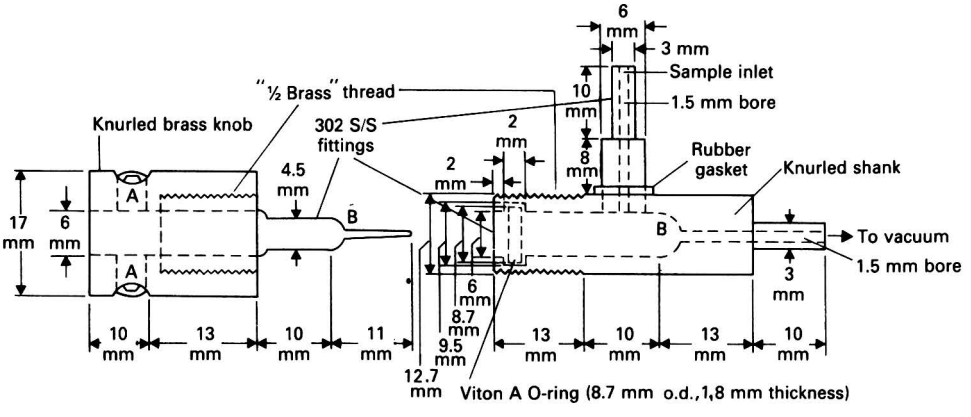

Fig. 1. 302 grade stainless-steel (S/S) vacuum control valve for controlling the flow of organic solvents through the microanalysis system. A, 2 × 2 mm grub-screws; B, ground stainless-steel surfaces.

Procedure

To a 15-ml aliquot of ashed plant extract or blank digest contained in a graduated culture tube, add 1.0 ml of sodium citrate solution and 1 drop of mixed indicator, and mix. Adjust the pH of the acidic extract to 5.6 $\pm$ 0.3 by adding 1.5 ml of 10 M sodium hydroxide solution and then either 2 M sodium hydroxide solution or 2 M hydrochloric acid dropwise until the extract is a golden orange colour.

Add 2.5 ml of combined tartrate and gluconate solution (pH 5.6) and mix. Add 0.5 ml of 30% *m/m* hydrogen peroxide and mix again. Re-adjust the pH of the extract to 5.6 $\pm$ 0.3 before adding 0.5 ml of 2NN solution and allowing it to stand for 1 h.

Add 3 ml of carbon tetrachloride and shake vigorously for 5 min on a horizontal shaker. Allow the carbon tetrachloride layer to settle and remove the upper aqueous layer by suction. Shake the organic layer with 15 ml of 1 M hydrochloric acid for 5 min, allow to stand for 2 min and remove the upper layer by suction. Add 4 ml of 12 M hydrochloric acid, re-seal the culture tube and agitate vigorously for 20 s. Add 10 ml of distilled water and remove the hydrochloric acid solution by suction. Shake the carbon tetrachloride layer with 10 ml of 1 M sodium hydroxide solution for 5 min and allow it to settle before filtering it through a 9-cm Whatman No. 1 phase-separation (PS) paper into a small culture tube fitted with a screw-cap and PTFE liner. Cobalt determinations are delayed until the next day so that trace amounts of water, if present in the filtrate, can separate from the organic layer. The cobalt - 2NN complex in carbon tetrachloride is stable for 48 h. Cobalt is determined spectrophotometrically at 307 nm. Up to 100 cobalt determinations can be carried out in 2 d.

Recommended Method for Molybdenum Determination

Reagents

Hydrochloric acid, 36 and 32% m/m.

Thiourea (T) solution, 10% *m/V.*

Ascorbic acid, 18% *m/V - citric acid,* 7.5% *m/V (AACA).* Prepare the daily requirement of T - AACA solution by mixing 1 volume of T with 1 volume of AACA.

Alkaline toluene-3,4-dithiol (dithiol) solution, 1.0% *m/V.* As prepared from dithiol by Bingley.[16]

Potassium sulphate solution, 7.5% *m/V.*

Carbon tetrachloride. AnalaR grade.

Standards

Prepare a 100 mg l^{-1} molybdenum stock solution, according to the method of Bingley.[16] Dilute 1 + 99 *V/V* with distilled water to prepare a 1 mg l^{-1} molybdenum standard and then transfer 0–5-ml aliquots (0–5 μg of molybdenum) into graduated culture tubes. Add 4 ml of 32% *m/m* hydrochloric acid and 5 ml of 7.5% *m/V* potassium sulphate solution to each culture tube and dilute to approximately 22 ml with distilled water. The working range can be extended to 20 μg of molybdenum with the method outlined here, but the calibration graph starts to curve above 10 μg of molybdenum.

Apparatus

As described for cobalt determination.

Procedure

Add 2 ml of 36% *m/m* hydrochloric acid to a 20-ml aliquot of the ashed plant extract or blank extract contained in a graduated culture tube and mix on a vortex mixer. Add 1 ml of T - AACA solution and re-mix, then add 0.5 ml of the dithiol solution, re-mix and allow 15-min reaction time before adding 3 ml of carbon tetrachloride. Shake vigorously on a horizontal shaker for 5 min and allow the carbon tetrachloride layer to settle before filtering through a 9-cm Whatman No. 1 PS paper into a small culture tube fitted with a screw-cap and PTFE liner. The molybdenum - dithol complex remains stable in carbon tetrachloride for 2 d. Molybdenum is determined spectrophotometrically at 680 nm. Sixty extracts can be analysed in 1 d.

Results and Discussion

Testing the Dry-ashing Procedure

Previous studies have shown that the complete oxidation of large amounts of plant material by dry-ashing procedures and the extraction of inorganic constituents from plant ash are promoted by the addition of chemical ashing aids such as sulphuric or nitric acid and by increasing the supply of oxygen in the ashing chamber.[5,6] In addition, the extraction of cobalt[9] and molybdenum[10] from soils containing organic matter has been facilitated by fusing and dry ashing the soil with potassium hydrogen sulphate.[8]

The effects of various levels of the chemical ashing aids potassium hydrogen sulphate and nitric acid with oxygen enrichment were assessed from observations during ashing and by assays of cobalt and molybdenum in three plant samples containing a naturally high level of either silicon, calcium or chlorine (Table I). Clearly, measured concentrations of cobalt and molybdenum in samples ashed in the absence of the chemical aids were lower than those for samples ashed in their presence. Moreover, it was observed that in the absence of the chemical aids dark ashes were formed, indicating the ashing was incomplete for these samples. Varying the amount of potassium hydrogen sulphate added from 0.5 to 1.0 g and nitric acid from 1 to 3 ml did not affect the determinations of cobalt or molybdenum in these samples. However, it was observed that adding less than 0.75 g of potassium hydrogen sulphate sometimes increased carbonate effervescence on the addition of hydrochloric acid to the final ash and also increased the adsorption of silica on the walls of the ashing vessel. In contrast, adding more than 0.75 g of potassium hydrogen sulphate sometimes caused salt precipitation, which made phase separations more difficult during the cobalt and molybdenum assay procedures.

In a separate study, the effects on cobalt and molybdenum determinations of high levels of added silicon, calcium and chlorine (up to a total of 4% *m/m*), either individually or in combination, were examined in a range of plant materials. Negligible differences were measured.

TABLE I

EFFECT OF ASHING AIDS ($KHSO_4$ AND HNO_3) ON THE DETERMINATIONS OF Co AND Mo IN PLANT SAMPLES

4 g of plant material were ashed in the presence of 100 ml min^{-1} of oxygen at 550 °C with the prescribed amounts of $KHSO_4$ and HNO_3 for 10 h and H_2SO_4 and HNO_3 (0.75 ml of each) for 10 h.

Ashing treatment		Concentration in plant material*					
		Barley glumes†		Barley straw		Lucerne pasture 1	
$KHSO_4$/g	HNO_3/ml	Co/ ng g^{-1}	Mo/ mg kg^{-1}	Co/ ng g^{-1}	Mo/ mg kg^{-1}	Co/ ng g^{-1}	Mo/ mg kg^{-1}
0	0	24	0.32	29	0.42	229	0.42
0.5	0	27	0.36	35	0.46	236	0.45
0.5	1	31	0.37	35	0.47	252	0.49
0.5	2	30	0.37	36	0.48	254	0.49
0.5	3	32	0.36	37	0.48	250	0.50
0.75	0	28	0.38	36	0.47	232	0.47
0.75	1	32	0.38	36	0.48	257	0.50
0.75	2	31	0.38	35	0.49	255	0.50
0.75	3	30	0.39	36	0.48	250	0.50
1.0	0	30	0.37	35	0.48	240	0.48
1.0	1	32	0.37	37	0.49	259	0.50
1.0	2	30	0.38	35	0.48	257	0.50
1.0	3	30	0.37	36	0.48	254	0.51
L.S.D.‡ (P = 0.05)	..	1.4	n.s.	n.s.	n.s.	n.s.	n.s.

* Mean of three determinations.

† Concentrations of potentially interfering elements Si, Cl or Ca: barley glumes 4.6% *m/m* Si; barley straw 1.8% *m/m* Cl; lucerne pasture 1 1.9% *m/m* Ca.

‡ L.S.D. = least significant difference (P = 0.05); the effects of the chemical treatments during ashing were not significant (n.s.); the nil $KHSO_4$ - nil HNO_3 treatment for respective samples was excluded from the analysis of variance.

Also, the form of silica (dried powder, acid-washed plant silica or silicic acid) added to the plant material at levels up to 4% *m/m* before ashing did not affect the determinations of either element (unpublished work).

The provision of oxygen at 100 ml min^{-1} during ashing produced ashes that were noticeably whiter after 20 h than when oxygen was not supplied. Slightly higher levels of cobalt and molybdenum were also measured in samples ashed in the presence of oxygen (Table II). Higher flow-rates of oxygen and longer ashing times (Table II) did not improve the efficiency of ashing or the determinations of cobalt and molybdenum. However, it was observed that some samples (*e.g.*, lucerne pasture 1) were ashed completely within 15 h, although 20 h were required for ashing other samples.

Varying the amount of sample ashed from 1 to 6 g did not significantly affect the determinations of cobalt and molybdenum by the ashing and analytical procedures described (Table III), although it was observed that the amount of occluded carbon was sometimes increased by dry ashing samples weighing more than 4 g. As a consequence, 4-g sub-

TABLE II

EFFECT OF OXYGEN ENRICHMENT DURING DRY ASHING AND ASHING TIME ON THE DETERMINATIONS OF CO AND MO IN PLANTS

	Concentration in plant material*					
	Barley glumes		Lucerne pasture 1		Wheaten hay	
Ashing treatment	Co/ ng g^{-1}	Mo/ mg kg^{-1}	Co/ ng g^{-1}	Mo/ mg kg^{-1}	Co/ ng g^{-1}	Mo/ mg kg^{-1}
Effect of ashing treatment†—						
With oxygen enrichment‡ ..	30	0.38	235	0.46	55	0.22
No oxygen added	26	0.36	230	0.45	52	0.20
Effect of ashing time§—						
Total ashing time/h						
15	27	0.36	235	0.45	52	0.20
20	30	0.38	235	0.46	55	0.22
25	29	0.38	237	0.46	57	0.22

* Mean of three determinations.

† 4 g of plant material were ashed at 550 °C with $KHSO_3$ (0.75 g) and HNO_4 (1.5 ml) for 10 h and H_2SO_4 and HNO_3 (0.75 ml each) for 10 h.

‡ Oxygen added at 100 ml min^{-1} during ashing.

§ Time of ashing varied during the second ashing phase.

TABLE III

EFFECT OF SAMPLE MASS ON THE DETERMINATIONS OF CO AND MO IN PLANTS

The plant material was ashed in the presence of 100 ml min^{-1} oxygen at 550 °C with $KHSO_4$ (0.75 g), HNO_3 (1.5 ml) for 10 h and H_2SO_4 and HNO_3 (0.75 ml each) for 10 h.

	Concentration in plant material*					
	Barley glumes		Lucerne pasture 1		Wheaten hay	
Sample mass/g	Co/ng g^{-1}	Mo/mg kg^{-1}	Co/ng g^{-1}	Mo/mg kg^{-1}	Co/ng g^{-1}	Mo/mg kg^{-1}
2	31	0.39	237	0.44	55	0.21
3	30	0.40	235	0.46	55	0.22
4	30	0.38	236	0.46	56	0.22
5	28	0.39	234	0.47	54	0.21
6	27	0.37	229	0.45	54	0.20

* Mean of three determinations.

samples of plant material were selected for all subsequent tests, so that the analytical precision was maintained in assays of samples with low cobalt and molybdenum concentrations. The selected ashing procedure therefore consisted of ashing 4-g sub-samples of plant material at

550 °C in the presence of 100 ml min^{-1} of oxygen with 0.75 g of potassium hydrogen sulphate and 1.5 ml of nitric acid for 10 h and with 0.75 ml of both nitric and sulphuric acids for an additional 10 h.

Comparison with Other Procedures

Triplicate determinations of cobalt and molybdenum for nine cereal and pasture samples and for Bowen's kale[17] were made after dry ashing 4-g samples by the analytical procedures described. The determinations were compared with triplicate determinations of the same materials by wet digestion and the analytical procedures of Simmons[18] for cobalt (0.5 g digested; final volume 10 ml; electrothermal atomic-absorption spectrophotometry) and of Bingley[16] for molybdenum (1.0 g digested; final volume 20 ml; spectrophotometric assay). Close agreement ($P \ll 0.05$) between the determinations for both elements by the two methods was obtained for all materials, which varied appreciably in concentrations of cobalt, molybdenum and other elements (Table IV).

TABLE IV

COMPARISON OF Co AND Mo CONCENTRATIONS IN PLANT SAMPLES DETERMINED BY THE DRY-ASHING AND WET-DIGESTION PROCEDURES

Sample	Digestion procedure*	Concentration in plant material† Co/ng g^{-1}	Mo/mg kg^{-1}
Barley glumes	W.D.	21	0.36
	D.A.	30	0.38
Barley straw	W.D.	38	0.47
	D.A.	36	0.48
Wheaten hay	W.D.	65	0.22
	D.A.	55	0.22
Lucerne pasture 1	W.D.	245	0.46
	D.A.	235	0.46
Lucerne pasture 2	W.D.	188	0.98
	D.A.	179	0.98
Medic pasture	W.D.	50	0.23
	D.A.	50	0.22
Lupin straw	W.D.	131	0.61
	D.A.	142	0.59
Subterranean clover, ryegrass pasture 1	W.D.	285	0.51
	D.A.	274	0.54
Subterranean clover, ryegrass pasture 2	W.D.	201	0.30
	D.A.	189	0.30
Bowen's kale[17]	W.D.	67	2.27
	D.A.	64	2.31

* Co and Mo analyses by Simmons'[18] and Bingley's[16] wet-digestion procedures (W.D.), respectively; D.A. = dry ashing.
† Mean of three determinations.

Recovery of Added Cobalt and Molybdenum

To three 4-g sub-samples of ten different plant materials were added 200 ng g^{-1} of cobalt and to another three of each were added 1.5 mg kg^{-1} of molybdenum. All samples were then ashed and analysed for cobalt and molybdenum by the methods described. The mean recoveries varied from 94.5 to 106.0% for cobalt and from 93 to 99% for molybdenum (Table V).

Procedures for Assaying Cobalt and Molybdenum

Various attributes of previously published procedures and other specific modifications have been combined in the recommended procedures for extracting and assaying cobalt and molybdenum in ashed plant materials. By these procedures the determination of both elements was as sensitive (detection limit = 4 ng g^{-1} for both cobalt and molybdenum) and as reproducible as other currently available techniques such as electrothermal atomic-absorption spectrophotometry.[18,19]

TABLE V

RECOVERY OF Co AND Mo ADDED TO PLANT SAMPLES

Sample	Concentration in plant material: Co — Added sample concentration*/ng g^{-1}	Co — Mean analysis†/ng g^{-1}	Co — Mean recovery, %	Mo — Added sample concentration*/mg kg^{-1}	Mo — Mean analysis†/mg kg^{-1}	Mo — Mean recovery, %
Barley glumes	0	30		0	0.35	
	200	242	106.0	1.5	1.75	93
Barley straw	0	35		0	0.49	
	200	241	103.0	1.5	1.94	97
Wheaten hay	0	56		0	0.20	
	200	250	97.0	1.5	1.65	97
Lucerne pasture 1	0	236		0	0.50	
	200	435	99.5	1.5	1.98	99
Lucerne pasture 2	0	176		0	0.99	
	200	370	97.0	1.5	2.40	94
Medic pasture	0	51		0	0.23	
	200	255	102.0	1.5	1.72	99
Lupin straw	0	135		0	0.58	
	200	324	94.5	1.5	2.04	97
Subclover, ryegrass pasture 1	0	279		0	0.53	
	200	469	95.0	1.5	1.93	93
Subclover, ryegrass pasture 2	0	195		0	0.30	
	200	386	95.5	1.5	1.73	95
Bowen's kale[17]	0	64		0	2.30	
	200	269	102.5	1.5	3.75	97

* Co and Mo equivalent to 200 ng g^{-1} of Co and 1.5 ng g^{-1} of Mo as $Co(SO_4)_2.7H_2O$ and Na_2MoO_4, respectively, were added to 4-g sub-samples of plant material, which was then ashed by the recommended dry-ashing procedure.
† Mean of three determinations.

The modifications provided certain advantages. Firstly, rapid separation of the organic and aqueous layer was achieved by filtering the organic phase through a phase-separation paper; this provided an organic layer free from water, insoluble residues and ionised chemicals, which might have subsequently interfered in the spectrophotometric determination of both elements (unpublished work). Secondly, potential interferences from other elements during the spectrophotometric procedures have been prevented. These will now be discussed in detail.

Cobalt assay

Ni, Hg, Ag, Fe^{3+}, Cu^{2+} and Cr^{3+} ions form complexes with 2NN that interfere in the spectrophotometric determination of the cobalt - 2NN complex at pH 5–5.5[15] to produce an artificially high reading. These effects were effectively prevented during the assay procedure by chelating the interfering ions with citrate, tartrate and gluconate in the aqueous phase. Recoveries ranging from 97 to 101 and 97 to 100% were obtained when 200 ng g^{-1} of cobalt were added as $CoSO_4.7H_2O$ to 4-g sub-samples of lucerne pasture 1 and wheaten hay, respectively, before ashing and in the presence of 50 mg kg^{-1} concentrations of one or all of the potentially interfering elements described above.

Interferences from Fe^{2+} and Fe^{3+} ions (up to 1500 mg kg^{-1} of Fe) were prevented by oxidising Fe^{2+} with hydrogen peroxide[11,20] and complexing the Fe^{3+} in the acidic trichelate medium. Moreover, the addition of gluconate to ashed plant extracts containing up to 4% *m/m* of plant-equivalent calcium prevented the precipitation of calcium salts, which otherwise caused handling difficulties during the washing steps.

During the development and testing of the cobalt assay procedures artificially high values (more than a 30% increase) were measured for samples containing high levels of manganese (over 250 mg kg^{-1}) when gluconate and the 12 M hydrochloric acid washing steps were omitted from the recommended procedure. When tartrate was also omitted, artificially high values (more than a 20% increase) were detected for cobalt assays of plant material containing manganese concentrations of 50 mg kg^{-1}. However, this type of interference was not observed in samples containing up to 500 mg kg^{-1} of manganese when assayed by the recommended procedure. For example, when manganese as $MnCl_2.4H_2O$ was added with the acidified potassium hydrogen sulphate ashing solution to 4-g sub-samples of lucerne pasture 1 and wheaten hay to adjust their total plant concentration to 500 mg kg^{-1} of manganese prior to ashing, negligible differences (+2, −3) from the respective control values of 235 and 57 ng g^{-1} of cobalt were observed. Individual determinations were also highly

reproducible in the presence of citrate, tartrate and gluconate but analytical errors were markedly higher when one or more of these chelating agents were excluded (Table VI). The cobalt assay in the presence of the three chelating agents was also shown to be an accurate measure of cobalt concentration in lucerne pasture 1 by the close agreement between assays with this and the Simmons[18] procedure (Tables IV and VI) and the high percentage recovery of added cobalt in Table V.

Finally, more rapid and accurate analysis was made possible by maintaining the pH of the cobalt - 2NN reaction at 5.6 ± 0.3 with simple colour indicators[18] and by simplifying the washing and extraction steps of the assay procedure. The sensitivity measured as the molar absorptivity (5.1×10^4 l mol^{-1} cm^{-1}) was greatest when the cobalt determinations were read at 307 mm[11] compared with previously published wavelengths of 367 mm[21] and 530 nm.[15]

TABLE VI

EFFECT OF CHELATING AGENTS ON THE DETERMINATION OF Co IN LUCERNE PASTURE 1

Chelating agent(s) added	Concentration in plant material*/ng g^{-1}
Citrate (C)	284 ± 18
Tartrate (T)	279 ± 16
Gluconate (G)	302 ± 23
C + T	278 ± 6
C + G	265 ± 8
T + G	282 ± 10
C + T + G†	242 ± 3

* Mean concentration ± standard error (n = 9).
† Recommended procedure.

Molybdenum assay

Previous studies have shown that the quantitative determination of molybdenum as the molybdenum - dithiol complex in carbon tetrachloride is rapid, sensitive and reproducible. In the method described here, the concentration and combining of chemicals permitted an over-all reduction in the sample volume and the number of analytical steps prescribed for some other methods[16,22] and improved the ease and speed of the assay. Quantitative determinations of up to 20 mg kg^{-1} of molybdenum in plant materials were possible with this procedure.

Interference from tin was eliminated by increasing the acidity of the extract to 2 N with respect to hydrochloric acid.[12] The addition of the combined T - AACA reagent, containing thiourea and ascorbic and citric acids, in the recommended procedure also prevented interferences from ionic species such as Cu^{2+}, W, Sn^{4+} and Fe^{3+} [12,16] at concentrations up to 50 mg kg^{-1} for the first three and 2000 mg kg^{-1} for Fe^{3+}.

Recoveries ranging from 98 to 102 and 96 to 103% were obtained when 1.5 mg kg^{-1} of molybdenum were added as Na_2MoO_4 to 4-g sub-samples of lucerne pasture 1 and wheaten hay, respectively, before ashing and in the presence of the above elements at the concentrations listed.

Analytical Efficiency and Versatility

The sub-sampling of ashed plant extracts and up to 100 cobalt and molybdenum determinations can be performed per week by one analyst using the modified spectrophotometer. In addition, other elements such as copper, zinc and manganese can also be determined on separate aliquots of the ashed plant extract,[13] which is a further advantage of this dry-ashing technique.

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NOTE—Reference 13 is to Part II of this series.

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Determination of Trace Elements in Plant Materials by a Dry-ashing Procedure

Part II.* Copper, Manganese and Zinc

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A method is described for determining copper, manganese and zinc in ashed plant extracts by flame atomic-absorption spectrophotometry after cobalt and molybdenum have been assayed on separate aliquots of the same plant extracts. Ashing aids were necessary to maintain accuracy in the determinations. Concentrations of up to 3.5% *m/m* of silicon and calcium and 4% *m/m* of chlorine in the plants did not affect the determinations, but in some instances lower concentrations were determined in plant samples containing equal or higher levels of both added silica and calcium.

Potential interference was prevented during assays for copper by automatic background correction, for manganese and zinc by diluting the extract, and for all three analytes by selectively matching their chemical matrices with hydrochloric acid and potassium sulphate concentrations in respective working standards. By these procedures, assays for the three elements were similar to determinations by flame atomic-absorption spectrophotometry on samples digested by a standard wet-digestion method.

Keywords: *Dry ashing*; *plant material*; *copper, manganese and zinc determination*; *flame atomic-absorption spectrophotometry*

In Part I[1] a dry-ashing procedure for preparing ashed plant extracts and for determining cobalt and molybdenum in aliquots of the extract was described and evaluated. This paper describes methods for determining copper, manganese and zinc in separate aliquots of the same extract. It also examines possible interferences that might occur during the extraction of these elements from the ash and during the assay by flame atomic-absorption spectrophotometry.

Dry Ashing and Extract Preparation

Oven-dried and ground plant samples were dry ashed with potassium hydrogen sulphate, nitric acid and sulphuric acid in the presence of oxygen and extracted into 55 ml of 1 M hydrochloric acid as described in Part I.[1] Cobalt and molybdenum determinations were performed on 35 ml of the extract and the remainder was used for the determinations of copper, manganese and zinc.

Copper concentrations were determined in an aliquot of the extract by flame atomic-absorption spectrophotometry, the absorbance readings being corrected for non-atomic absorption with a hydrogen continuum lamp.[2] Another aliquot, after dilution 1 + 3 *V/V* with distilled water, was analysed for manganese and zinc by flame atomic-absorption spectrophotometry. Blank extracts were treated similarly.

Apparatus

A Varian 175 atomic-absorption spectrophotometer, equipped with a hydrogen continuum lamp for automatic background correction and a 100-mm laminar-flow burner for high solid flow, was used. Readings after scale expansion and integration over a 3-s period were automatically converted into units of concentration.

Reagents

Separate stock solutions containing 100 mg l^{-1} of copper, manganese and zinc were prepared by dissolving 0.3929 g of $CuSO_4.5H_2O$, 0.4060 g of $MnSO_4.4H_2O$ and 0.1245 g of ZnO, respectively, in 20 ml of 1 M hydrochloric acid and diluting to 1 l with distilled water.

* For Part I of this series, see p. 172.

Standards in the range 0–2 mg l^{-1} of copper were prepared in 1 M hydrochloric acid containing 1.5% *m/V* of potassium sulphate. Standards containing 0–5 mg l^{-1} of manganese and 0–2 mg l^{-1} of zinc were prepared in 0.25 M hydrochloric acid and 0.4% *m/V* potassium sulphate.

Results and Discussion

Testing the Dry-ashing Procedure

Samples ashed in the absence of the chemical ashing aids, potassium hydrogen sulphate and nitric acid, but in the presence of oxygen produced lower concentrations of copper, manganese and zinc than in comparable samples ashed with the chemical aids (Table I). Varying the additions of potassium hydrogen sulphate from 0.5 to 1.0 g and nitric acid from 1 to 3 ml had negligible effects on the determinations of the three elements in three samples that varied in elemental composition (Table I).

Oxygen enrichment during ashing increased the concentrations of the elements determined, and slightly higher concentrations were sometimes assayed in samples ashed for 20 h compared with 15 h (Table II).

In general, these findings on the effects of ashing aids support those previously reported for determinations of cobalt and molybdenum in other aliquots of the same plant extract.[1] Thus, for all samples examined, the addition of the ashing aids improved the efficiency of ashing and the extraction of all five elements. In the tests described hereafter 4-g samples were ashed in the presence of 100 ml min^{-1} of oxygen with 0.75 g of potassium hydrogen sulphate and 1.5 ml of nitric acid for 10 h and 0.75 ml each of sulphuric and nitric acids for a further 10 h.

Effect of High Levels of Silicon, Chlorine and Calcium

Other studies[3,4] have shown that high levels of silicon and chlorine in plant materials can lower the measured concentrations of copper, manganese and zinc in plant extracts. These losses are caused by adsorption of the metals on to insoluble residues or the ashing vessel,[4]

TABLE I

EFFECT OF THE ASHING AIDS $KHSO_4$ AND HNO_3 ON THE DETERMINATIONS OF CU, MN AND ZN IN PLANT SAMPLES WHEN OXYGEN IS SUPPLIED

4 g of plant material were ashed in the presence of 100 ml min^{-1} of oxygen with $KHSO_4$ (0.75 g) and HNO_3 (1.5 ml) for 10 h and H_2SO_4 and HNO_3 (0.75 ml each) for 10 h.

Ashing treatment		Concentration in plant material*/mg kg^{-1}								
		Barley glumes†			Barley straw			Lucerne pasture 1		
$KHSO_4$/g	HNO_3/ml	Cu	Mn	Zn	Cu	Mn	Zn	Cu	Mn	Zn
0	0	2.3	46.6	8.4	4.6	35.9	20.9	7.6	30.7	39.3
0.5	0	2.9	47.8	9.0	5.9	37.8	22.2	8.8	33.8	42.0
0.5	1	3.4	51.5	9.7	6.8	39.5	27.0	9.3	34.5	42.9
0.5	2	3.3	51.3	9.9	6.8	39.6	27.2	9.4	34.5	43.1
0.5	3	3.3	51.3	9.8	6.8	39.5	27.2	9.4	34.6	42.9
0.75	0	3.2	48.6	9.2	6.5	39.8	27.0	9.3	34.4	42.4
0.75	1	3.4	51.5	9.8	6.8	39.7	27.2	9.3	34.4	42.9
0.75	2	3.3	51.4	10.0	6.6	39.8	27.1	9.4	34.3	43.1
0.75	3	3.4	51.6	10.0	6.8	39.7	27.2	9.4	34.6	43.1
1.0	0	3.3	49.1	9.5	6.6	39.5	27.0	9.3	34.4	42.9
1.0	1	3.3	51.3	9.7	6.7	39.7	27.3	9.3	34.5	42.8
1.0	2	3.4	51.6	10.0	6.8	39.8	27.0	9.4	34.3	43.2
1.0	3	3.4	51.4	9.8	6.8	39.6	27.2	9.3	34.5	42.9
L.S.D.‡ (P = 0.05)		0.1	0.5	0.2	n.s.	0.5	0.3	0.2	0.3	n.s.

* Mean of three determinations.

† Concentrations of potentially interfering elements Si, Cl or Ca: barley glumes 4.6% *m/m* Si; barley straw 1.8% *m/m* Cl; lucerne pasture 1 1.9% *m/m* Ca.

‡ L.S.D. = least significant difference (P = 0.05); the effects of the chemical treatments during ashing were not significant (n.s.); the nil $KHSO_4$ - nil HNO_3 treatment for respective samples was excluded from the analyses of variance.

TABLE II

EFFECT OF OXYGEN ENRICHMENT DURING ASHING AND ASHING TIME ON THE DETERMINATIONS OF CU, MN AND ZN IN PLANTS

Ashing treatment	Concentration in plant material*/mg kg^{-1}								
	Barley glumes			Lucerne pasture 1			Wheaten hay		
	Cu	Mn	Zn	Cu	Mn	Zn	Cu	Mn	Zn
Effect of ashing treatment†—									
With oxygen enrichment‡ ..	3.4	51.3	9.8	9.3	34.4	42.9	3.1	31.4	11.0
No oxygen added	3.1	49.3	8.9	8.5	32.5	41.7	2.8	28.9	9.9
Effect of ashing time§—									
Ashing time/h									
15	3.2	50.4	9.5	9.1	34.2	42.5	2.8	30.7	10.7
20	3.4	51.3	9.8	9.3	34.4	42.9	3.1	31.4	11.0
25	3.4	51.1	9.8	9.2	34.6	42.8	3.0	31.3	10.9

* Mean of three determinations.
† 4 g of plant material were ashed at 550 °C with $KHSO_4$ (0.75 g) and HNO_3 (1.5 ml) for 10 h and H_2SO_4 and HNO_3 (0.75 ml each) for 10 h.
‡ Oxygen added at 100 ml min^{-1} during ashing.
§ Time of ashing varied during the second ashing phase.

formation of volatile chlorides[3,4] (*e.g.*, $ZnCl_2$) and incomplete ashing and extraction of elements in plants containing high concentrations of alkalis[5] and fusable salts.[6] In addition, high calcium levels interfere in copper assays undertaken with flame atomic-absorption spectrophotometry.[7]

Two different plant materials containing added silicon (as powdered silica) and calcium (as $CaSO_4.2H_2O$) in factorial combination and at a constant chloride concentration of 4% *m/m* (added as powdered sodium chloride), were dry ashed and aliquots of the plant extracts were analysed for copper, manganese and zinc. Concentrations of silica and calcium tested included values up to 4% *m/m*, which are considered extreme for plants. Three replicates of each treatment were used.

Negligible differences in the concentrations of all three elements were measured for plant samples to which silicon and calcium had been added at concentrations up to and including 3.0% *m/m* and at concentrations of 4% *m/m* of chlorine (Table III).

When silicon concentrations in plants were held constant and the calcium concentration was increased to 4% *m/m*, the measured copper and zinc concentrations tended to decrease. Conversely, when calcium was held constant and silicon was increased the measured concentrations of copper and zinc tended to decrease also. However, in general, the effects of silicon and calcium on copper and zinc determinations were small except for silicon and calcium concentrations exceeding 3.5% *m/m* in some plants. Concentrations of manganese were significantly depressed by up to 10 mg kg^{-1} (or 33% *m/m*) in wheaten hay and 4.3 mg kg^{-1} (13% *m/m*) in lucerne pasture 1 in samples containing more than 3.5% *m/m* of both silicon and calcium. Basson and Böhmer[3] reported manganese assays lowered by up to 47% for rice samples containing lower calcium (0.73% *m/m*) and chlorine (0.9% *m/m*) concentrations than those reported here, for two dry-ashing methods. They concluded that during dry ashing manganese is lost by the formation of insoluble residues and the embedding of manganese in the ashing vessel's surface as previously proposed by Gorsuch[4] and Palfrey *et al.*[8]

Preventing Interference During Assay

High concentrations of salts in plant extracts can contribute to inaccurate assays by flame atomic-absorption spectrophotometry because of non-atomic absorption and chemical interference.[9] For example, copper assays were up to 1.2 mg kg^{-1} higher when uncorrected for non-atomic absorption, and manganese and zinc assays, after correction for non-atomic absorption, were up to 44 and 11% *m/m* lower, respectively, for ashed plant extracts of plant samples described in Table IV (unpublished work). Errors in manganese and zinc assays

TABLE III

EFFECT OF SI AND CA CONCENTRATIONS ON THE QUANTITATIVE DETERMINATIONS OF CU, MN AND ZN IN TWO PLANT SAMPLES

Ashing treatment†		Concentration in plant material*/mg kg^{-1}					
		Lucerne pasture 1			Wheaten hay		
Total Si, % *m/m*	Total Ca, % *m/m*	Cu	Mn	Zn	Cu	Mn	Zn
Control‡	Control	9.1	34.3	42.8	3.1	30.4	12.1
	3.0	9.3	33.9	42.7	3.1	30.0	11.9
	3.5	8.9	33.7	42.2	3.1	29.7	11.8
	4.0	8.8	33.7	42.1	3.1	29.0	11.7
3.0	Control	8.8	33.9	42.3	3.1	30.2	11.7
	3.0	8.8	33.7	42.2	3.1	29.5	11.7
	3.5	8.5	32.2	42.3	3.0	27.0	11.4
	4.0	8.0	30.5	41.5	3.1	25.9	11.6
3.5	Control	8.9	34.2	42.6	3.0	29.7	11.5
	3.0	8.7	33.8	42.3	3.1	29.5	11.2
	3.5	8.6	32.4	41.3	3.1	26.6	11.2
	4.0	8.8	30.9	40.3	2.9	25.1	11.0
4.0	Control	8.7	33.7	41.6	3.0	29.7	11.5
	3.0	8.6	32.9	42.0	3.2	26.9	10.5
	3.5	8.3	31.9	41.5	3.0	22.8	10.1
	4.0	8.0	30.0	40.2	2.9	20.4	9.8
F-test§	Si	***	***	**	n.s.	***	**
	Ca	***	***	**	n.s.	***	***
	Si × Ca	n.s.	n.s.	n.s.	n.s.	***	n.s.
L.S.D.¶ ($P = 0.05$)		0.4	1.3	1.4	0.2	1.1	0.7

* Mean of three determinations.

† Powdered SiO_2 and $CaSO_4.2H_2O$ were added to 4-g sub-samples of plant material to give the prescribed concentrations of Si and Ca. NaCl was added to all samples except the control treatments to provide 4% *m/m* Cl concentrations.

‡ The natural levels of Si and Ca were 0.2 and 1.9% *m/m* in lucerne pasture 1 and 0.8 and 0.2% *m/m*, respectively, in wheaten hay.

§ *** significant ($P \leqslant 0.001$); ** significant ($P \leqslant 0.01$); n.s. = not significant.

¶ L.S.D. = least significant difference ($P = 0.05$) for Si × Ca interaction.

were overcome by diluting the extract 1 + 3 *V/V* and by matching the hydrochloric acid and potassium sulphate matrices of the working standards with the diluted plant extracts as closely as possible. With this procedure corrections for non-atomic absorption were not required for the determination of manganese and zinc in plant samples described in Table IV and containing up to 4% *m/m* of calcium and chlorine.

The detection limits by the outlined procedures for copper, manganese and zinc in plants were 0.25, 0.23 and 0.05 mg kg^{-1}, respectively.

Comparisons with Wet-digestion Procedures

The accuracy of the dry-ashing technique was compared with that of a standard wet-digestion procedure.[9] Three replicates of nine different plant samples were dry ashed (4-g samples; 55 ml of extract) and their copper, manganese and zinc determinations were compared with determinations on the same materials digested by a standard wet-digestion procedure[9] (1-g samples; final volume 20 ml).

There was close agreement (Table IV) between the methods for all elements and for all materials containing a wide range of silicon, chlorine and calcium concentrations, and differences between replicates were not significant ($P \leqslant 0.05$).

Recovery of Added Copper, Manganese and Zinc

To three 4-g sub-samples of nine different plant materials were added copper, manganese and zinc at concentrations of 10, 100 and 50 mg kg^{-1}, respectively. All samples were

subsequently ashed and analysed for these three elements by the recommended method. The mean recoveries varied from 96 to 100% for copper, 95 to 101% for manganese and 96 to 99% for zinc (Table V).

TABLE IV

COMPARISON OF Cu, Mn AND Zn CONCENTRATIONS IN PLANT SAMPLES AS DETERMINED BY THE DRY-ASHING AND WET-DIGESTION PROCEDURES

Sample	Concentration in plant material*, % *m/m*			Digestion procedure†	Concentration in plant material‡/mg kg^{-1}		
	Si	Cl	Ca		Cu	Mn	Zn
Barley glumes	4.6	0.5	0.2	W.D.	3.6	50.9	10.0
				D.A.	3.4	51.3	9.8
Barley straw	1.0	1.8	0.4	W.D.	7.0	38.7	27.1
				D.A.	6.8	39.7	27.2
Wheaten hay	0.8	0.8	0.2	W.D.	3.3	31.0	10.8
				D.A.	3.1	31.4	11.0
Lucerne pasture 1	0.2	1.2	1.9	W.D.	9.2	34.1	43.5
				D.A.	9.3	34.4	42.9
Medic pasture	0.1	0.8	1.1	W.D.	7.6	16.6	26.5
				D.A.	7.4	17.2	26.7
Lupin straw	0.2	0.2	1.8	W.D.	4.5	137	20.4
				D.A.	4.4	140	20.0
Subclover, ryegrass pasture 1	0.4	0.7	0.6	W.D.	7.0	166	26.5
				D.A.	6.9	164	26.4
Mixed legume and fog-grass pasture	3.1	4.0	3.5	W.D.	10.5	43.7	58.5
				D.A.	10.2	43.3	58.0
Bowen's kale[10]	0	0.3	4.0	W.D.	4.9	14.2	32.4
				D.A.	4.6	14.5	32.5
				Published values§	5.0	14.9	32
F-test (replicates)¶					n.s.	n.s.	n.s.
F-test (treatments)¶					n.s.	n.s.	n.s.

* By X-ray fluorescence analysis.
† Cu, Mn and Zn analyses by Allen's[9] wet-digestion procedure (W.D.); D.A. = dry ashing.
‡ Mean of three determinations.
§ Data from Bowen.[10]
¶ n.s. = not significant.

In a separate study, the recoveries of copper, manganese and zinc were examined by using the recommended dry-ashing procedure after adding silicon (0–4), chlorine (0–4), calcium (0–4), potassium (5), magnesium (2) or phosphorus (1% *m/m*) prior to ashing, to acidified potassium hydrogen sulphate solution containing the trace elements as sulphate salts at plant equivalent concentrations of 10, 100 and 50 mg kg^{-1} of copper, manganese and zinc, respectively. None of the macro-elements in the simulated ash solutions interfered seriously in the dry-ashing procedure. Recoveries of copper, manganese and zinc were between 95 and 100% when silicon as SiO_2, calcium as $CaSO_4.2H_2O$ and chlorine as NaCl were added together in various proportions of up to 4% *m/m*. Similar recoveries of 97–100% and 95–98% of total copper, manganese and zinc were measured for the treatments with potassium as K_2SO_4 and phosphorus as KH_2PO_4, respectively.

Conclusion

Accurate assays for copper, manganese and zinc concentrations in the test plant materials can be carried out by the dry-ashing procedure. Interferences are negligible except at concentrations of silicon and calcium that are extreme for plants (above 3.5%).

Moreover, the absence of interferences in recovery tests with both simulated and ashed plant extracts containing added copper, manganese and zinc and the close agreement between assays by wet-digestion and the dry-ashing procedures demonstrate the efficiency of the latter technique for extracting and measuring copper, manganese and zinc concentrations in plant material.

TABLE V

RECOVERY OF Cu, Mn AND Zn ADDED TO PLANT SAMPLES

Sample	Concentration of Cu in plant material			Concentration of Mn in plant material			Concentration of Zn in plant material		
	Added sample concentration*/mg kg^{-1}	Mean analysis†/ mg kg^{-1}	Mean recovery, %	Added sample concentration*/mg kg^{-1}	Mean analysis†/ mg kg^{-1}	Mean recovery, %	Added sample concentration*/mg kg^{-1}	Mean analysis†/ mg kg^{-1}	Mean recovery, %
Barley glumes	0	3.4	97	0	51.0		0	9.8	
	10	13.1		103	154.4	100	50	57.8	96
Barley straw	0	6.9	96	0	39.3		0	27.5	
	10	16.5		103	139.2	97	50	76.1	97
Wheaten hay	0	3.0	97	0	30.8		0	31.6	
	10	12.7		103	129.6	96	50	79.4	96
Lucerne pasture 1	0	9.2	99	0	34.6		0	42.9	
	10	19.1		103	134.1	97	50	92.0	98
Medic pasture	0	7.3	99	0	17.1		0	26.4	
	10	17.2		103	117.5	97	50	74.2	96
Lupin straw	0	4.4	99	0	138		0	20.9	
	10	14.3		103	240	99	50	69.8	98
Subclover, ryegrass pasture 1	0	7.1	99	0	167		0	25.8	
	10	17.0		103	271	101	50	75.4	99
Mixed legume and fog-grass pasture	0	10.4	100	0	43.5		0	57.8	
	10	20.4		103	146.8	100	50	106.7	98
Bowen's kale[10]	0	4.6	99	0	14.6		0	32.5	
	10	14.5		103	112.9	95	50	81.2	97

* Cu, Mn and Zn equivalent to 10 mg kg^{-1} Cu, 100 mg kg^{-1} Mn and 50 mg kg^{-1} Zn as $CuSO_4.5H_2O$, $MnSO_4.4H_2O$ and $ZnSO_4.7H_2O$, respectively, were added to 4-g sub-samples of plant material, which were then ashed by the recommended dry-ashing procedure.
† Mean of three determinations.

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Assessment of Phosphorescence Spectroscopy for Crude Oil Identification

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The application of phosphorescence techniques to crude oil identification has been studied. Conventional phosphorescence spectra are insufficiently structured to offer adequate discrimination between the crude oil samples investigated. Synchronous excitation and total contour spectra of a series of crude oils have been compiled. The latter techniques show promise as a method for differentiation between unweathered crude oils. Spectral discrimination is enhanced by the use of an external "heavy atom" quencher.

Lifetime measurements of phosphorescence decay offer limited scope, with respect to crude oil characterisation, owing to the complex nature of the observed decay rates.

Keywords: *Crude oil identification*; *phosphorescence*

Current oil spill identification strategies are based on a battery of analytical techniques,[1] the extent of which is dependent on several considerations ranging from the weathered condition of the sample to availability of equipment. It is desirable to investigate the means of improving the specificity of each analytical technique in an attempt to reduce the amount of "corroborative evidence" required from complementary techniques and thence to reduce both the range of analytical instrumentation required and the time employed to perform analyses.

The application of luminescence techniques to the analysis of complex mixtures is particularly attractive owing to the inherent high sensitivity coupled with the relatively short time scales over which analyses can be performed. In the latter context the direct analysis of samples in aqueous solution is a feature that should encourage continued development of luminescence methods.[2] The fact that most of the fluorescent components of crude oils, such as the polynuclear aromatic hydrocarbons, are relatively insoluble in water and involatile means that the fluorescence emission is comparatively stable to the effects of weathering of the oils.

The advantages of luminescence methods in analyses of complex mixtures has led to a considerable growth of the relevant literature in recent years. Citation of references here will be limited to the most relevant publications; more comprehensive bibliographies can be found in reviews by Bentz[1] and Giering.[3]

The simplest form of emission spectroscopic analysis involves the examination of a conventional emission spectrum using a fixed wavelength of excitation. This method is extremely limited in application to the analysis of complex mixtures. The virtually identical fluorescence profiles of a variety of crude oils of widely different origin has been demonstrated.[4] Some degree of discrimination is afforded by the employment of a variety of excitation wavelengths. This approach forms the basis of an accepted fluorescence analytical procedure for oil spill identification.[5] The scope of conventional luminescence analyses may be considerably increased through the increased structure of emission profiles observed at low temperatures.[6] From the examination of about 80 crude oil samples in our laboratory, we have concluded that conventional spectroscopy at room temperature is capable of subdivision of the samples examined into about ten main categories of spectral response.[2] Differentiation between two crude oils within a given category is extremely difficult and would require the use of a prohibitively large number of excitation wavelengths.

The technique of synchronous excitation fluorimetry, initiated by Lloyd,[7] was first applied to crude oil identification by John and Soutar.[4] In this technique both excitation and analysis monochromators are scanned in a synchronous manner such that a constant wavelength increment, $\Delta\lambda$, is maintained. The frequency range over which a given component of the fluorescent mixture may emit is compressed to an extent dependent on the magnitude of $\Delta\lambda$. Further, "automatic selection" of optimum excitation wavelengths of the various components of the

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sample is achieved. The net effect is to produce a more structured profile relative to a conventional spectrum that is characteristic of the sample. Vo-Dinh[8] has suggested that the use of small wavelength increments ($\Delta\lambda \approx 3$ nm) would allow the identification of the individual components of moderately complex mixtures. However, the general applicability of the technique to the identification of components has been disputed.[9–11] For the purpose of identification of the components of complex mixtures, in a "finger-printing" sense, the employment of $\Delta\lambda$ values of 15–25 nm produces optimum results by matching $\Delta\lambda$ to the energy difference between the excitation and emission maxima of most of the fluorescent constituents.[4]

Maximisation of the information obtainable through conventional fluorescence spectroscopy is obtained through examination of the total luminescence surface as a function of both excitation and emission wavelength. Two-dimenional representation in the form of a contour diagram is convenient for the purpose of display and comparison of data. Freegard *et al.*[12] proposed the use of contour plots of fluorescence data for the characterisation of oil samples. Hornig and co-workers[13,14] have been particularly active in the development of total luminescence spectroscopic techniques in the analysis of crude oils and other systems. Giering[3] has cited examples of the applications of total fluorescence spectroscopy by this group of workers. Frank[15] has proposed a simpler approach to the problem of sampling the three-dimensional emission surface. Johnson *et al.*[16] have described the development of a video fluorimeter for total luminescence data display.

Despite considerable use of phosphorimetry in a variety of analytical situations (*e.g.*, see reference 17) there has been little interest in the application of phosphorescence techniques to the identification of oils.[18] The potential of time-resolved phosphorimetry for the analysis of mixtures has been demonstrated.[19,20] Although the evolution of time-resolved spectra through synthesis from a series of decay curves offers undoubted advantages in the resolution of mixtures containing relatively few components with similar excitation and emission spectra,[19] it is unlikely that the method will prove valuable in the analysis of complex mixtures of hydrocarbons such as crude oils.

In this paper we report on an investigation into the application of phosphorescence techniques in order to assess their importance to crude oil identification and it represents an extension to the work of Eastwood and Hanks.[18] In particular, data concerning the application of conventional phosphorimetry, synchronous excitation phosphorimetry and total phosphorescence spectroscopy in both the absence and presence of phosphorescence enhancement through heavy atom effects will be presented.

Experimental

Materials

Diethyl ether (May and Baker, AnalaR grade) was dried and distilled over sodium metal in the complete absence of light. Isopentane (BDH) was subjected to extraction with concentrated sulphuric acid and washed with water prior to distillation over metallic sodium. Ethanol (BDH, spectroscopic grade) was refluxed over calcium oxide prior to fractional distillation. Iodomethane was purified by fractional distillation.

Crude oil samples were obtained from Warren Spring Laboratory and used as received. Eight crude oils were studied. They were of three geographical origins: African (Brega, Gamba, Hassi Messaoud), Middle East (Kuwait, Qatar, Zakum) and North Sea (Ecofisk, Forties).

Spectroscopy

Phosphorescence spectra were obtained on a Perkin-Elmer 44A fluorescence spectrometer equipped with a "rotating can" phosphorescence accessory. Emission spectra were recorded at right-angles to the excitation beam from solid solutions of crude oils in diethyl ether - isopentane - alcohol (ethanol) (2 + 5 + 5 volume ratio) (EPA) glasses maintained at 77 K in an optical Dewar vessel. Phosphorescence decays were displayed, following cessation of excitation, on a Telequipment DM64 oscilloscope.

Total phosphorescence contour maps were generated "off-line" from a series of phosphorescence emission spectra recorded at 10-nm excitation wavelength intervals (Burroughs 5700 computer) using the CALCOMP GPCP contouring program. Emission spectra were uncorrected for the wavelength dependence of the instrument response.

Results and Discussion

Conventional Phosphorimetry

Conventional phosphorescence emission spectra were obtained for each of the eight crude oils over a range of concentrations from 50 to 2000 μl l^{-1} in EPA glass at 77 K. Spectra were generated at fixed excitation wavelengths over the range 240–370 nm at intervals of 10 nm. Optimum resolution was obtained using excitation and emission slit widths corresponding to 10 nm and concentrations of 200 μl l^{-1} in crude oil. Maximum differentiation between emission spectra occurred upon excitation at 260 nm, although the spectral profiles contain little structure even under optimised conditions such as those illustrated in Fig. 1.

Although the spectra offer some discrimination between crude oils, the lack of structural features in the emission envelopes renders the use of conventional phosphorescence spectra in oil identification difficult, even if the spectra are examined at a variety of excitation wavelengths. However, the limited degree of discrimination between oils offered by phosphorescence spectra could prove useful as additional evidence for differentiation between oils such as those of North Sea origin, which are difficult to discriminate solely on the basis of fluorescence spectra.[21]

Iodomethane was added (10% by volume) to the EPA solvent to enhance phosphorescence relative to fluorescence through the influence of the external heavy atom effect.[22] The addition of the heavy atom quencher of the excited singlet states has three major effects on the emission observed from the triplet states of the aromatics in the crude oil:

(*a*) an increase in phosphorescence emission intensity, at a given excitation wavelength, through enhanced inter-system crossing from the excited singlet states and increased rate of radiative deactivation of the triplet level;

(*b*) a shift in wavelength corresponding to maximum excitation efficiency of about 40–50 nm and a lesser shift in the emission spectral maximum; and

(*c*) a slightly enhanced spectral structure.

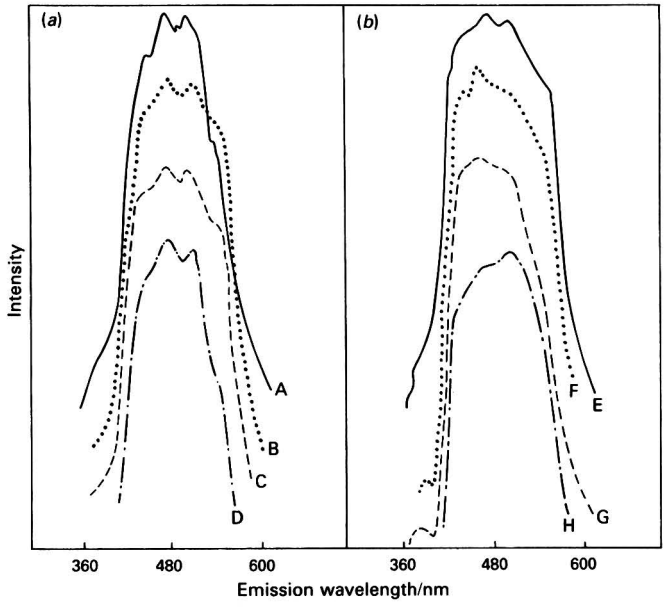

Fig. 1. Phosphorescence emission spectra in EPA glass at 77 K. Excitation wavelength = 260 nm; crude oil concentration = 200 μl l^{-1}. (*a*) Crude oil: A, Ecofisk; B, Forties; C, Hassi Messaoud; and D, Gamba. (*b*) Crude oil: E, Brega; F, Qatar; G, Zakum; and H, Kuwait.

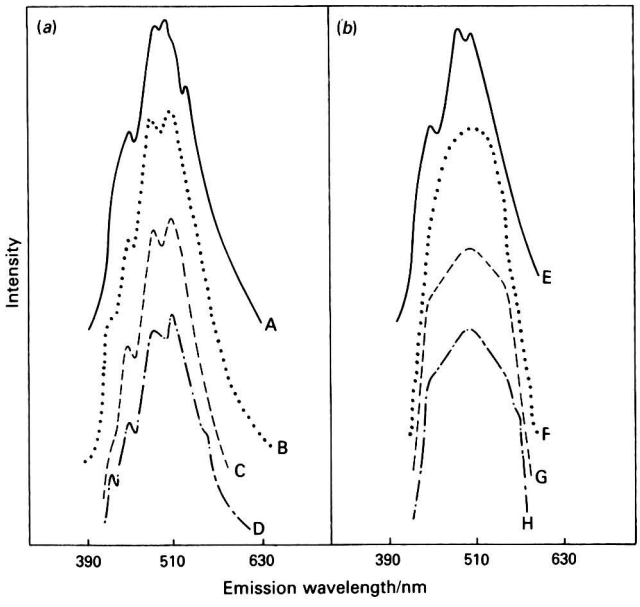

Fig. 2. Effect of heavy atom quencher: phosphorescence emission spectra in IEPA glass (EPA containing 10% by volume of iodomethane) at 77 K. Excitation wavelength = 300 nm; crude oil concentration = 200 μl l^{-1}. (*a*) Crude oil: A, Ecofisk; B, Forties; C, Hassi Messaoud; and D, Gamba. (*b*) Crude oil: E, Brega; F, Qatar; G, Zakum; and H, Kuwait.

The phosphorescence spectra obtained upon excitation at 300 nm are shown in Fig. 2. The photophysical parameters governing effect (*b*) are discussed below with respect to total phosphorescence contour diagrams. Enhancement of spectral structure in the presence of a singlet quencher (bromoform) has been observed previously at lower quencher concentrations by Eastwood and Hanks.[18]

Despite the additional information that may be obtained through use of phosphorescence emission spectra in the presence or absence of heavy atom species, it is unlikely that the technique will find extensive application in oil spill identification except to augment data for samples that have extremely similar fluorescence spectra.

Synchronous Excitation Phosphorimetry

The success of synchronous excitation fluorescence in oil identification[1,21–24] and other analyses (*e.g.*, references 7, 8, 27 and 28) acts as an indicator of possible enhanced discrimination between oils using synchronous excitation of phosphorescence. The use of synchronously excited phosphorescence in the separation of emission bands of individual components in relatively simple mixtures has been reported by Vo-Dinh and Gammage.[29]

Synchronous excitation spectra were obtained over a range of "spectroscopic windows," $\Delta\lambda$, from 80 to 300 nm in increments of 20 nm. The synchronous spectra were broad and featureless and showed little difference between samples except for those obtained with $\Delta\lambda$ in the region of 100 nm. Fig. 3 shows the synchronously excited phosphorescence emission profiles of Kuwait and Forties crude oils for wavelength increments of 80, 100 and 120 nm. The addition of 10% of iodomethane resulted in the production of less distinctive spectral profiles than those obtained in pure EPA glass.

Synchronous excitation of phosphorescence appears to offer a method by which increased detail may be obtained for augmentation of the information obtained by fluorescence methods regarding the identity of a crude oil sample.

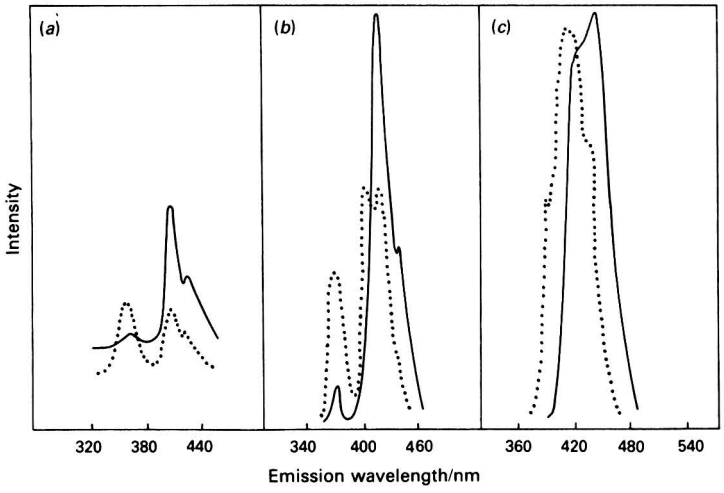

Fig. 3. Synchronously excited phosphorescence spectra (EPA glass, 77 K; crude oil concentration = 200 μl l^{-1}). (*a*) $\Delta\lambda$ = 80 nm; (*b*) $\Delta\lambda$ = 100 nm; and (*c*) $\Delta\lambda$ = 120 nm. Solid line, Kuwait crude oil; and broken line, Forties crude oil.

Total Phosphorescence Spectra

Two-dimensional representation of the three-dimensional dependence of phosphorescence intensity on excitation and emission wavelengths was achieved through a contour mapping procedure. Data were sampled at 10-nm emission wavelength intervals across emission profiles recorded at successive excitation wavelength increments of 10 nm. Peak intensities of the emission - excitation - intensity matrix for each oil were normalised prior to computer generation of the contour diagrams.

Contour diagrams were generated for each of the eight oils studied. Figs. 4 and 5 show contour plots for the Forties and Kuwait samples. Although such plots appear similar, each map exhibits characteristic wavelength co-ordinates of peak maxima and spectral range. At a

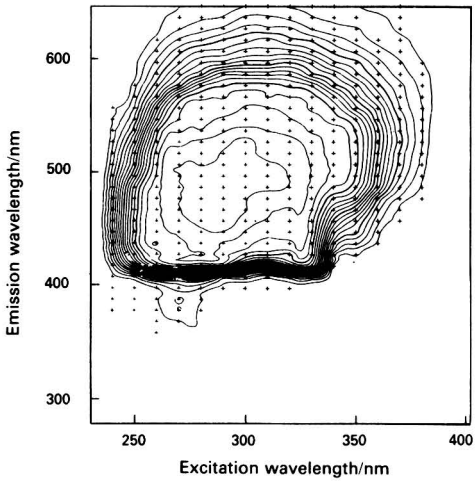

Fig. 4. Total phosphorescence contour diagram for Forties crude oil (100 μl l^{-1}, EPA glass, 77 K).

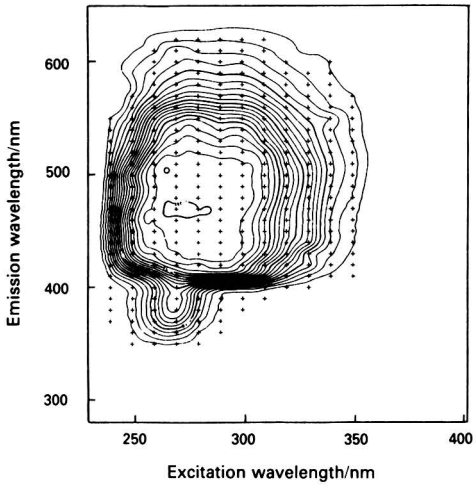

Fig. 5. Total phosphorescence contour diagram for Kuwait crude oil (100 μl l^{-1}, EPA glass, 77 K).

standard concentration in EPA glass at 77 K, each crude oil studied exhibited characteristic co-ordinates for the emission, $\lambda_{em.}^{max.}$, and excitation, $\lambda_{ex.}^{max.}$, wavelengths corresponding to the maximum phosphorescence peak intensity. This is illustrated in Table I. In certain instances, in both EPA and IEPA glasses, subsidiary peaks in the contour were observed but, clearly, this served to differentiate further between the spectra of the crude oils. The error limits represent the error in determining the values of $\lambda_{em.}^{max.}$ and $\lambda_{ex.}^{max.}$; duplicate analyses gave results within the quoted limits. The ranges of $\lambda_{em.}^{max.}$ and $\lambda_{ex.}^{max.}$ were about 40 and 20 nm, respectively, for the eight crude oils. The contour diagrams permit differentiation between the eight oils studied through either superposition or subtraction methods. The

TABLE I

EXCITATION AND EMISSION WAVELENGTHS FOR MAXIMUM PHOSPHORESCENCE INTENSITY, $\lambda_{ex.}^{max.}$ AND $\lambda_{em.}^{max.}$, FOR CRUDE OILS IN AN EPA MATRIX (200 μl l^{-1}) AT 77 K

Crude oil	Wavelength/nm $\lambda_{ex.}^{max.}$	Wavelength/nm $\lambda_{em.}^{max.}$
Brega	281±2	472±5
Gamba	292±2	464±5
Hassi Messaoud	280±2	475±2*
	280±2	500±2†
	300±2	438±2‡
	300±2	466±2‡
	300±2	500±2†
Kuwait	291±2	496±2
Qatar	300±2	492±2
Zakum	280±2	458±2
Ecofisk	291±2	478±2
Forties	280±2	480±5

* Most intense peak.
† Minor peaks.
‡ Identical peak intensities.

principles of such an identification procedure can be illustrated by simple visual examination of the contour diagrams of two oils such as Kuwait and Forties, which are considerably different in chemical composition (*cf.*, Figs. 4 and 5):

(*a*) there is a difference in the area of optimum emission - excitation;

(*b*) the contour diagram for the Kuwait crude is much more extensive than that of the Forties sample, reflecting the differences in aromatic composition of the two species and particularly the larger proportion of high relative molecular mass aromatic components in the Kuwait oil; and

(*c*) overlay of the sample contours would result in multiple crossing of contour lines in accord with distinctions between the chemical compositions of the oils afforded by the total emission spectral method.

Figs. 6 and 7 show the effects of the addition of 10% by volume of the iodomethane heavy atom quencher. The resultant contour plots are shifted to longer wavelengths of both excitation and emission for both samples relative to those characteristic of unquenched samples (*cf.*, Figs. 4 and 5). This trend was observed for all of the oils studied.

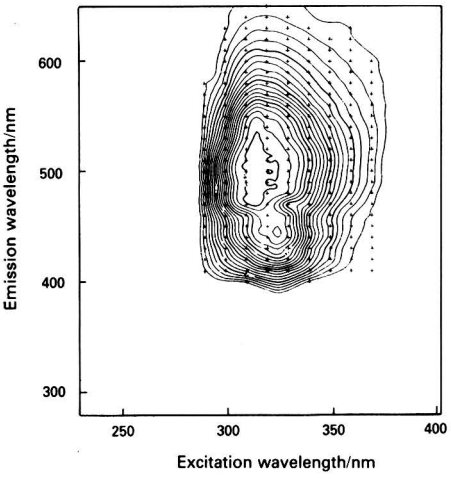

Fig. 6. Total phosphorescence contour diagram for Forties crude oil in IEPA glass (100 μl l^{-1}, 77 K).

Further, there was a concomitant increase in the phosphorescence emission intensity even in the reduced spectral range at longer wavelengths. Methyl iodide absorbs strongly at wavelengths below 320 nm under the conditions of the experiment. Thus, re-absorption of the phosphorescence emission by methyl iodide is negligible although the observed shift to longer wavelength may be explained, in part, by absorption of the excitation radiation by the quencher. The enhanced phosphorescence quantum yield at longer wavelengths demonstrates that "inner filter" effects are not solely responsible for the observed frequency shift. Hood and Winefordner[30] have observed increased phosphorescence intensities of higher aromatic components in ethanol glasses at 77 K resulting from the addition of ethyl iodide. The overall effect is to produce compression of the whole contour diagram relative to that obtained in the absence of heavy atom species whilst retaining the characteristic features necessary for differentiation between the various oils studied.

Upon initial consideration of the enhancement of phosphorescence emission from the more condensed aromatic components of the mixture, it is tempting to explain the effect solely in terms of enhanced inter-system crossing rates for higher aromatics relative to species of lower relative molecular mass. However, the situation is more complex. The effect of heavy atom quenchers on a given polynuclear aromatic molecule is to enhance the rates of radiative and radiationless deactivation of the $^3M^*$ states in addition to promotion of the $^3M^* \leftarrow {}^1M^*$

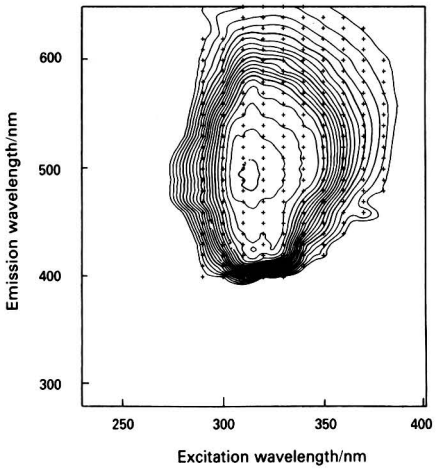

Fig. 7. Total phosphorescence contour diagram for Kuwait crude oil in IEPA glass (100 μl l^{-1}, 77 K).

transition by inter-system crossing.[22,31,32] The observed effect on the relative importance of radiative deactivation for a series of difference aromatic molecules will depend upon variations in the individual rate parameters k_{TM}(inter-system crossing from ^{1}M*), k_{PT} (radiative decay of ^{3}M*) and k_{GT} (radiationless deactivation of ^{3}M*). The notation used for the designation of rate coefficients is that of Birks.[33]

The data presented here are not sufficient to determine the dominant factor in the determination of the increased efficiency of phosphorescence from the larger polynuclear aromatics in the mixture relative to the species of lower relative molecular mass upon addition of a heavy atom quencher. However, it has been observed that the presence of a heavy atom quencher produces a similar lowering of the relative intensity in the higher energy emission range of the low-temperature fluorescence of crude oils.[21] Consequently, the enhancement of lower energy phosphorescence emissions evident in Figs. 6 and 7 is not dependent on increased inter-system crossing rates, k_{TM}, as such a relative depopulation of the singlet level could lead to a decreased fluorescence emission from the species of higher relative molecular mass. It is likely that the enhanced phosphorescence of the higher aromatics is a consequence of a greater influence of the external quencher on k_{PT} in the larger aromatic molecules because, for naphthalenes at least, k_{GT} is little affected by external heavy atom species.[32]

Fig. 8 shows the total phosphorescence contour diagram for a sample of Forties crude oil of concentration 2000 μl l^{-1}. Comparison with Fig. 4 reveals that the general effect of increased concentration is the expansion of the spectral range of the contour plot. It was noted that as the crude oil concentration was increased over the range 50–2000 μl l^{-1} for each sample the spectra became less structured. The effects of concentration may be rationalised in terms of energy transfer processes of both a radiationless and a radiative nature, which will increase in importance at higher concentrations.

In summary, total phosphorescence spectroscopy performed in the presence and absence of heavy atom quenchers allows discrimination between the crude oils studied in this work and would prove valuable in the provision of spectroscopic data to augment those obtained by fluorescence at room temperature.

Phosphorescence Transient Decay Studies

Rayner and Szabo[34] have shown that studies of fluorescence decay curves of oils can allow discrimination between samples. The use of phosphorescence decay data in this context has not been reported so far.

Phosphorescence temporal profiles were studied for several crude oils at a fixed excitation wavelength of 320 nm and analysed at 440, 460 and 480 nm. Under fixed emission wavelength (460 nm) conditions the variation of the decay profile at excitation wavelengths of 300, 320 and 340 nm was studied.

The decays displayed marked deviations from exponential behaviour, which is not unexpected for aromatic mixtures. Although the characterisation of such decays by a "rate constant" or "a lifetime" ($1/e$ or $\frac{1}{2}$) is not meaningful, the following generalisations are apparent:

(*a*) not unexpectedly, the decay rate for a given oil is dependent upon excitation and emission wavelengths;

(*b*) the decay rate at a fixed wavelength varies from one oil to another; and

(*c*) although the use of decay data to complement steady-state intensity data appears feasible, the complexity of decay characterisation of the number of variables involved will probably result in the neglect of such a procedure in oil spill identification.

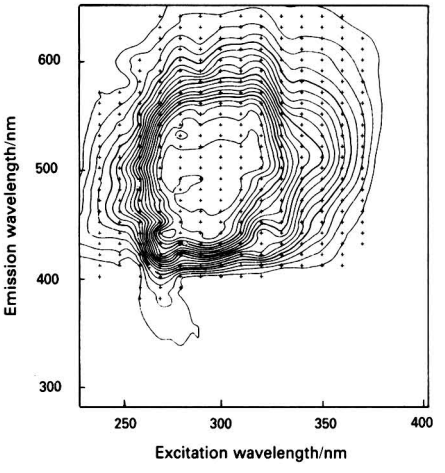

Fig. 8. Total phosphorescence contour diagram for Forties crude oil (2000 μl l^{-1}, EPA glass, 77 K).

Conclusion

It has been shown that phosphorescence techniques, notably total phosphorescence spectroscopy, discriminate between the unweathered crude oil samples investigated. Although the acquisition of sufficient spectra for subsequent data processing to produce a total emission contour is time consuming, the advantages over conventional and synchronously excited phosphorescence spectra are evident. The advent of spectrometers interfaced to minicomputers will allow the rapid composition of total luminescence spectra. Such instrumentation has recently become commercially available.[35] It remains to be shown if the technique will discriminate between a wider range of both weathered and unweathered crude oils.

Although phosphorescence could conceivably prove of value in the assignment of a crude oil to a series of suspect samples, the stringent purity requirements for solvent glasses extend the over-all analysis time relative to room temperature fluorescence measurements. Moreover, the phosphorescence techniques cannot capitalise on a major advantage of fluorimetry, namely, direct determination in an aqueous medium. As a consequence, it is likely that phosphorescence spectroscopy will be resorted to only in instances where conventional, synchronous or total fluorimetry fails to provide unambiguous evidence as to the identity of the source of an oil pollution incident.

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Simultaneous Spectrophotometric Determination of Palladium(II) and Gold(III) with Methiomeprazine Hydrochloride: Analysis of Alloys and Minerals*

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Methiomeprazine hydrochloride (MMH) is proposed as a reagent for the simultaneous spectrophotometric determination of palladium(II) and gold(III). The reagent instantaneously forms an orange - red 1 : 1 complex with palladium(II) and a blue species with gold(III) in hydrochloric acid - sodium acetate buffer. The palladium - MMH complex has an absorption maximum at 480 nm with a molar absorptivity of 3.6×10^3 l mol^{-1} cm^{-1}. The blue species has an absorption maximum at 630 nm with a molar absorptivity of 1.3×10^4 l mol^{-1} cm^{-1}. Beer's law is obeyed for the range 0.4–21 p.p.m. of palladium(II) and 0.2–10 p.p.m. of gold(III). Molar excesses of 4-fold and 35-fold of the reagent are required for palladium(II) and gold(III), respectively. The concentration of two metals in an unknown mixture can be simultaneously determined in the range of 0.5–15 p.p.m. of palladium(II) and 1–10 p.p.m. of gold(III) using the following equations:

$$[\mathrm{Pd}] \times 10^4 = 3.023A_{480} - 0.8403A_{630}$$

$$[\mathrm{Au}] \times 10^4 = 0.8177A_{630} - 0.1959A_{480}$$

The method has been used successfully for the determination of palladium and gold in alloys and minerals.

Keywords: *Palladium and gold determination*; *spectrophotometry*; *methiomeprazine hydrochloride reagent*

Palladium and gold are found in certain minerals and alloys. Although many methods for the spectrophotometric determination are known, one has not previously been proposed for the simultaneous determination of palladium and gold. A literature survey showed that no attempt had been made to study the colour reactions of palladium(II) and gold(III) with methiomeprazine hydrochloride (MMH), 10-[3-(*NN*-dimethylamino)-2-methylpropyl]-2-methylthiophenothiazine hydrochloride. This paper describes the investigation of the colour reaction of MMH with palladium(II) and gold(III) for the rapid spectrophotometric determination of palladium and gold.

Experimental

Reagents

Palladium(II) solution. Prepare a stock solution of palladium(II) by dissolving about 1 g of palladium(II) chloride (Johnson Matthey Chemicals) in 1 l of 0.1 M hydrochloric acid and standardise gravimetrically by the dimethylglyoxime method.[1]

Gold(III) solution. Prepare a stock solution of gold(III) by dissolving the requisite amount of gold(III) chloride (Baird and Tatlock) in hydrochloric acid and diluting to 1 l to give a solution 1 M with respect to hydrochloric acid; standardise gravimetrically by the hydroquinone method.[2]

Methiomeprazine hydrochloride. Prepare a stock solution of 0.5% *m*/*V* MMH (Rhone-Poulenc) in doubly distilled water and store in an amber-glass bottle in a refrigerator.

Prepare solutions of hydrochloric acid - sodium acetate buffer and foreign ions of suitable concentrations by using analytical-reagent grade reagents. The buffer was prepared by mixing 50 ml of 1 M sodium acetate solution with between 10 and 100 ml of 1 M hydrochloric acid. This mixture was diluted to 250 ml to give solutions of pH 0.65–5.2.

* Presented at the 5th SAC International Conference on Analytical Chemistry, Lancaster, July 20–26th, 1980.

Instrument

A Beckman, Model DB, spectrophotometer with matched 1-cm silica cells was used for absorbance measurements.

Procedure for the Determination of Palladium or Gold

Transfer aliquots of the stock solutions containing 0.4–21 p.p.m. of palladium(II) or 0.2–10 p.p.m. of gold(III) into a series of 25-ml calibrated flasks. Add 5 ml of hydrochloric acid - sodium acetate buffer of pH 1.4 and 5 ml of 0.5% MMH solution to each flask and dilute to the mark with doubly distilled water. Mix well and measure the absorbance at 480 nm for palladium(II) solutions and at 630 nm for gold(III) solutions, against the corresponding reagent blank. The graph of absorbance *versus* the concentration of palladium or gold is a straight line passing through the origin. Determine the absorbance of the sample containing palladium or gold by the method described above and then deduce the palladium or gold concentration of the sample solution from the calibration graph.

Procedure for the Determination of Gold in Gold - Copper - Silver Alloy

Transfer an accurately weighed amount of gold alloy (approximately 0.1 g) into a covered 50-ml beaker and treat with 5 ml of aqua regia. Heat the solution gently to dissolve the alloy and then carefully add 10 ml of concentrated hydrochloric acid in 2-ml portions and evaporate to dryness on a steam-bath after each addition. Dissolve the residue in 10 ml of 1 N hydrochloric acid and filter off the silver chloride. Wash both the beaker and the silver chloride well with 1 N hydrochloric acid. Dilute the filtrate and washings to 500 ml with doubly distilled water in a calibrated flask. Use aliquots of this solution for reaction with the MMH as described in the procedure for the determination of gold. Measure the absorbance at 630 nm and read the gold concentration from the calibration graph. The results for three samples of gold alloy are given in Table I.

Procedure for the Simultaneous Determination of Palladium and Gold

Transfer an aliquot of the solution containing 0.5–15 p.p.m. of palladium(II) together with an aliquot of the 1–10 p.p.m. of gold(III) solution into a series of 25-ml calibrated flasks. Add 5 ml of hydrochloric acid - sodium acetate buffer of pH 1.4 and 5 ml of 0.5% MMH solution to

TABLE I

DETERMINATION OF GOLD IN GOLD - COPPER - SILVER ALLOYS

Sample	Certified composition, %*	Gold content of solution, p.p.m.	
		Certified value	Found†
1	Au, 43.4; Cu, 49.35; Ag, 7.25	2.0	1.98
		4.0	4.02
		6.0	6.04
		8.0	7.98
2	Au, 40; Cu, 55.5; Ag, 4.5	2.5	2.45
		5.0	5.04
		7.0	6.96
		9.0	9.04
3	Au, 60; Cu, 35; Ag, 5	3.0	2.94
		5.5	5.40
		7.5	7.56
		9.5	9.40

* These values were given by the suppliers of the samples.

† A known mass of sample was dissolved and aliquots of the solution diluted to give solutions containing four different levels of gold. The values, calculated from the gold content certified by the supplier, are expressed as certified values. The results quoted as "Found" are the means of five determinations on each solution.

each flask. Shake well, dilute to the mark and record the absorbances at 480 and 630 nm against the reagent blank prepared under similar conditions. Determine the amounts of the two metals in the solutions from the appropriate simultaneous equations.

Results and Discussion

MMH is soluble in water, giving a colourless solution. It readily forms an orange - red complex with palladium(II) and a blue oxidation product with gold(III) at room tmperature (27 °C) in hydrochloric, sulphuric, orthophosphoric or acetic acid, or in hydrochloric acid - sodium acetate buffer. The study of the orange - red complex and the blue species in hydrochloric, sulphuric, orthophosphoric and acetic acids is not recommended because they are less stable and many foreign ions interfere even at very low concentrations. Hydrochloric acid - sodium acetate buffer was therefore selected for further studies because of the greater stability of the coloured species and lower interference of diverse ions in this medium.

Effect of pH

The effective pH range for the formation of the orange - red complex and the blue species was found to be 1.4–4.1, as shown by the constancy of $\lambda_{max.}$ and absorbance readings within this pH range (Fig. 1). Below pH 1.4 the maximum intensity of the colour is not obtained and above pH 4.1 white turbidity is observed. A buffer medium of pH 2 was therefore selected for further studies.

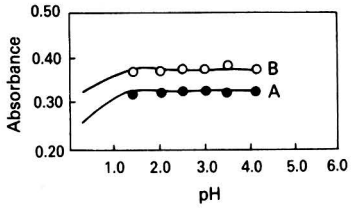

Fig. 1. Effect of varying pH on the absorbance of the palladium(II) - MMH complex and blue species. A, Palladium(II) - MMH complex, palladium(II) concentration 10 mg l^{-1} and MMH concentration 2.7×10^{-3} M, $\lambda_{max.}$ 480 nm; and B, blue species of MMH with gold(III), gold(III) concentration 5 mg l^{-1} and MMH concentration 2.7×10^{-3} M, $\lambda_{max.}$ 630 nm.

Absorption Spectra

The absorption spectra of the palladium - MMH complex, blue species, reagent blank, palladium(II) and gold(III) solutions are shown in Fig. 2. The complex exhibits maximum absorbance at 460–490 nm and the blue species at 620–640 nm. The absorption spectra of the palladium(II) and gold(III) solutions and the reagent blank show little absorbance at these wavelengths. All subsequent studies were made at 480 and 630 nm for the complex and the blue species, respectively.

Effect of Reagent Concentration

Investigations on the variation of the absorbance of the orange - red palladium(II) - MMH complex and the blue species with the reagent concentration were carried out by measuring the absorbance of solutions containing 8 mg l^{-1} of palladium(II) at 480 nm and solutions containing 4 mg l^{-1} of gold(III) at 630 nm with varying amounts of MMH in a buffer medium of pH 2.0. The absorbances were plotted against the molar excess of the reagent (Fig. 3). It was found that for the full development of the colour intensity a 4-fold molar excess of the reagent over palladium and a 35-fold molar excess of the reagent over gold were required. The optimum amount (5 ml) of 0.5% MMH reagent solution was adequate for the range covered in a final volume of 25 ml.

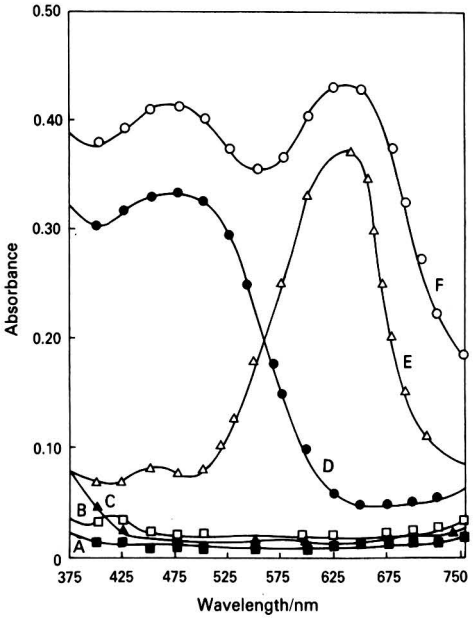

Fig. 2. Absorption spectra of palladium(II) - MMH complex, blue species, palladium(II), gold(III) solutions and reagent blank. A, Reagent blank, MMH concentration 2.7×10^{-3} M; B, palladium(II) concentration 10 mg l^{-1}, pH 2.0; C, gold(III) concentration 5 mg l^{-1}, pH 2.0; D, palladium(II) - MMH complex, pH 2.0, palladium(II) concentration 10 mg l^{-1} and MMH concentration 2.7×10^{-3} M; E, blue species of MMH with gold(III), pH 2.0, gold(III) concentration 5 mg l^{-1} and MMH concentration 2.7×10^{-3} M; F, palladium(II) - MMH complex plus blue species of MMH with gold(III), pH 2.0, palladium(II) concentration 10 mg l^{-1}, gold (III) concentration 5 mg l^{-1} and MMH concentration 2.7×10^{-3} M.

Effect of Time and Temperature

The maximum absorbance is obtained instantaneously after mixing the reagents. The palladium(II) - MMH complex is stable for about 8 d and the blue species for about 25 min. There was no appreciable change in the absorbance of the complex or the blue species if the order of addition of the reagents was varied. The formation of the complex and the blue species was investigated at temperatures ranging from 5 to 70 °C. The absorbance values were not affected by temperature over the range 5–50 °C for the complex and 5–40 °C for the blue species. At higher temperatures the absorbances gradually decreased with increase in temperature.

Calibration, Range, Sensitivity and Precision

Beer's law is obeyed over the range 0.4–21 p.p.m. of palladium(II) and 0.2–10 p.p.m. of gold(III). The optimum concentration range for the effective spectrophotometric determination, evaluated by Ringbom's method, is 0.6–20.5 p.p.m. for palladium(II) and 0.6–9.8 p.p.m. for gold(III). Sandell's sensitivity was determined to be 30 ng cm^{-2} for palladium(II) and 14.3 ng cm^{-2} for gold(III). The standard deviation calculated from 10 determinations on a solution containing 8 p.p.m. of palladium(II) and 3 p.p.m. of gold(III) is 0.0065 and 0.0016 p.p.m., respectively, and the relative error is less than 2%.

Stoicheiometry of Palladium - MMH Complex

The stoicheiometry of the complex was determined by Job's method of continuous variations,[3,4] the molar-ratio method[5] and the slope-ratio method.[6] The determinations were made in a medium of constant ionic strength (0.1 M sodium nitrate solution) at a pH value of 2.0 ± 0.1 and a temperature of 27 °C. The stoicheiometric ratio of the palladium - MMH complex was found to be 1:1. The apparent stability constant of the complex, evaluated by the molar-ratio method, was log $K = 4.7 \pm 0.1$ at 27 °C.

Nature of the Complex

The electrolytic nature of the palladium - MMH complex was found by ion-exchange experiments. When 25 ml of a 10^{-5} M solution of the palladium - MMH complex was passed through a Dowex 50W–X8 cation-exchange column (30 × 1.4 cm), the orange - red colour was completely adsorbed. The eluate was colourless. When a Dowex 1-X8 anion-exchange column was used the eluate was orange - red. This indicated that the palladium - MMH complex is cationic.

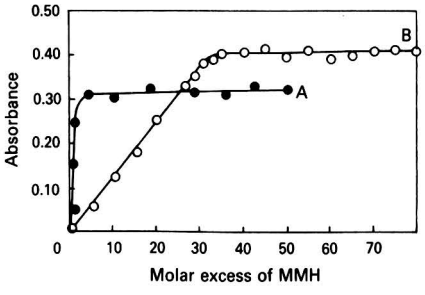

Fig. 3. Effect of reagent concentration on palladium(II) - MMH complex and blue species. A, Palladium(II) - MMH complex, palladium(II) concentration 10 mg l^{-1}, pH 2.0, $\lambda_{max.}$ 480 nm; and B, blue species of MMH with gold(III), gold(III) concentration 5 mg l^{-1}, pH 2.0, $\lambda_{max.}$ 630 nm.

Effect of Foreign Ions

In order to assess the possible analytical applications of the palladium - MMH complex or the blue species, the effect of some foreign ions that often accompany palladium or gold were examined. For these studies different amounts of ionic species were added to the sample solution containing 200 μg of palladium(II) or 100 μg of gold(III) in 25-ml calibrated flasks and the colour was developed as outlined in the standard procedure. An error of ± 2% in the absorbance readings was considered tolerable. Many cations and anions do not interfere. Interference from platinum metals and iron can be reduced by using a mxture of phosphate and tartrate as a masking agent. The interference due to silver(I) can be eliminated by removing silver as silver chloride with sodium chloride. Iodide, thiosulphate, EDTA, ruthenium(III), osmium(VIII), iron(III), tungsten(VI), rhodium(III), platinum(IV) and iridium(III) seriously interfere (Table II).

Simultaneous Determination of Palladium(II) and Gold(III)

The palladium - MMH complex and the blue species have maximum absorptions at 480 and 630 nm, respectively (Fig. 1) in the pH range 1.4–4.1. The molar absorptivities of the palladium - MMH complex are 3.6×10^3 l mol^{-1} cm^{-1} at 480 nm and 8.2×10^2 l mol^{-1} cm^{-1} at 630 nm. The molar absorptivities of the blue species of MMH with gold(III) are 3.4×10^3 l mol^{-1} cm^{-1} at 480 nm and 1.4×10^4 l mol^{-1} cm^{-1} at 630 nm. As the absorbance is

TABLE II

INTERFERENCE OF FOREIGN IONS IN THE DETERMINATION OF PALLADIUM AND GOLD

Ion added	Tolerance limit of ion, p.p.m.		
	Determination of Pd*	Determination of Au†	Determination of Pd plus Au‡
Cu(II)	1000	800	760
Zn(II)	2000	2400	2000
Cd(II)	3500	4000	3500
Fe(III)	0.5	0.4	0.1
Fe(III)	20§	14§	10.5§
Co(III)	200	200	160
Ni(II)	400	380	380
Al(III)	2000	1500	1370
As(III)	27	20	20
Pb(II)	2000	2000	1600
Ag(I)	2	1	0.5
Mn(II)	400	350	250
Mg(II)	4800	4800	4000
W(VI)	30	20	20
Mo(VI)	70	65	60
Ru(III)	0.6	0.8	0.5
Ru(III)	3§	3§	3§
Rh(III)	6	2	2
Rh(III)	17§	10§	10§
Os(VIII)	2	2	2
Os(VIII)	4§	3§	3§
Ir(III)	12	10	10
Ir(III)	14§	13§	12§
Pt(IV)	1	3	1
Pt(IV)	8§	6§	6§
Pd(II)	—	0.5	—
Au(III)	0.2	—	—
U(VI)	360	150	150
Te(VI)	4000	4000	4000
Sb(III)	100	120	100
Sulphate	4000	9000	3980
Nitrate	2000	400	390
Phosphate	1000	1500	800
Thiosulphate	0.2	0.15	0.1
Oxalate	5000	2000	1600
Citrate	2000	8000	1600
Tartrate	2000	2000	2000
EDTA	0.1	0.1	0.1
Fluoride	4000	4000	3800
Bromide	100	150	100
Iodide	0.2	0.1	0.1

* Concentration of palladium(II) was 8 p.p.m. and $\lambda_{max.}$ 480 nm.
† Concentration of gold(III) was 4 p.p.m. and $\lambda_{max.}$ 630 nm.
‡ Concentration of palladium(II) was 8 p.p.m. and of gold 4 p.p.m.; $\lambda_{max.}$ 480 and 630 nm
§ In the presence of 800 p.p.m. of phosphate and 2000 p.p.m. of tartrate.

directly proportional to the product of molar absorptivity and concentration and the absorbances of the complex and the blue species are additive, it is possible to set up two simultaneous equations:

$$A_{480} = \epsilon_{480}^{Pd}\,[Pd] + \epsilon_{480}^{Au}\,[Au] \quad .. \quad .. \quad .. \quad .. \quad (1)$$

$$A_{630} = \epsilon_{630}^{Pd}\,[Pd] + \epsilon_{630}^{Au}\,[Au] \quad .. \quad .. \quad .. \quad .. \quad (2)$$

where A and ϵ indicate absorbance and molar absorptivity values, respectively, the subscripts indicate wavelength and the superscripts the ion. The solution of these simultaneous

equations and substitution give the concentration of each metal ion in the synthetic mixtures as well as in the solutions of alloys and minerals.

$$[\text{Pd}] \times 10^4 = 3.023\ A_{480} - 0.8403\ A_{630} \quad .. \quad .. \quad .. \quad (3)$$

$$[\text{Au}] \times 10^4 = 0.8177\ A_{630} - 0.1959\ A_{480} \quad .. \quad .. \quad .. \quad (4)$$

Palladium(II) and gold(III) in a solution containing 0.5–15 p.p.m. of palladium and 1–10 p.p.m. of gold can be determined simultaneously using equations (3) and (4). This method can be used for the simultaneous determination of palladium and gold in synthetic mixtures (Table III), palladium - gold alloy and porpezite mineral (Table IV).

The interference of foreign ions on the simultaneous determination of palladium(II) and gold(III) is given in Table II. The precision and accuracy of the method were studied by analysing (five times) a sample containing known amounts of palladium and gold. The results are given in Table III.

TABLE III

SIMULTANEOUS DETERMINATION OF PALLADIUM AND GOLD IN SYNTHETIC MIXTURES

Mixture No.	Metal taken/ mg l^{-1}		Metal found*/ mg l^{-1}		Relative standard deviation, %	
	Pd	Au	Pd	Au	Pd	Au
1	1	10	1.02	9.99	2.30	0.16
2	1	1	1.00	0.99	0.40	1.50
3	5	5	4.99	4.96	0.44	0.46
4	10	5	10.02	4.97	0.28	0.64
5	10	2	10.05	1.97	0.18	2.20
6	12	4	12.23	3.94	0.23	0.77
7	15	1	15.20	0.98	0.05	2.10
8	15	10	15.00	10.07	0.02	0.12

* Average of five determinations.

Applications

Analysis of alloys and minerals of gold and palladium

The results in Table I show that copper in gold - copper - silver alloy does not interfere in the determination of gold. The values obtained with MMH compare favourably with the certified values for gold.

Alloys of jewellery metal (95.5% palladium and 4.5% ruthenium), palladium - gold (50% palladium) and the minerals stibiopalladinite (75% palladium and 25% antimony), sylvanite (7.64% gold, 32.4% silver and 59.96% tellurium) and porpezite (85–90% gold, 6–7% palladium and very small amounts of platinum, rhodium, iron, copper, zinc and lead) were not available for analysis. Hence, synthetic mixtures containing palladium, gold and other metals corresponding to the alloys and minerals were prepared. The palladium and gold contents were determined by following the standard procedure.

Conclusions

No reagent has previously been proposed for the simultaneous spectrophotometric determination of palladium and gold. The proposed MMH reagent has been shown to be suitable. The reagent is more sensitive than phenylthiourea,[7] α-benzyldioxime,[8] dimethylglyoxime and 8-mercaptoquinoline,[9] which can be used for palladium. It is more sensitive than aniline,[10] 1,4-diaminoanthraquinone-2,3-disulphonic acid,[11] hydrobromic acid,[12] nitron[13] and isonicotinic acid hydrazide,[14] which can be used for gold. The major advantage of the proposed method is that the maximum colour intensity is obtained almost instantaneously at room temperature. The proposed method offers the advantages of simplicity, rapidity, sensitivity and reasonable selectivity without the need for an extraction step or heating the solution, and is very useful for the analysis of gold - palladium alloys, which contain gold and palladium in the ratios 1:1 to 4:1.

TABLE IV

DETERMINATION OF PALLADIUM AND GOLD IN SYNTHETIC MIXTURES CORRESPONDING TO ALLOYS AND MINERALS

	Metal taken, p.p.m.		Metal ion added, p.p.m.									Metal found, p.p.m.*	
Mixture	Pd	Au	Ru	Sb	Te	Pt	Rh	Fe	Cu	Zn	Pb	Pd	Au
Jewellery alloy ..	3.0	—	0.13	—	—	—	—	—	—	—	—	3.04	—
	5.0	—	0.24	—	—	—	—	—	—	—	—	4.96	—
	10.0	—	0.48	—	—	—	—	—	—	—	—	9.90	—
	12.0	—	0.57	—	—	—	—	—	—	—	—	12.10	—
Stibiopalladinite mineral	2.0	—	—	0.68	—	—	—	—	—	—	—	1.96	—
	3.0	—	—	1.01	—	—	—	—	—	—	—	3.04	—
	6.0	—	—	2.02	—	—	—	—	—	—	—	5.90	—
	8.0	—	—	2.70	—	—	—	—	—	—	—	8.08	—
Sylvanite mineral ..	—	1.0	—	—	7.85	—	—	—	—	—	—	—	1.02
	—	2.0	—	—	15.70	—	—	—	—	—	—	—	1.96
	—	3.0	—	—	23.55	—	—	—	—	—	—	—	3.05
	—	4.0	—	—	31.40	—	—	—	—	—	—	—	3.92
Pd - Au alloy ..	1.0	1.0	—	—	—	—	—	—	—	—	—	0.98	1.02
	2.0	2.0	—	—	—	—	—	—	—	—	—	1.98	2.02
	3.0	3.0	—	—	—	—	—	—	—	—	—	2.98	2.96
	4.0	4.0	—	—	—	—	—	—	—	—	—	4.04	3.96
	5.0	5.0	—	—	—	—	—	—	—	—	—	4.90	5.04
Porpezite mineral ..	0.62	7.0	—	—	—	0.038	0.038	0.038	0.038	0.038	0.038	0.61	7.10
	0.70	9.0	—	—	—	0.050	0.050	0.050	0.050	0.050	0.050	0.69	9.06
	0.88	10.0	—	—	—	0.056	0.056	0.056	0.056	0.056	0.056	0.87	10.12

* Average of five determinations.

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Application of an Automatically Triggered Digital Integrator to Flame Atomic-absorption Spectrometry of Copper Using a Discrete Nebulisation Technique

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A signal integrator with an automatic trigger and a voltage to frequency (V/F) counter was applied to the flame atomic-absorption spectrometry of copper using a discrete nebulisation technique. When an injection volume larger than 50 μl was used, there was a good linear relationship between the absolute amount of copper and the integrated value, irrespective of the copper concentration and the injection volume. There was also a linear relationship between the ratio of the integrated value to the aspiration time and the copper concentration, irrespective of the injection volume. The relative standard deviation of the measurement was less than 4%, even with an injection volume of 20 μl. This method was applied to the determination of copper in standard biological samples (NBS-SRM 1577 Bovine Liver and 1566 Oyster Tissue). The results agreed well with the certified or reported values.

Keywords: *Automatic integration*; *atomic-absorption spectrometry*; *copper determination*; *biological standards*; *discrete nebulisation*

Flame atomic-absorption spectrometry with discrete nebulisation of sample solutions of less than 100 μl has been studied in detail by many workers[1–4] and applied successfully to the analysis of several elements in various samples.[4–6] However, measurement of the peak height is time consuming and the sensitivity is often too low to determine trace amounts of metals. Thus, it is preferable to integrate automatically the peak area of the spike-like signal. However, the precision was not as good as that in peak-height measurements.[7] Recently, it was claimed that satisfactory results were obtained by automated multiple flow injection analysis,[8] where, however, an additional device was necessary for inserting the sample solution into a continuous solvent stream.

In our laboratory, a digital integrator automatically triggered by the electrical conductivity of the nebulised sample solution was constructed.[9] This paper deals with the application of the integrator to the determination of copper in small amounts of biological samples. The effects of injection volumes and sample flow-rates on peak height, aspiration time, integrated value and the ratio of the integrated value to the aspiration time were investigated.

Experimental

Apparatus

A Hitachi, Model 518, atomic-absorption spectrophotometer was used, with a burner head of 10 cm for the air - acetylene flame. The signal intensity was recorded on a strip-chart recorder (Rikadenki B-25L, Mark II), which had a fast response. The flow-rates of the nebuliser and auxiliary air were controlled separately, with a total fixed flow-rate of 16.0 l min^{-1}. Therefore the sample flow-rate could be varied with a fixed nebuliser. The wavelength used was 324.8 nm. The concentration of copper was measured, under normal operating conditions, with an air - acetylene flame: flow-rate of acetylene, 3.0 l min^{-1} (0.5 kg cm^{-2}); flow-rate of air, 4.5 l min^{-1} (1.5 kg cm^{-2}) for the nebuliser and 11.5 l min^{-1} (1.5 kg cm^{-2}) auxiliary; burner height, position 2.5; and sample flow-rate, 4.1 ml min^{-1}. A small PTFE funnel with a platinum electrode is coupled directly to the nebuliser needle (platinum tube), which is the counter electrode (Fig. 1). The sample solution injected into the funnel is thus nebulised into the flame. When the channel between the platinum electrodes was filled with the sample solution,

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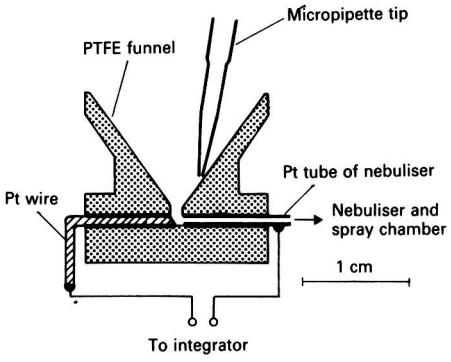

Fig. 1. PTFE funnel and platinum electrode.

the electrical conductivity between both the electrodes acts as an automatic trigger.[9] The signal was integrated with a V/F counter and the value (counts) was displayed digitally. The integration time was pre-set so as to include the aspiration time (Fig. 2). The aspiration time, which is the time required for the sample solution to pass through the channel, was also measured and displayed digitally. Micropipettes (Gilson P5000, P1000 and P200) were used for dilution and injection.

Reagents

Copper stock solution (2000 p.p.m. *in* 0.5 M *nitric acid.* Copper metal of greater than 99.999% purity from Mitsuwa Pure Chemicals Co. Ltd. was dissolved in concentrated nitric acid and diluted to 100 g with doubly distilled water. Working solutions were prepared by diluting the stock solution by mass, with doubly distilled water, nitric acid and perchloric acid using micropipettes, to appropriate concentrations in 10-ml polypropylene bottles. These solutions should contain the same concentrations of nitric and perchloric acids as the sample solution. A small amount of sample was decomposed with an acid mixture (nitric acid plus perchloric acid) in a sealed PTFE bomb and diluted to an appropriate mass.[6]

Results and Discussion

Effect of Injection Volume on Integration Value

Complete integration of the area of the spike-like signal was initiated by the automatic trigger; an analogue signal was pre-amplified to a suitable level, the signal converted into a corresponding series of pulses by a voltage to frequency counter and then the pulses were counted with a digital counter. The background signal, caused by the flame without sample

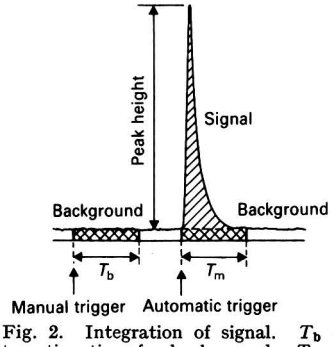

Fig. 2. Integration of signal. T_b = Integration time for background; T_m = integration time for total signal (= T_b).

aspiration, was integrated in the same manner using a manual starting switch. A net integrated value of the signal was obtained by subtracting the background value from the total signal value (Fig. 2).

Fig. 3 gives the results together with the aspiration times. The peak height is also plotted against the injection volume (broken line).

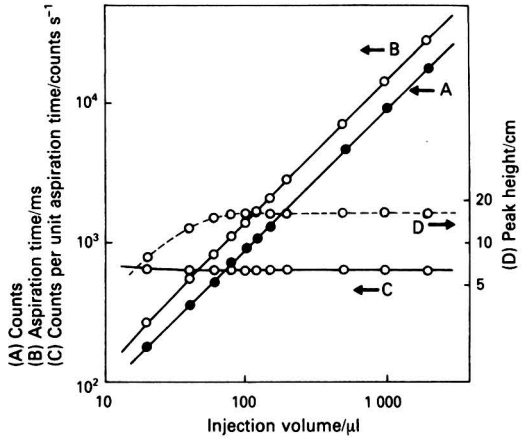

Fig. 3. Effect of injection volume on four variables. Sample flow-rate, 4.1 ml min^{-1}; copper concentration, 3.0 p.p.m. (0.1 M nitric acid).

The integrated value (counts) increases linearly with increase in the injection volume of a constant concentration standard copper solution. When a constant injection volume is used, there is a linear relationship between the integrated value and the copper concentration, as shown in Fig. 4(*a*), the lines passing through the origin. As expected, the slopes of the calibration graphs increase with increase in the injection volume. The straight-line graphs indicate that the integrated value is proportional to the absolute amount of copper.

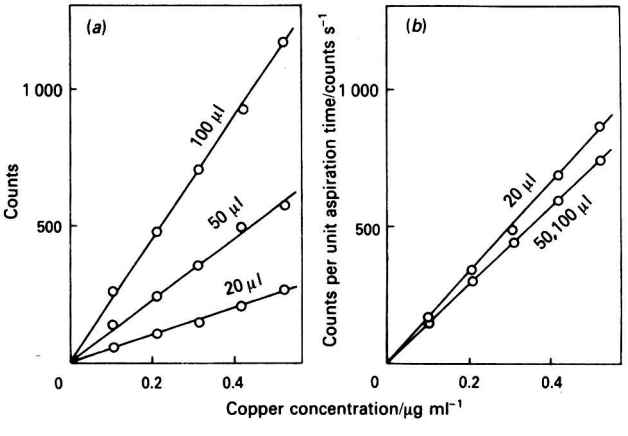

Fig. 4. Calibration graphs for copper. (*a*), Integrated value; and (*b*), ratio of integrated value to aspiration time.

Effect of Injection Volume on the Ratio of the Integrated Value to the Aspiration Time

The aspiration time increases linearly with increase in the injection volume (Fig. 3), as does the integrated value. Therefore, the ratio of the integrated value to the aspiration time is constant, irrespective of the injection volume when it is more than about 50 μl. In other words, this ratio is dependent only upon the concentration of copper in the sample solutions. With an appropriate injection volume of larger than 50 μl, a single linear calibration graph is obtained, which passes through the origin, as shown in Fig. 4(*b*). A linear calibration graph, which passes through the origin, is also obtained when a fixed injection volume of less than 50 μl is used, regardless of the fact that the integrated value and the aspiration time are not linear with respect to the injection volume.

Reproducibility

Table I shows the reproducibility of measurements of the variables studied, obtained with injection volumes of 20, 50 and 100 μl and copper concentrations of 4.0 and 0.5 p.p.m. The relative standard deviation of each variable obtained with injection volumes of 50 and 100 μl is small and better than that obtained by continuous aspiration. Even with an injection volume of 20 μl, the relative standard deviation of each variable is less than 4%. Hence, the method is reproducible and satisfactory. Whenever a suitable automatic sample injector is used instead of manual injection, the reproducibility will be improved further.

TABLE I

REPRODUCIBILITY OF VARIABLES

The number of measurements of each variable was 15.

Variable	20-μl injection volume	RSD*, %	50-μl injection volume	RSD*, %	100-μl injection volume	RSD*, %
Copper 4.0 p.p.m.; *no signal expansion*—						
Aspiration time/ms	259	3.7	655	1.4	1328	0.8
Peak height/mm	111	3.5	198	1.1	225	0.5
Counts	265	3.5	574	0.9	1163	0.5
Counts per unit aspiration time/counts s^{-1}	1021	1.5	877	0.8	876	0.6
Copper 0.5 p.p.m.; *signal expansion* ~ ×7—						
Aspiration time/ms	292	1.9	753	1.7	1517	1.9
Peak height/mm	126	1.7	187	1.3	198	1.1
Counts	236	2.3	592	0.5	1159	1.0
Counts per unit aspiration time/counts s^{-1}	811	3.4	786	1.4	764	1.2

* RSD = relative standard deviation.

Effect of Sample Flow-rate

The sample flow-rate was varied by changing the flow-rate of air for the nebuliser, as already described. The effect of sample flow-rate on the four variables was investigated using a constant injection volume of 100 μl. The results are shown in Fig. 5. With increase in the sample flow-rate, the peak height increases but the aspiraton time decreases. The integrated value is almost constant over the whole range of sample flow-rates, 2.1–4.6 ml min^{-1}. The value is highest and essentially constant in the range 3.5–4.0 ml min^{-1}. This is mainly due to the nebulisation of the same absolute amount of copper. Thus, the ratio of the integrated value to the aspiration time increases with increase in the sample flow-rate, because of the decrease in the aspiration time. These results show that both the peak height and the ratio of the integrated value to the aspiration time increase with the increase in sample flow-rate, irrespective of the injection volume. It is also seen that the integrated value depends only on the absolute amount of the copper, irrespective of the sample flow-rate and the injection volume.

Determination of Copper in Standard Biological Samples

The method was applied to the determination of copper in NBS-SRM 1577 Bovine Liver and 1566 Oyster Tissue. The sample was decomposed in a sealed PTFE vessel with a mixture of nitric and perchloric acids (5 + 1) for 3 h at 130 °C and diluted, by mass, with water. The

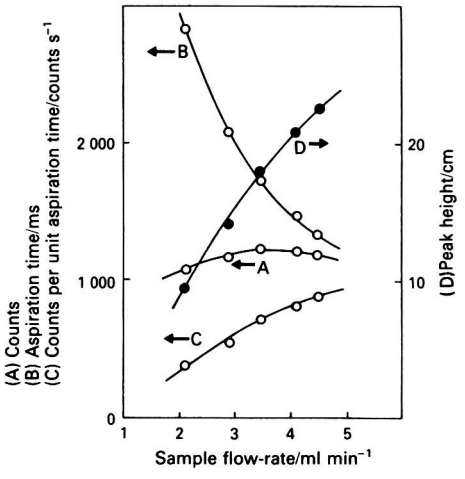

Fig. 5. Effect of sample flow-rate on four variables. Injection volume, 100 μl; copper concentration, 4 p.p.m.; integration time, 3 s; signal expansion, zero.

details of sample and standard solution preparation were given in a previous paper.[6] The results are given in Table II with the data obtained for various sample sizes. The results agree well with one another and also with the certified value, or the previously reported value.[6] When different injection volumes are used the results are still in good agreement with one another.

Application of the Absolute-amount Method

As already mentioned, the integrated value is proportional to the absolute amounts of analyte in the injection volume. Thus, the integrated value is constant when the product of the concentration and the injection volume is constant, even if both factors vary with each other. The results are given in Table III. This fact enables the copper content in the sample solutions to be determined when the concentration is below the lower limit of the calibration graph, by increasing the injection volume and consulting the same calibraton graph. A typical example is shown in Fig. 6. If a 0.0155 p.p.m. copper solution is injected (a on calibration graph B in Fig. 6) the copper content cannot be determined by the peak-height method,

TABLE II

DETERMINATION OF COPPER IN BIOLOGICAL STANDARDS

Sample	Sample mass/mg	Final mass/g	Injection volume/μl	Copper content found, p.p.m. Peak-height method	Count method	Counts per unit aspiration time method
NBS Bovine Liver ..	1.0	0.5	100	186	188	186
	1.0	0.5	50	190	187	185
	2.5	2.5	100	189	191	189
	5.0	2.5	100	189	190	19.1
NBS Oyster Tissue ..	1.0	0.5	100	62.7	62.2	61.3
	1.0	0.5	50	63.7	61.9	59.9
	1.0	0.5	20	65.0	64.0	—
	5.0	2.5	100	61.0	60.4	59.8

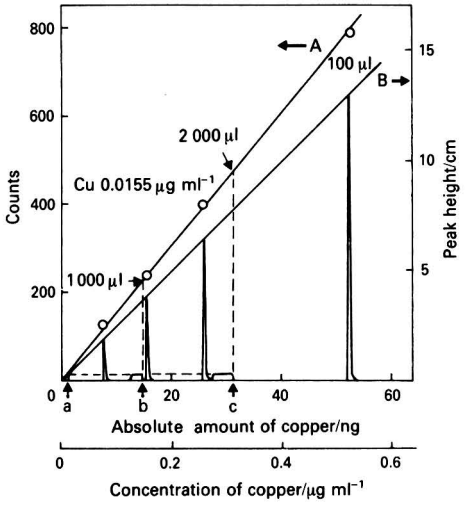

Fig. 6. Determination of copper by absolute amount method. Sample flow-rate, 4.1 ml min^{-1}. A, Counts *vs.* absolute amount of copper; and B, peak height *vs.* concentration of copper.

but it is determined exactly by the integrated-value method (absolute-amount method) by use of a 1000- or 2000-µl solution (b or c on calibration graph A in Fig. 6). This method was applied to the determination of copper in some biological standards. The results are given in Table IV.

The results obtained with different injection volumes are in good agreement with one another for the copper standards. The same results are also obtained from the calibration graph constructed with the different injection volumes. The results for the biological standards are also in good agreement with the certified value or the value previously reported.[6]

Conclusions

The integration of the peak area is more useful and faster than the peak-height measurements usually carried out. It is possible in practice to carry out 360 determinations per hour with the injection of 100 µl of sample solution, and 10–50 determinations per millilitre of

TABLE III

INTEGRATED VALUES WITH CONSTANT ABSOLUTE AMOUNT OF COPPER

Copper concentration, p.p.m.	Injection volume/µl	Integrated value for 156 ng of copper	Integration time/s
0.0781	2000	678	33
0.156	1000	672	17
0.260	600	672	9
0.521	300	675	7
0.779	200	678	6
1.04	150	676	6
1.56	100	675	4
2.09	75	669	4
2.60	60	665	4
3.14	50	659	4

TABLE IV

DETERMINATION OF COPPER BY ABSOLUTE-AMOUNT METHOD

The values in parentheses are standard deviations.

Sample	Copper concentration, p.p.m.	Copper concentration found, p.p.m.		Calibration graph	
		2000-μl injection volume	1000-μl injection volume	Concentration, p.p.m.	Injection volume/μl
Copper standard solution	0.0155	0.0157	0.0149	0–0.8	100
	0.0781	0.079	—	0–3	100
	0.156	—	0.154	0–3	100
	0.053	0.054	—	0–4	50
		0.058	—	0–4	100
		0.054	—	0–4	200
NBS Bovine Liver, 5 mg → 2.5 g‡ ..	0.386* (±0.020)		0.394 (±0.003)	0–4	100
NBS Oyster Tissue, 5 mg → 2.5 g‡ ..	0.126†		0.128 (±0.002)	0–4	100

* The value calculated from the certified value.
† The value calculated from the previous data (see reference 6).
‡ Sample of 5 mg was decomposed and diluted to a final solution mass of 2.5 g.

sample solution with injection volumes of 20–100 μl. Whenever an automatic sample injector and automatically triggered digital integrator and a data processing device are combined with the conventional atomic-absorption spectrophotometer, a rapid, reproducible and automatic atomic-absorption spectrometric determination would be possible using the discrete nebulisation technique.

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Determination of "Inorganic" Arsenic(III) and Arsenic(V), "Methylarsenic" and "Dimethylarsenic" Species by Selective Hydride Evolution Atomic-absorption Spectroscopy

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A technique for the determination of "inorganic" arsenic(III) and arsenic(V), "methylarsenic" and "dimethylarsenic" species is described which is based on the trapping of arsines and selective volatilisation into a heated quartz atomiser tube situated in the optical path of an atomic-absorption spectrometer. Improved reproducibility is obtained by the use of a continuous flow reduction stage and detection limits are approximately 0.25 ng (based on twice the standard deviation of 10 blank measurements). For a typical sample volume of 10 ml this corresponds to a detection limit of 0.025 ng ml^{-1} of arsenic. Interferences were investigated and depression of results was observed in the presence of silver(I), gold(III), chromium(VI), iron(II), iron(III), germanium(IV), molybdenum(VI), antimony(III), antimony(V), tin(II), manganese(VII) and nitrite. Various approaches to overcoming such interferences were investigated, and for general use masking with EDTA is advocated. The choice of extraction procedures for speciation analysis is discussed.

Keywords: *Arsenic determination*; *"methylarsenic" species*; *atomic-absorption spectroscopy*; *hydride generation*; *environmental analysis*

Arsenic is a relatively common element, existing in natural systems in a variety of chemical forms, including "inorganic" arsenic(III) and arsenic(V), and several mono-, di- and trimethylated arsenic compounds, frequently described as "methylarsenic," "dimethylarsenic" and "trimethylarsenic."[1] The development of techniques for the separate analysis of these species is necessary owing to the widely differing toxicities of the arsenic species,[2] and a need to understand the metabolic processes involved in the interconversion of such forms.

The differentiation of "inorganic" arsenic(III) and arsenic(V) can be achieved by exploitation of the pH sensitivity of the reduction of arsenic compounds by sodium tetrahydroborate(III), as adapted to analyses by atomic-absorption spectroscopy[3] and the silver diethyldithiocarbamate spectrophotometric procedure.[4] An alternative to pH control of arsine production involves suppresssion of arsenic(V) reduction by the addition of dimethylformamide to the reduction mixture.[5] Separation of arsenic(III), arsenic(V) or "organic" arsenic by solvent extraction has been described by Yasui *et al.*[6]

The analysis of the methylarsenicals necessitates the use of chromatographic or distillation procedures to separate the compounds of interest, linked to sensitive and specific detection methods. A method for the separation of arsenicals by ion-exchange chromatography has been described by Yamamoto[7] for the determination of organoarsenic compounds found in water and sediment extracts. Typical gas-chromatographic methods involve conversion of "inorganic" and methylated arsenic compounds into their diethyldithiocarbamate complexes[8] or trimethylsilyl derivatives.[9,10] The approach of Braman and co-workers,[11,12] however, involves the trapping of arsines formed by reduction with sodium tetrahydroborate(III), and their subsequent sequential volatilisation into an electric discharge emission detector. The arsine trapping procedure has been modified by other workers to include detection by graphite tube furnace[13] and flame[14] atomic-absorption spectroscopy.

The techniques reported to date for the differentiation of "inorganic" arsenic(III), arsenic(V), "methylarsenic" and "dimethylarsenic" are typically insensitive, of poor reproducibility or require the construction of sophisticated detection systems. Although the trapping and

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sequential evolution of arsines is an elegant procedure for the isolation and separation of the fragments of interest, precision is frequently poor owing to the manual addition of reagents. Additionally, information on interferents is limited although many procedures for the determination of "total" arsenic by hydride generation atomic-absorption spectroscopy have been found to suffer from severe interference problems occurring in the arsine generation step.[15,16]

This paper describes the development of an analysis for "inorganic" arsenic(III), arsenic(V), "methylarsenic" and "dimethylarsenic" based on sodium tetrahydroborate(III) reduction, trapping and detection by atomic-absorption spectroscopy using a quartz-tube atomiser. Good reproducibility at low levels is achieved by replacing manual mixing by a pumped reagent system. Interferences were investigated and methods for overcoming such effects are discussed.

Experimental

Reagents and Glassware

All chemicals were of analytical-reagent grade unless otherwise stated. Glassware was soaked in nitric acid (1 + 10), rinsed with distilled water and dried before use.

Stock solutions containing 1000 μg ml^{-1} of arsenic were prepared from arsenic(III) oxide, disodium hydrogen arsenate (Na_2HAsO_4), methylarsonic acid (disodium salt) and dimethylarsinic acid (sodium salt) and were checked for arsenic content by flame atomic-absorption spectroscopy using an air - acetylene flame. Solutions of lower concentration were prepared by dilution of the stock solutions immediately before use. Laboratory-reagent grade sodium tetrahydroborate(III) was used to prepare an unstabilised 2% *m/V* solution in distilled water, which was filtered before use. Buffer solution (pH 5.0) was prepared by adjusting sodium acetate solution (0.1 M) to the appropriate pH with acetic acid. A solution (0.002 M) of ethylenediaminetetraacetic acid (EDTA), as the disodium salt, was prepared in 0.1 M acetic acid.

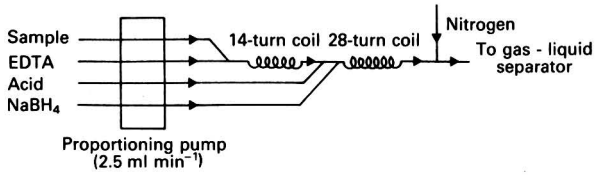
Apparatus

The apparatus can be conveniently considered to consist of three distinct sections: the arsine generator, the trapping section and the detector. The arsine generator is constructed mainly from standard Technicon AutoAnalyzer components and is illustrated in Fig. 1(*a*). Sample solution is mixed with acid or buffer and is then reduced with sodium tetrahydroborate(III) solution during passage through a mixing coil (Technicon, Part No. 105–0083). The resultant gas - liquid mixture is transported by a carrier gas stream of nitrogen to a custom-made gas - liquid separator from where the gas stream passes through a lead acetate scrubber, to remove residual hydrogen sulphide, and then through drying agent to the trap [Fig. 1(*b*)]. The addition of EDTA to the sample solution is achieved by the insertion of an additional 14-turn mixing coil (Technicon, Part No. 105–0082) and reagent line into the system prior to the acidification of the sample. The trap is constructed from borosilicate glass, the wide-bore arm of which is filled with glass beads (approximately 40-mesh). Design and preliminary silanisation of the trap components with trimethylchlorosilane are crucial to the sensitivity and resolution of the system.

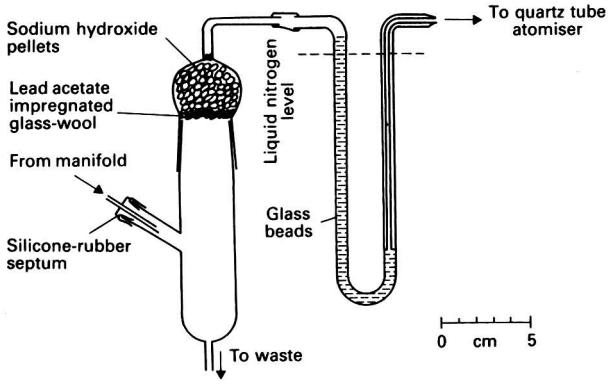
Following condensation of arsines at −196 °C, the trap is allowed to warm to room temperature when the arsines volatilise in order of increasing boiling-point and are swept into the atomisation cell. The atomiser is a quartz tube (Fig. 2) heated by a conventional 10-cm path length air - acetylene flame[17] aligned in the light path of a background-corrected atomic-absorption spectrometer (Varian Techtron AA175AB fitted with an EMI 9783B photomultiplier giving extended response to ultraviolet radiation). The atomiser is of a T-piece design with the inlet tube protected from heat and accidental breakage by a concentric quartz tube, which is left open to the atmosphere to prevent pressure build-up. The following spectrometer conditions were used throughout the work: light source, arsenic hollow-cathode lamp; wavelength, 193.7 nm; spectral band pass, 1 nm; and damping time constant, 1.5 s. The spectrometer output was monitored on a chart recorder (Tekman TE200) having a response time of 0.3 s (full-scale deflection).

Instrumental Operation

With the carrier gas flow-rate set to 200 ml min^{-1}, the trap is cooled to −196 °C by submersion in liquid nitrogen for 2 min. The sampling tube is placed in the sample and the peristaltic pump is started. When the sample solution has been completely consumed, the sampling



(*a*) Reduction manifold



(*b*) Gas - liquid separator and trap

Fig. 1. Apparatus for the generation and trapping of arsines by reduction with sodium tetrahydroborate(III).

tube is placed in distilled water and pumping is continued for 7–8 min. The trap is then allowed to warm to room temperature whilst the absorbance of the evolved gases is monitored at 193.7 nm. During the evolution of the trapped arsines it is possible to stop the pump to conserve reductant solution. In the event of such action, however, the sensitivity of the analysis is reduced as poor atomisation results from the absence of air - hydrogen flames at the ends of the atomisation tube. The volatilisation is insensitive to the temperature fluctuations encountered in the normal laboratory environment.

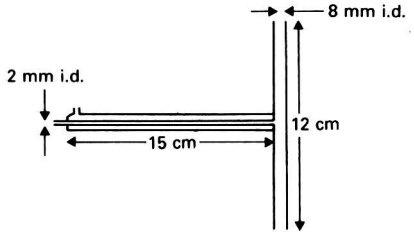


Fig. 2. Construction details of quartz atomiser cell.

Sample Digestion

The sample (approximately 1 g) is weighed into a boiling-tube (15 × 2.5 cm i.d.) and concentrated hydrochloric acid (35% *m/m*) (10 ml) is added. The mixture is heated overnight at 65–70 °C, filtered (Whatman GF/A glass-fibre filter-papers) and made up to 25 ml with concentrated hydrochloric acid.

Caution—Care should be exercised when shaking the calibrated flasks containing concentrated hydrochloric acid owing to the possible build-up of excessive gas pressure.

Results

Reduction Conditions

The conditions required for the efficient reduction of arsenic compounds to their corresponding arsines have been discussed previously.[17] Using a pumping rate of 2.5 ml min^{-1} for sample reagents, effective reduction was achieved with a 2% *m/V* sodium tetrahydroborate(III) solution in the presence of hydrochloric acid (1 M). The system was purged by pumping distilled water through the sample input tube for 7 min between samples, this time being governed by the time required to cool and warm the trap during the volatilisation step. Additionally, the extended wash period overcomes potential cross-contamination of samples. Under such conditions the analysis time is approximately 10 min per sample.

The reduction of "inorganic" arsenic(III) and arsenic(V) species can be controlled by suitable adjustment of the pH under which the reduction is performed.[18] At pH 5 arsine is produced solely from arsenic(III), whilst in the presence of 1 M hydrochloric acid, arsine is produced from both arsenic(III) and arsenic(V). By performing the reduction at two pH values it is therefore possible to determine "inorganic" arsenic(III) and "total inorganic arsenic," and hence "inorganic" arsenic(V) by difference. Analysis of 12 synthetic mixtures containing 5–50 ng of arsenic(III) and arsenic(V) gave recoveries of 98.0 ± 1.5% and 99.0 ± 1.0% for arsenic(III) and arsenic(V), respectively.

Carrier Gas

The carrier gas flow-rate influences both the sensitivity and resolution of the analysis by affecting the steady-state arsenic concentration in the atomiser cell and the rate of arsine volatilisation. The effect on sensitivity of varying carrier gas flow-rate over the range 0–450 ml min^{-1} is demonstrated in Fig. 3. The peak height for arsenic (III) or -(V), "methylarsenic"

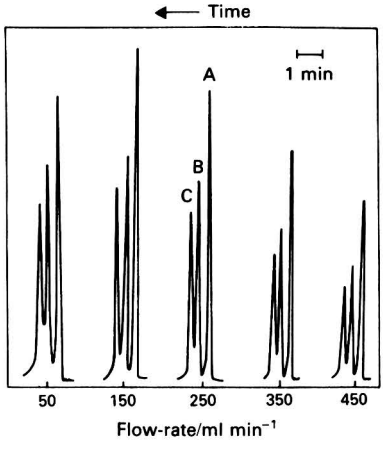


Fig. 3. Typical arsine signals under conditions of varying carrier-gas flow-rates: (A) arsenic(III) or arsenic(V); (B) "methylarsenic"; and (C) "dimethylarsenic."

and "dimethylarsenic" decreased with increasing flow-rate whereas instrument stability and resolution improved with increasing gas flow-rates. The optimum flow-rate was considered to be 150 ml min^{-1}.

In order to prevent excessive condensation of water in the trap it was found necessary to dry the gas stream before it entered the trap. Of the various drying agents that were tried (orthophosphoric acid, sulphuric acid, silica gel, calcium oxide, magnesium perchlorate and sodium hydroxide) only sodium hydroxide did not absorb the arsines. Following replacement of the desiccant and drying of the trap, poor recovery of the arsines is experienced, and the apparatus must be conditioned by use until it functions reproducibly (normally after three or four sample injections).

Sample Volume

Over the studied range of sample sizes (2.5–20 ml) the response for all arsenic species was found to drop slightly with increasing volume (Fig. 4). In view of this, calibrants and samples were of a measured standard volume for each set of analyses.

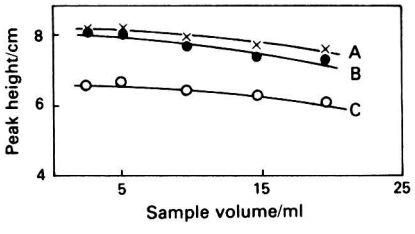


Fig. 4. Influence of sample volume on peak heights for arsenic (10 ng) as (A) arsenic(III) or arsenic(V), (B) "methylarsenic" and (C) "dimethylarsenic."

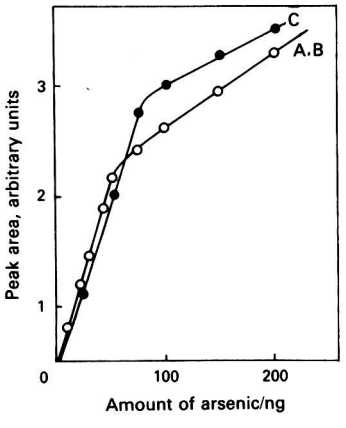


Fig. 5. Typical calibration graphs for (A) arsenic(III) or arsenic(V), (B) "methylarsenic" and (C) "dimethylarsenic."

Calibration, Reproducibility and Detection Limits

Calibration is linear for all four species over the range 0–50 ng of arsenic (corresponding to concentrations of 0–10 ng ml^{-1} of arsenic for a 5-ml sample) (Fig. 5). Above 50 ng, however, an unexplained transition is evident to a region in which peak area is still linearly related to arsenic concentration, but with reduced sensitivity. Based on twice the standard deviation of 10 blank measurements the detection limit of the technique is approximately 0.25 ng of arsenic as arsenic(III), arsenic(V), "methylarsenic" or "dimethylarsenic." Reproducibility was studied by the replicate analyses of 25 standard solutions containing 10 and 50 ng of arsenic as the four arsenic species (Table I).

TABLE I

DETECTION LIMIT AND REPRODUCIBILITY

Arsenic species	Detection limit/ng	Relative standard deviation, %	
		10 ng of arsenic	50 ng of arsenic
Arsenic(III) (pH 5) ..	0.62	7.3	3.2
Arsenic(V)	0.23	6.9	2.8
"Methylarsenic"	0.26	3.5	2.7
"Dimethylarsenic"	0.25	3.8	1.9

Interferences

The influence of foreign ions on the analysis was assessed by the omission of EDTA from the reaction mixture and the determination of 20 ng of arsenic in the presence of 5 ml of standard solutions of the potential interferent. Depression of results was observed in the presence of silver(I), gold(III), chromium(VI), iron(II), iron(III), germanium(IV), molybdenum(VI), antimony(III), antimony(V), tin(II), manganese(VII) and nitrite (Table II). Those elements which gave rise to a signal depression of less than one standard deviation of the expected response were deemed not to interfere. No effects were observed for up to 10 μg ml^{-1} of aluminium(III), bismuth(III), calcium(II), cadmium(II), cobalt(II), copper(II), mercury(II), manganese(II), magnesium(II), sodium(I), lead(III), selenium(IV), selenium(VI), vanadium(IV) and tellurium(IV); up to 1000 μg ml^{-1} of perchlorate, bromide, iodide, nitrate, sulphate or cysteine hydrochloride did not interfere.

TABLE II

SPECIES INFLUENCING ARSINE EVOLUTION FROM 20 ng OF ARSENIC AS ARSENIC(III) AND ARSENIC(V), "METHYLARSENIC" AND "DIMETHYLARSENIC"

Ion	Concentration of ion/μg ml^{-1}	Relative arsenic response, %			
		As(III)	As(V)	"Methyl-arsenic"	"Dimethyl-arsenic"
Ag(I)	10	63	66	63	2
Au(III)	1	53	58	26	35
Cr(VI)	10	99	100	73	33
Fe(II)	10	100	100	88	78
Fe(III)	10	98	100	86	81
Ge(IV)	1	29	31	21	30
Mo(VI)	10	101	99	90	94
Ni(II)	1	89	89	54	73
Pt(IV)	1	38	43	22	35
Sb(III)	1	87	91	39	68
Sb(V)	1	75	88	52	69
Sn(II)	10	100	99	62	81
MnO_4^-	1000	98	100	38	14
NO_2^-	100	101	100	98	73

All observed interferences, except those due to antimony, gold(III) and nitrite, were masked by the pumped addition of 0.02 M EDTA (the pH adjusted to 3 with dilute hydrochloric acid) to the sample prior to the reduction step (Table III). In the presence of a 5000-fold excess of iron(III), however, depression of the dimethylarsenic signal cannot be completely restored by EDTA (Table IV). Extraction of the sample solution (at pH 2.0) with a 0.005 M solution of dithizone (diphenylthiocarbazone) in dichloromethane overcomes interferences due to silver(I),

TABLE III

ASSESSMENT OF METHODS FOR OVERCOMING INTERFERENCE EFFECTS

Interferent	Relative arsenic response, %											
	Dithizone extraction				Chelex 100				EDTA masking			
	As(III)	As(V)	"Methyl-As"	"Dimethyl-As"	As(III)	As(V)	"Methyl-As"	"Dimethyl-As"	As(III)	As(V)	"Methyl-As"	"Dimethyl-As"
Ag(I)	100	100	99	100	99	100	99	100	100	100	99	101
Au(III)	99	99	99	98	99	99	100	100	26	22	20	31
Cr(VI)	99	100	99	99	100	99	99	100	100	98	100	99
Fe(II)					84	89	88	78	100	100	100	101
Fe(III)					87	88	86	81	99	100	98	98
Ge(IV)	19	23	17	39	102	99	100	100	98	98	98	99
Mo(VI)	99	98	100	99	98	99	99	99	101	101	99	99
Ni(II)	89	89	54	73	100	99	100	99	99	99	100	100
Pt(IV)	100	100	58	62	98	99	98	98	100	100	99	99
Sb(III)					99	100	100	101	65	85	21	53
Sb(V)					99	100	98	98	62	83	27	48
Sn(II)	100	102	101	100	101	99	99	100	100	101	100	99

gold(III), chromium(VI), molybdenum(VI) and tin(II). An alternative to removal of gold(III) by extraction with the dithizone solution involves masking by the addition of thiosemicarbazide solution to make the sample (at pH 3) 0.01 M in thiosemicarbazide.

TABLE IV

EFFECT OF 5000-FOLD EXCESS OF IRON ON THE DETERMINATION OF 10 ng OF ARSENIC

	Relative arsenic response, %			
	Unmasked		EDTA masking	
	Fe(II)	Fe(III)	Fe(II)	Fe(III)
As(III)	91	102	95	98
As(V)	93	98	97	97
"Methylarsenic"	81	15	99	98
"Dimethylarsenic"	0	0	103	71

An attractive approach to the removal of interferents involves the use of the chelating ion-exchange resin Chelex 100. With the exception of iron(II) and iron(III) all identified cationic interferents can be overcome by passing the sample solution (at pH 4.0) through a column (15 × 1 cm i.d.) of Chelex 100 resin (100–200 mesh, Na^+ form). The ubiquitous nature of iron, however, precludes the application of such a procedure in most practical circumstances (see also below).

Analysis of Samples

The described procedure has been applied to the speciation analysis of arsenic in estuarine samples, following the hydrochloric acid digestion procedure described previously. Some typical results are given in Table V, which clearly demonstrate the marked distinction of arsenic species present in environmental samples. It is to be noted that in molluscs and algae the predominant species is dimethylarsenic due to biological methylation reactions occurring in the food chain. The water samples, however, reflect the contrasting geological surroundings of the sampling sites together with a small concentration of dimethylarsenic derived from planktonic conversion and decaying organic material.

Discussion and Conclusions

The instrument described provides a simple extension of the range of applicability of a standard atomic-absorption spectrometer to the analysis of methylarsenic compounds. The modification is simply carried out using standard auto-analysis components, a custom-made gas - liquid separator and a quartz atomiser tube. At low pH, arsines are quantitatively produced from "inorganic" arsenic(III) and arsenic(V), "methylarsenic' and "dimethylarsenic" compounds, whereas at pH 5 with arsenic(III) and arsenic(V), only the former is reduced. It is therefore possible to determine both arsenic(III) and arsenic(V) together with the studied methylarsenicals. Detection limits of approximately 0.25 ng for "inorganic" arsenic(III) and arsenic(V), "methylarsenic" and "dimethylarsenic," reproducibility and availability of apparatus compare favourably with alternative techniques.[12,19] The pumped reagent system gives rise to improved precision and convenience when compared with methods depending on the manual addition of reductant to the sample solution.

TABLE V

ANALYSIS OF ESTUARINE SAMPLES

Results are in micrograms per gram unless otherwise stated.

Sample type	Description	Source	Total arsenic(III) and arsenic(V)	As(III)	As(V)	"Methyl-arsenic"	"Dimethyl-arsenic"
Mollusc	*Nucella lapillus*	Brean Down, Somerset	0.99 ± 0.04	—	—	0.26 ± 0.01	1.26 ± 0.04
	Patella vulgata	Brean Down, Somerset	0.17 ± 0.01	—	—	0.03 ± 0.01	0.27 ± 0.01
	Littorina littorea	Southsea, Hampshire	1.59 ± 0.07	—	—	0.29 ± 0.01	1.74 ± 0.04
Algae	*Fucus vesiculosis*	Hurst Spit, Dorset	0.20 ± 0.01	—	—	0.05 ± 0.01	2.79 ± 0.08
River water ..		Beaulieu River, Hampshire	—	0.10 μg l^{-1}	1.02 μg l^{-1}	0.06 μg l^{-1}	0.23 μg l^{-1}
		Restronguet Creek, Cornwall	—	1.6 μg l^{-1}	19.4 μg l^{-1}	<0.2 μg l^{-1}	<0.2 μg l^{-1}

Several ions have been found to influence the analysis of arsenic compounds by arsine generation techniques. Depression of results can occur as a consequence of consumption of reductant by the interferent, slowing of reduction kinetics or effects resulting from the formation of other volatile hydrides, such as hydrogen selenide. The pumped reagent system maintains continuous mixing of the sample with a concentrated reductant solution, hence suppressing depression of results that may arise from consumption of reductant by interfering species. The delay between mixing of sample with reductant and the trapping of arsines ensures efficient reduction of arsenic compounds in the presence of interferents that give rise to reduced reduction rates.

Several procedures have been investigated for overcoming interferences, the most generally applicable of which is the pumped addition of EDTA to the sample solution before the reduction stage. The use of the chelating ion-exchange resin Chelex 100 for the removal of interfering species is limited by the ability of the resin to retain a proportion of the arsenic from solutions containing iron. Solvent extraction of interferents as their dithizonates offers little improvement over masking with EDTA and leads to a significant increase in the time required for sample preparation.

One of the most important stages in the speciation analysis of arsenic compounds is the extraction of the arsenicals from the sample. Oxidising acids and dry-ashing procedures are to be avoided as they fail to retain the original speciation of "inorganic" arsenic(III) and arsenic(V), whilst care must be taken to minimise losses of arsenic(III) chloride when using hydrochloric acid. Within such constraints, the most viable available alternative is wet ashing of biological material with warm hydrochloric acid. Under such conditions there is no evidence of de-methylation of the simpler methylarsenic anions, but until the structures of naturally occurring organoarsenicals have been elucidated the effect of acid treatment on the compounds of interest cannot be assumed. With samples requiring more vigorous digestion procedures, such as sediments, arsenic speciation can only be currently assessed by acidic extraction, as more thorough conventional digestion procedures destroy the original speciation of the element.

In conclusion, it is possible to construct a highly sensitive instrument for the determination of "inorganic" arsenic(III) and arsenic(V), "methylarsenic" and "dimethylarsenic" by simple modification of a conventional atomic-absorption spectrometer. In use, the apparatus, being convenient to operate and highly reproducible at trace levels, has proved to be a valuable tool in the investigation of arsenic metabolism and speciation in the marine environment.

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Studies in Chemical Phase Analysis

Part II.* Determination of the Solubilities of Carbides, Nitrides, Oxides and Sulphides in Certain Organic Solvent - Bromine Mixtures

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As part of a study on the quantitative separation of carbide, nitride, oxide and sulphide inclusions from metals, the solubilities of five carbides, seven nitrides, sixteen oxides and eleven sulphides have been determined at 25 °C in methyl acetate - and acetonitrile - bromine mixtures (10 + 1 *V/V*) both after shaking at room temperature and after refluxing. Aluminium nitride and the carbides and nitrides of chromium, niobium, titanium and vanadium have low or very low solubilities, particularly in methyl acetate - bromine at room temperature, but iron carbide and iron and manganese nitrides are extensively decomposed, with the iron and manganese passing into solution. None of the oxides is more than sparingly soluble but most of the sulphides are appreciably soluble. Appropriate conditions are suggested for achieving a clean separation of oxides and stable carbides and nitrides from metals.

Keywords: *Solubilities of carbides, nitrides, oxides and sulphides*; *methyl acetate - bromine*; *acetonitrile - bromine*

For the isolation of non-metallic inclusions from metals the ideal solvent is one that will readily dissolve the metallic matrix and leave the inclusions unattacked. The use of ester - bromine mixtures for the quantitative isolation of aluminium nitride from steels was pioneered by Beeghly.[1] In steels that contained an excess of aluminium over nitrogen plus oxygen and that had been appropriately heat treated, he showed that virtually all of the nitrogen was recovered as insoluble aluminium nitride after dissolution of the steel in bromine - methyl acetate solvent. The nitrogen as aluminium nitride was determined as ammonia after a Kjeldahl distillation from alkaline solution. The method has since been widely used to determine aluminium nitride in steels.

The usefulness of bromine - methyl acetate mixtures to isolate aluminium nitride quantitatively from steels prompted investigations on the possibility of using such solvents to isolate other inclusions from steels. The results of these investigations are reported in several papers and reviews.[2–5] Of course, for success in these types of phase separations with steels, iron must be appreciably soluble in the solvent, and alloying metals at least moderately so. In Part I,[6] solubility studies were made using organic solvent - bromine mixtures (10 + 1 *V/V*) with refluxing. The organic solvents were methyl acetate, butyl acetate and acetonitrile and the solubilities of aluminium, chromium, cobalt, copper, iron, lead, manganese, molybdenum, nickel, niobium, phosphorus, silicon, sulphur, tin, titanium, tungsten and vanadium were determined at 25 °C in the mixtures after refluxing. All of the elements except lead, molybdenum, silicon and tungsten were appreciably or very soluble in these solvent mixtures. Molybdenum was sparingly soluble and the others almost insoluble.

The inclusions of most interest are carbides, nitrides, oxides and sulphides. The general consensus is that most oxides are "insoluble" in ester - bromine mixtures, as are the more thermodynamically stable carbides and nitrides, but that sulphides are extensively decomposed by the solvents. With less stable nitrides and carbides such as Cr_2N[7,8] and Fe_3C[3,9] and with a few oxides such as FeO and MnO[5,10] there are conflicting reports as to whether these phases are soluble, partly soluble or insoluble. Therefore, it was felt that quantitative solubility data were needed for many of these compounds and this information is supplied in this paper.

The solubilities of certain carbides, nitrides, oxides and sulphides, which are known to occur as inclusions in metals, in organic solvent - bromine mixtures (10 + 1 *V/V*) are reported. Because methyl acetate - bromine and acetonitrile - bromine systems were over-all slightly better solvents for the elements than butyl acetate - bromine, it was decided to employ only

* For details of Part I of this series, see reference list, p. 226.

the former solvent mixtures in future work and to investigate the solubilities of the compounds at 25 °C both after refluxing and without refluxing. The potential of these solvent mixtures for the quantitative isolation of inclusions is also discussed.

Experimental

Reagents

Acetonitrile. Laboratory-reagent grade, Fisons.

Methyl acetate. Laboratory-reagent grade, Fisons.

Bromine. AnalaR grade, Fisons.

Metal compounds. The following were used: aluminium nitride, AlN, 99%, Koch-Light; aluminium oxide, Al_2O_3, AnalaR grade, BDH Chemicals; aluminium sulphide, Al_2S_3, technical grade, BDH Chemicals; chromium carbide, Cr_3C_2, Alpha Division; chromium nitride, Cr_2N, Metals Research; chromium(III) oxide, Cr_2O_3, laboratory-reagent grade, Fisons; chromium(III) sulphide, Cr_2S_3, 99%, Metals Research; cobalt(II) oxide, CoO, laboratory-reagent grade, Fisons; cobalt oxide, Co_3O_4, laboratory-reagent grade, BDH Chemicals; copper(I) oxide, Cu_2O, laboratory-reagent grade, BDH Chemicals; copper(II) oxide, CuO, AnalaR grade, BDH Chemicals; copper(II) sulphide, CuS, laboratory-reagent grade, Prestons; iron carbide, Fe_3C, 99%, Metals Research; iron nitride, $Fe_2N + Fe_4N$, Metals Research; iron(II) oxide, FeO, prepared by heating iron(II) oxalate in a stream of argon at 900 °C; iron(III) oxide, Fe_2O_3, laboratory-reagent grade, BDH Chemicals; iron(II) sulphide, FeS, technical grade, BDH Chemicals; manganese nitride, Metals Research (chemical analysis indicated that this was a mixture of Mn_2N and Mn_4N); manganese(II) oxide, MnO, Diamond Shamrock; manganese(IV) oxide, MnO_2, laboratory-reagent grade, Fisons; manganese sulphide, MnS, 99.9%, Metals Research; nickel(II) oxide, NiO; nickel sulphide, NiS, technical grade, BDH Chemicals; niobium carbide, NbC, high purity, Koch-Light; niobium nitride, NbN, 99.5%, Metals Research; niobium(V) oxide, Nb_2O_5, Specpure, Johnson Matthey; niobium sulphide, NbS, 99.8%, Metals Research; tin(II) oxide, SnO, laboratory-reagent grade, BDH Chemicals; tin(IV) oxide, SnO_2, laboratory-reagent grade, BDH Chemicals; tin(II) sulphide, SnS, laboratory-reagent grade, BDH Chemicals; tin(IV) sulphide, SnS_2, laboratory-reagent grade, BDH Chemicals; titanium carbide, TiC, 99.8%, Koch-Light; titanium nitride, TiN, 99%, Alpha Division; titanium(IV) oxide, TiO_2, laboratory-reagent grade, Fisons; titanium(IV) sulphide, TiS_2, 99.8%, Metals Research; vanadium carbide, VC, Alpha Products; vanadium nitride, VN, 99%, Metals Research; vanadium(III) sulphide, V_2S_3, 99.8%, Metals Research; vanadium(V) oxide, V_2O_5, laboratory-reagent grade, Fisons.

Any material not in the form of powder was crushed to powder before use. All oxides were heated in an oven at 105–120 °C for 1 h before use to ensure that they were dry.

Purification and Drying of the Solvents

Acetonitrile was purified according to the method of Walter and Ramaley[11] and methyl acetate by using a standard procedure.[12]

Bromine was purified by shaking equal volumes of bromine and concentrated sulphuric acid in a separating funnel, separating the bromine and storing it in a stoppered bottle.

Determination of the Solubilities of Compounds in Organic Solvent - Bromine Mixtures

In all instances the organic solvent - bromine ratio was 10 : 1 *V*/*V*. Solubilities are reported later as masses of metallic elements from the compounds in 100-ml volumes of solution. These masses were determined by atomic-absorption spectrophotometry.

Without refluxing

Place 30 ml of solvent mixture in a 100-ml round-bottomed flask, which with its stopper is fitted with glass hooks. Fit a reflux condenser and a calcium chloride drying tube to the flask to prevent the ingress of moist air. Add about 300 mg of compound to the flask and note any reaction. If a vigorous reaction is observed, control this reaction by immersing the flask in ice-cold water. When any reaction has subsided, add a further 300 mg of compound. If all of the material dissolves, continue to add 300-mg amounts of compound until all noticeable reaction ceases.

Remove the condenser and drying tube, stopper the flask, attach springs to the hooks and place the flasks in an electric shaker for 15 min, then place the flask in a thermostatically controlled water-bath at 25 °C overnight.

Quickly filter the solution through a disc of Whatman Glass Microfibre paper, type GF/F, in a dry Millipore filtration apparatus under reduced pressure. Pipette an appropriate volume of the filtrate in the range 10–20 ml into a beaker and evaporate the solvent by heating on a steam-bath. Dissolve the residue with a suitable acid and transfer the solution quantitatively into a calibrated flask for subsequent analysis by atomic-absorption spectrophotometry, after removing by filtration any small residue of insoluble organic material that may remain.

With refluxing

Employ the procedure that was used for determining the solubility of elements in the organic solvent - bromine mixtures as reported in Part I.[6]

Results

Reactivities of Compounds with Methyl Acetate - Bromine (10 + 1 V/V)

Manganese(II) sulphide reacted violently with the formation of a yellow solid. Iron carbide reacted vigorously and the sulphides of iron(II), tin(II), tin(IV), titanium(IV) and vanadium-(III) dissolved readily in the cold. Iron and manganese nitrides dissolved slowly in the cold. For the other compounds there was no noticeable reaction in the cold but some showed reaction on refluxing.

Reactivities of Compounds with Acetonitrile - Bromine (10 + 1 V/V)

Manganese(II) sulphide reacted violently with the formation of a yellow solid. Iron carbide reacted vigorously and manganese nitride and the sulphides of copper(II), iron(II), nickel, tin(II), tin(IV) and vanadium(III) reacted readily in the cold. Iron nitride and titanium(IV) sulphide dissolved slowly in the cold. Other compounds showed little reaction in the cold but some started to react when the mixture was heated under reflux.

Solubilities

The solubilities of the compounds are shown in Tables I–IV. These are expressed as solubilities of metallic elements from the compounds at 25 °C either after reaction at or near room temperature or after refluxing with the solvents. The metallic elements dissolve as anhydrous bromides when the compound is decomposed.

Discussion

It can be seen from Tables I and II that aluminium nitride and the carbides and nitrides of chromium, niobium, titanium and vanadium have very low solubilities in bromine - methyl acetate and can be described as "insoluble." Frequently they are slightly more soluble in bromine - acetonitrile and, as expected with both solvents, less compound dissolves at room temperature than after refluxing. On the other hand, iron carbide and nitride and manganese

TABLE I

SOLUBILITIES OF CARBIDES IN ORGANIC SOLVENT - BROMINE MIXTURES

	Solubility at 25 °C/g per 100 ml			
	Acetonitrile		Methyl acetate	
Compound	After refluxing	No refluxing	After refluxing	No refluxing
Chromium carbide, Cr_3C_2	0.007	0.002	0.005	0.001
Iron carbide, Fe_3C	5.5	4.7	2.5	1.9
Niobium carbide, NbC	0.03	<0.01	0.03	<0.01
Titanium carbide, TiC	0.016	0.008	0.016	0.008
Vanadium carbide, VC	0.24	0.04	0.05	0.02

TABLE II

SOLUBILITIES OF NITRIDES IN ORGANIC SOLVENT - BROMINE MIXTURES

Compound	Solubility at 25 °C/g per 100 ml			
	Acetonitrile		Methyl acetate	
	After refluxing	No refluxing	After refluxing	No refluxing
Aluminium nitride, AlN	0.009	0.007	0.008	0.003
Chromium nitride, Cr_2N	0.01	0.009	0.008	0.006
Iron nitride, $Fe_2N + Fe_4N$	3.2	1.8	1.3	1.1
Manganese nitride, $Mn_2N + Mn_4N$	2.3	1.6	2.1	1.1
Niobium nitride, NbN	0.03	<0.01	0.03	<0.01
Titanium nitride, TiN	0.010	0.008	0.010	0.008
Vanadium nitride, VN	0.04	0.03	0.03	0.02

nitride are decomposed in both solvents and the metallic moieties pass into solution as bromides. Presumably carbide is converted into carbon on decomposition and nitride into elemental nitrogen.

From Table III it can be seen that the solubilities of all oxides are low and none is more than sparingly soluble. Solubilities are often slightly greater in bromine - acetonitrile than in bromine - methyl acetate. This is probably because acetonitrile is a better co-ordinating solvent than methyl acetate. Again the solubilities are greater after refluxing. Obviously bromine - methyl acetate is a good solvent for isolating oxide inclusions from metals but slight solubility, particularly after refluxing, can be noticed with copper(I) and -(II) oxides, iron(II) oxide, manganese(IV) oxide and nickel(II) oxide.

As can be seen from Table IV, most sulphides have appreciable solubility in the solvent mixtures and those of copper(II), iron(II), manganese(II), nickel(II), tin(II) and tin(IV) are considerably more soluble in bromine - acetonitrile than in bromine - methyl acetate. Again this is because of the better co-ordinating ability of acetonitrile compared with methyl acetate. Only chromium(III) sulphide and niobium sulphide have low solubilities in these solvents. Metal sulphides dissolve to produce solutions of the metal and sulphur bromides. Clearly these organic solvent - bromine mixtures cannot be used to isolate most sulphide inclusions but they could be used to isolate oxides and many carbides and nitrides free from most sulphides. The solubility of manganese(II) sulphide appears to be low, particularly in bromine - methyl

TABLE III

SOLUBILITIES OF OXIDES IN ORGANIC SOLVENT - BROMINE MIXTURES

Compound	Solubility at 25 °C/g per 100 ml			
	Acetonitrile		Methyl acetate	
	After refluxing	No refluxing	After refluxing	No refluxing
Aluminium oxide	0.013	0.007	0.008	0.003
Chromium(III) oxide	0.003	0.002	0.004	0.003
Cobalt(II) oxide	0.03	0.02	0.09	0.04
Cobalt oxide, Co_3O_4	0.005	0.004	0.06	0.04
Copper(I) oxide	0.32	0.13	0.18	0.09
Copper(II) oxide	0.23	0.11	0.21	0.02
Iron(II) oxide	0.17	0.13	0.16	0.13
Iron(III) oxide	0.003	0.0006	0.002	0.0001
Manganese(II) oxide	0.09	—	0.06	—
Manganese(IV) oxide	0.30	0.09	0.18	0.08
Nickel(II) oxide	0.30	0.10	0.12	0.10
Niobium(V) oxide	0.03	<0.01	0.03	<0.01
Tin(II) oxide	0.04	0.02	0.01	0.008
Tin(V) oxide	0.016	0.013	0.008	0.005
Titanium(IV) oxide	0.006	0.004	0.006	0.004
Vanadium(V) oxide	0.06	0.02	0.07	0.02

TABLE IV

SOLUBILITIES OF SULPHIDES IN ORGANIC SOLVENT - BROMINE MIXTURES

	Solubility at 25 °C/g per 100 ml			
	Acetonitrile		Methyl acetate	
Compound	After refluxing	No refluxing	After refluxing	No refluxing
Aluminium sulphide	0.33	0.29	0.30	0.28
Chromium(III) sulphide	0.20	0.02	0.02	0.01
Copper(II) sulphide	1.4	1.0	0.23	0.10
Iron(II) sulphide	4.7	3.5	1.8	0.95
Manganese sulphide	2.0	1.2	0.22	0.02
Nickel sulphide	2.6	2.3	0.63	0.23
Niobium sulphide, NbS	0.06	0.03	0.04	0.03
Tin(II) sulphide	2.6	1.7	0.93	0.35
Tin(IV) sulphide	3.9	2.3	1.0	0.53
Titanium(IV) sulphide	0.72	0.40	1.5	0.68
Vanadium(III) sulphide	1.3	1.1	1.9	1.3

acetate without refluxing. However, as mentioned earlier, there is a violent reaction between manganese(II) sulphide and the solvent to produce a yellow solid of low solubility, which has not yet been characterised, and the solvent mixture cannot be used to isolate manganese(II) sulphide.

Considering all the solubilities in Tables I–IV, bromine - methyl acetate is preferred to bromine - acetonitrile for isolating inclusions. The total concentration of inclusions in a metal can be very low, sometimes not exceeding 0.01%. At first sight, the solubilities given in Tables I–III appear not to be low enough for achieving quantitative isolation of inclusions of oxides and the more stable carbides and nitrides. However, these solubilities were determined using conditions designed to encourage dissolution. Appreciable amounts of finely divided solids were shaken or refluxed with organic solvent - bromine mixtures (10 + 1 *V*/*V*). During the whole of the dissolution period the concentration of bromine remained high and the compounds and bromine were in contact for at least 3 h and often overnight.

In actual practice, the following conditions should apply.

(*i*) Calculate the amount of bromine required for the dissolution of the metallic phases in the mass of material taken and use an appropriate volume of methyl acetate plus bromine so that there is not more than a 10% excess of bromine but that enough methyl acetate is present to retain the soluble metal bromides in solution. Do not exceed a bromine to methyl acetate ratio of 1:10 *V*/*V*.

(*ii*) Carry out the dissolution at as low a temperature as possible in order to discourage dissolution of oxide and most carbide and nitride inclusions.

(*iii*) Filter the solution to collect the inclusions as soon as the metallic matrix has dissolved and wash the inclusions with pure solvent to remove trace amounts of bromine and bromides.

Under these conditions the solubilities of the "insoluble" oxides, carbides and nitrides should be sufficiently low to ensure their virtually quantitative isolation.

It is felt that the solubility data presented in this paper are a guide to which quantitative separations should be possible and which are unlikely to be successful in the field of chemical phase analysis. Until reliable methods for quantitative phase determinations have been devised using the solids themselves and microscopic techniques, chemical phase analysis following separation will continue to be necessary. However, it must be appreciated that the solubilities of carbides, nitrides and oxides, whether as inclusions or as powdered compounds, will be dependent on the size, surface area and density of the particles. No two materials are likely to be identical in all of these respects and a solubility determined for a particular sample of a compound is only a rough guide to the solubility for inclusions of the same compound in metals. Even so, the solubilities reported here should be helpful to those using methods of phase separation based on the dissolution of metals using organic solvent - bromine mixtures. With these data it should be possible to devise new separations and to confirm or view with caution some of the statements on solubilities that have appeared in the chemical literature.

It will be appreciated that only binary compounds as models for binary inclusions have been investigated in this study. Many binary inclusions exist in metals but many others contain more than two elements, for example, manganese silicon nitride, silicates, carbonitrides and carbosulphides. Because titanium carbide and nitride have been found to have very low solubilities in methyl acetate - bromine mixture, it can be assumed that titanium carbonitride will also have low solubility in this mixture. Similar predictions can be made with other carbonitrides. However, it is not possible to predict the solubilities of manganese silicon nitride or titanium carbosulphide from the data that have been presented. The solubilities of such compounds require further study.

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NOTE—Reference 6 is to Part I of this series.

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Determination of Microgram Amounts of Calcium in Small Biological Samples by EDTA Titration Using Patton and Reeder's Indicator

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An EDTA titration method has been developed for the determination of calcium in small biological samples. Fresh or dried material is extracted with 1 N nitric acid and the extract is alkalinised to pH 13.4 and titrated with EDTA in the presence of Patton and Reeder's indicator and sodium tartrate. Barium, magnesium, manganese, iron, lead, zinc, oxalate, phosphate and sulphate do not interfere owing to the high pH and the presence of tartrate. Copper interferes, but up to 5 μg of copper can be masked with cyanide if the copper concentration does not exceed one tenth the calcium concentration. This method permits the quantification of calcium in the range 0.5–40 μg and with a reproducibility of ±2.5% or better.

Keywords: *Calcium microdetermination*; *EDTA titration*; *biological samples*

The EDTA titration method described here for the determination of calcium is based on the method of Allen *et al.*[1] The procedure has been scaled down, the titration medium made more alkaline and tartrate added in order to minimise interferences from other electrolytes. In this form the method has been used for determination of calcium in chloragosomes and calciferous gland from the earthworm *Lumbricus terrestris*.[2] However, the original indicator, glyoxal bis(2-hydroxyanil), has a diffuse end-point colour change from red to orange to yellow, which makes the indicator difficult to use at low calcium concentrations. Further, the indicator is extremely unstable at high pH.

A search was made to find a more suitable indicator, and we finally adopted Patton and Reeder's indicator [2-hydroxy-1-(2-hydroxy-4-sulpho-1-naphthylazo)-3-naphthoic acid]. This metallochrome was originally proposed for the titration of calcium by Patton and Reeder.[3] With its very distinct end-point colour change from red to blue, it was found to be nearly perfect for this titration method, permitting detection of very small amounts of calcium. We found, for instance, that the calcium contents of as few as 25 individuals of the amoeba *Chaos carolinensis* could be determined (0.5–1 μg). We therefore decided to investigate this final procedure more extensively in order to determine whether the method might be generally useful.

Experimental

Reagents

Analytical-reagent grade chemicals and de-ionised (or glass distilled) water are used throughout. If necessary all glassware and sample vials are rinsed with dilute acid and de-ionised water before use. Stock solutions, except sodium tartrate, are stable for at least 1 year if stored in polyethylene containers.

Calcium solutions. Dissolve 1.000 g of dry calcium carbonate in 1 N nitric acid and dilute to 1000 ml with 1 N nitric acid. Dilute this solution further with 1 N nitric acid to make standard solutions covering the range 2.5–40.0 μg ml^{-1}.

EDTA solutions. Dissolve 0.4655 g of disodium ethylenediaminetetraacetate in water and dilute to 1000 ml (0.1 ml ≡ 5 μg of calcium). For titration in the range 0–5 μg of calcium dilute the EDTA stock solution 2–5-fold with water.

Indicator solution. Dissolve 0.1 g of Patton and Reeder's indicator [2-hydroxy-1-(2-hydroxy-4-sulpho-1-naphthylazo)-3-naphthoic acid] (Sigma) in 100 ml of ethanol.

Sodium hydroxide solution, 1 N. Dissolve 40 g of sodium hydroxide in water and dilute to 1000 ml.

Sodium tartrate solution. Dissolve 0.2 g of sodium tartrate in 10 ml of water. This solution must be freshly prepared.

Preparation of Samples

Biological material can be extracted directly with 1 N nitric acid, or it can be dried to constant mass before extraction. For small samples the undissolved material may remain in the nitric acid extract. For larger samples the extract can be cleared by filtration (ash-free filter-paper) or by centrifugation and decantation. The nitric acid extracts can be stored in polycarbonate or polyethylene tubes at 0–5 °C. For wet-ashed material the oxidants (hydrogen peroxide, chlorate) are boiled off or decomposed and the final acid concentration is adjusted to about 1 N with 10 N sodium hydroxide solution.

Analytical Procedure

To 500 μl of 1 N sodium hydroxide solution add 100 μl of nitric acid extract or calcium standard. Add 200 μl of sodium tartrate solution and swirl. Add 25 μl of indicator solution and titrate immediately. For larger numbers of samples delay the final addition of indicator until immediately before titration. The degree of redness of the sample can be used to judge whether the stock or a dilute EDTA solution should be used for titration.

For the burette use a 1-ml syringe graduated at 0.01-ml intervals. We made a satisfactory titration syringe by insertion of two lengths of polyethylene tubing in the tip of a disposable 1-ml polypropylene syringe (see Fig. 1). With training 0.005-ml intervals can be read.

In the analysis of biological materials a suitable range of calcium standards should always be included in order to ensure detection of method errors or calcium contamination. Nitric acid blank runs should always be performed and should show negligible or no calcium. A positive blank indicates that the rinsing of the equipment was insufficient.

Patton and Reeder's indicator is unstable in aqueous solution and the authors[3] therefore added it as a dispersion in dry sodium sulphate. We found, however, that the indicator is stable for several months when dissolved in ethanol. The ethanolic stock solution is simpler to use than a dry dispersion and permits a more rapid and reproducible titration.

As the indicator is very unstable at high pH (about 13.4), the titration should be finished within 1 min after the addition of the indicator. For very small amounts of calcium the titration sample should be compared with a reagent blank in order to determine the exact end-point for the titration. A very dilute aqueous solution of Naphthalene Black B (Colour Index No. 20470; BDH Chemicals), which is stable, can conveniently be used instead of the unstable reagent blank.

A background consisting of a milk-glass screen lit from behind with a strong tungsten lamp improves the determination of the titration end-point.

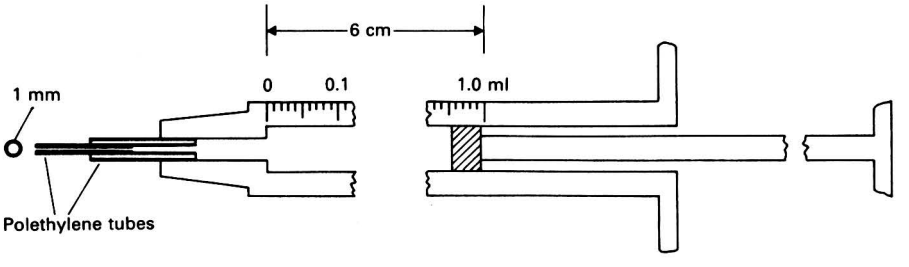

Fig. 1. Titration syringe made from a 1-ml disposable polypropylene syringe by insertion of two polyethylene tubes in the tip of the syringe.

Interferences

Various amounts of diverse ions were added to standard titration samples, in the form of sodium salts (anions) or chlorides (metal ions). Interferences from magnesium were investigated in both the absence and presence of tartrate.

Results

Calibration graphs covering the range 0–40 μg or 0–5 μg were used. The day-to-day reproducibility was good down to 2 μg and acceptable down to 0.5 μg (see Figs. 2 and 3).

Interferences

As shown in Fig. 4 (*cf.*, Fig. 3) moderate amounts of barium, iron, lead, magnesium, manganese, zinc, oxalate, phosphate or sulphate ions did not interfere seriously even at low calcium concentrations, indicating that the high pH and the presence of tartrate prevent the foreign ions from interacting with EDTA.

Up to 40 μg of magnesium could be added to a 20-μg calcium standard in the absence of tartrate without interfering in the titration, but in the presence of tartrate more than 200 μg could be added without interfering.

Up to 40 μg of zinc could be included in the 40-μg calcium standard. Inclusion of larger amounts of zinc led to an increased titration value because of a slight reaction between zinc and EDTA, even at pH 13.4, where zinc should be completely masked.[4]

Up to 25 μg of iron(III) could be included in the 40-μg calcium standard. Larger amounts led to a depression of the titration value. In all instances moderate (above 2–4 μg) amounts of iron(III) led to a grey instead of a blue end-point, probably because of oxidation of the free indicator. It is therefore important to titrate fairly quickly (within 30 s) in order to determine the proper titration end-point. Normally this can be done without difficulty.

Copper interferes seriously in the calcium titration, forming a stable complex with this indicator[3] and also with Eriochrome Black T.[4] Neither iodide, thioglycolate nor thiosulphate were able to mask copper at pH 13.5 but potassium cyanide was able partially to mask it. Provided that the cyanide was added to the titration medium before addition of the indicator, and provided that the copper concentration did not exceed one tenth the calcium concentration, up to 5 μg of copper could be masked. If copper was present at levels below one thousandth of the calcium concentration, the titration was not appreciably affected. Hence, inside the biological range of proportions of copper to calcium the presence of copper can be adequately masked.

Strontium reacts with the indicator and with EDTA so that quantification of calcium is impossible in the presence of strontium.

Up to 400 μg of phosphate could be included in the 40-μg calcium standard without affecting the titration end-point. However, above about 50 μg of phosphate it is necessary to titrate fairly slowly (2–4 min) and to shake the sample vigorously in order to release calcium from the calcium phosphate precipitate. The sample colour should be checked about 1 min after completion of the titration. If the sample has reverted to a reddish colour, the titration can be continued to the actual end-point.

Discussion

The calcium titration method described here is accurate even in the presence of zinc and very large amounts of magnesium and phosphate. The masking of zinc is due to the very high pH (13.4) of the titration medium,[4] and the masking of magnesium is due to the presence of

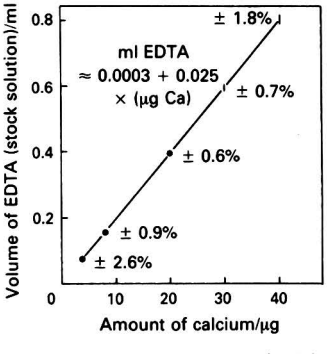

Fig. 2. Titration of 4–40 μg of calcium. Regression analysis of eight calcium standard titration series, each spanning over five steps.

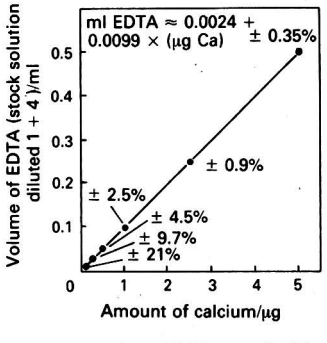

Fig. 3. Titration of 0.25–5 μg of calcium. Regression analysis of eight calcium standard titration series, each spanning over six steps. For titration the stock EDTA solution was diluted 1+4 with water.

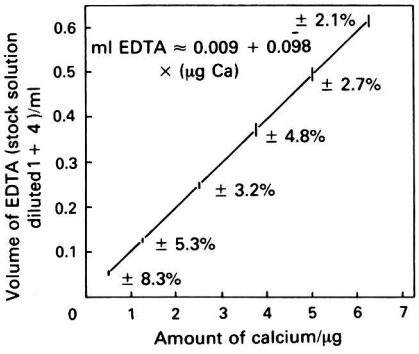

Fig. 4. Interference: regression analysis of the effects of fixed amounts of foreign ions in the calcium titration samples. For each foreign ion the titration series was performed twice. The values for any amount of calcium were too similar to permit individual curves to be drawn. Each titration series contained 0.5–6.25 μg calcium spanning over ten steps. Barium, lead, manganese, magnesium and zinc, 2.5 μg; oxalate, 10 μg; phosphate, 5 μg; and sulphate, 10 μg.

tartrate.[5] Probably tartrate acts by keeping the calcium hydroxide in solution, thus preventing it from being sequestered in the very massive magnesium hydroxide precipitate.

Patton and Reeder[3] pointed out that the inclusion of a small amount of magnesium in the titration sample facilitated the determination of the calcium titration end-point and suggested that the indicator is adsorbed on the magnesium hydroxide precipitate. However, we did not find any favourable effect of magnesium in determining the titration end-point, even when tartrate was not included in the titration medium. Possibly in this method the pH is too high for such an effect to occur.

In conclusion, this calcium titration method can be used on most biological materials without serious problems. Even large amounts of magnesium, zinc, phosphate and oxalate do not interfere. The concentrations of copper and iron(III) in biological materials are generally too low to affect the calcium titration.

Only very small amounts of extract need be used, permiting the determination of other inorganic constituents in the same extract, even when the biological sample is small; a 1-ml extract permits duplicate or several determinations of calcium, zinc, iron and phosphate.[2] The method is highly reproducible and accurate down to 1 μg of calcium and can be used satisfactorily down to 0.25 μg. The method is less sensitive than electrothermal atomic-absorption spectrophotometry, but is rapid, reliable, virtually free from interferences and inexpensive.

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Automatic Titration by Stepwise Addition of Equal Volumes of Titrant

Part VI.* Further Extension of the Gran I Method for Calculation of the Equivalence Volume in Acid - Base Titrations

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In Part V of this series a very simple method for the calculation of the equivalence volume (V_e) from titration data was presented. It was based on the method of stepwise addition of equal volumes of titrant combined with an extended version of the Gran I method. The method presented had some limitations, however, as it could not be used for acids with log K between 2 and 4.2 or for very weak acids (log $K > 7$).

In this paper equations of a more complicated nature are given, which can be used for monoprotic acids with log $K \leqslant 10$. The equations contain, besides titrant volumes and hydrogen ion concentration, values for K and K_w, which, however, need not be known but are calculated from the titration data at the same time as V_e. The same type of calculation can be used in the calculation of V_e for most di- and triprotic acids.

A discussion is given of possible causes for errors in the equivalence volume, calculated using the equations presented. When accurate automatic pipettes are used for adding the titrant the uncertainty in the determination of the hydrogen ion concentration is the main source of error.

Keywords: *Gran I method*; *acid - base titration*; *automatic titration*; *equivalence volume calculation*; *potentiometric titration*

In Part V[1] of this series an extended version of the Gran I method[2] was used to deduce equations for the calculation of the equivalence volume in acid - base titrations. These equations are simple and they can be handled with a simple pocket calculator. On the other hand, the equations have certain limitations with regard to the ranges of acid stability constants for which they are valid.

The equations given in Part V cannot be used for acids with $2 \lesssim \log K \lesssim 4.2$ and $\log K \gtrsim 7$. In this paper some equations will be presented, which are valid for a wider range of stability constants but require a programmable calculator with a not too limited number of memories.

Four examples will be treated: (*a*) monoprotic acids of intermediate strength ($\log K = 3 \pm 1.5$); (*b*) very weak monoprotic acids ($\log K > 7$); (*c*) conditionally strong acids,[3] where the salt of an acid weaker than acetic acid is used as a medium; and (*d*) di- and triprotic acids.

Theoretical

Monoprotic Acids of Intermediate Strengths

If V_0 ml of a solution of a monoprotic acid HA is titrated with a strong base of concentration C_B, equation (1) [equation (3) in Part III[4]] applies after addition of V ml of titrant solution:

$$V + K\left\{V[\mathrm{H}] + \frac{V_0 + V}{C_B}([\mathrm{H}]^2 - K_w) + \right\}\frac{V_0 + V}{C_B}\left([\mathrm{H}] - \frac{K_w}{[\mathrm{H}]}\right) - V_e = 0 \qquad (1)$$

where K is the stability constant of the acid, K_w is the ionic product of water and V_e is the equivalence volume. K and K_w are concentration constants. [H] is therefore the hydrogen ion concentration, not the activity.

In acid solutions the term $(V_0 + V)K_w/(C_B[\mathrm{H}])$ may be neglected. The term $K(V_0 + V)K_w/C_B$ can also be neglected if the acid is of intermediate strength.

For details of Part V of this series, see reference list, p. 242.

In Part V[1] it was shown that for any monoprotic acid with log $K < 6$ the following equation is valid:

$$V_e = V_j + \frac{V_j - V_i}{\frac{KC_B V_i + (V_o + V_i)(1 + K[H]_i)}{K C_B V_j + (V_o + V_j)(1 + K[H]_j)} \cdot \frac{[H]_i}{[H]_j} - 1} \quad .. \quad .. \quad (2)$$

where V_i and V_j are the volumes of two additions of titrant and $[H]_i$ and $[H]_j$ are the hydrogen ion concentrations measured after these additions. Equation (2) contains the stability constant K, which so far is unknown. For strong acids it can be assumed to be zero but this is not so for moderately strong acids. K can be calculated from

$$K = \frac{C_B (V_e - V) - [H](V_o + V)}{C_B [H]V + [H]^2 (V_o + V)} = \frac{C_B V_e - [C_B V + [H](V_o + V)]}{[H][C_B V + [H](V_o + V)]} \quad (3)$$

where V is any addition of titrant before the equivalence point and [H] the corresponding hydrogen ion concentration. Equation (3), however, requires the knowledge of V_e. This dilemma can be solved by an iteration technique, *e.g.*, by starting with an even very approximate value for V_e and calculating a value for K by using equation (3) and then using this value for K in equation (2) for calculating V_e. One or two iterations are usually enough in order to obtain two consecutive values for V_e, which do not differ by more than, for example, 10^{-3} or 10^{-4} ml.

If the titrations are performed using an accurately calibrated electrode couple, the V_e values found in this way will be very close to the true value and the K value is also obtained with a high degree of accuracy.

With less well calibrated electrodes, V_e values may deviate somewhat from the true value. In such instances a regression analysis will lead to accurate values, as will be explained later.

Very Weak Monoprotic Acids

The equations hitherto discussed are restricted to acids with log $K \leqslant 7$, because the hydroxide ion concentration has been neglected {the term $(V_o + V)K_w/([H]C_B)$}. In this and the next section some equations will be given for titration of acids with higher stability constants.

Equation (1) can be slightly rewritten to read

$$C_B (V_e - V) = C_B KV[H] + (K[H] + 1)(V_o + V)\left([H] - \frac{K_w}{[H]}\right) \quad .. \quad (4)$$

If two such expressions for additions V_i and V_j are divided and 1 is subtracted from each quotient the following equation is obtained after rearrangement:

$$V_e = V_j + \frac{V_j - V_i}{\frac{C_B KV_i[H]_i + (K[H]_i + 1)(V_o + V_i)\left([H]_i - \frac{K_w}{[H]_i}\right)}{C_B KV_j[H]_j + (K[H]_j + 1)(V_o + V_j)\left([H]_j - \frac{K_w}{[H]_j}\right)} - 1} \quad .. \quad (5)$$

The stability constant can be calculated from

$$K = \frac{C_B V_e - \left[C_B V + (V_o + V)\left([H] - \frac{K_w}{[H]}\right)\right]}{[H]\left[C_B V + (V_o - V)\left([H] - \frac{K_w}{[H]}\right)\right]} \quad .. \quad .. \quad (6)$$

where V is any titrant addition before the equivalence volume.

An acceptable value for K_w can be calculated according to the definition of K_w:

$$K_w = [H][OH] \quad .. \quad .. \quad .. \quad .. \quad .. \quad (7)$$

where the hydroxide ion concentration can be calculated approximately from

$$[\mathrm{OH}] = \frac{V_\mathrm{N} - V_\mathrm{e}}{V_\mathrm{o} + V_\mathrm{N}} \cdot C_\mathrm{B} \quad .. \quad .. \quad .. \quad .. \quad (8)$$

where preferably $V_\mathrm{N} > 1.5V_\mathrm{e}$.

Equations (7) and (8) give

$$K_\mathrm{W} = \frac{V_\mathrm{N} - V_\mathrm{e}}{V_\mathrm{o} + V_\mathrm{N}} \cdot C_\mathrm{B} \cdot [\mathrm{H}]_\mathrm{N} \quad .. \quad .. \quad .. \quad .. \quad (9)$$

A more accurate value for the hydroxide ion concentration can be obtained from the charge balance

$$[\mathrm{OH^-}] + [\mathrm{A^-}] = [\mathrm{H^+}] + [\mathrm{Na^+}] \quad .. \quad .. \quad .. \quad .. \quad (10)$$

where

$$[\mathrm{A}] = C_\mathrm{HA} - [\mathrm{HA}] = C_\mathrm{HA} - K[\mathrm{H}][\mathrm{A}] = \frac{C_\mathrm{HA}}{1 + K[\mathrm{H}]}$$
$$= \frac{V_\mathrm{e}C_\mathrm{B}}{(V_\mathrm{o} + V)(1 + K[\mathrm{H}])} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (11)$$

$$[\mathrm{Na}] = V C_\mathrm{B}/(V_\mathrm{o} + V) \quad .. \quad .. \quad .. \quad .. \quad .. \quad (12)$$

C_HA is the total concentration of the acid. This gives

$$K_\mathrm{W} = \frac{C_\mathrm{B}[\mathrm{H}]_\mathrm{N}}{V_\mathrm{o} + V_\mathrm{N}}\left(V_\mathrm{N} - \frac{V_\mathrm{e}}{1 + K[\mathrm{H}]_\mathrm{N}}\right) + [\mathrm{H}]_\mathrm{N}^2 \quad .. \quad .. \quad .. \quad (13)$$

In order to calculate V_e it is necessary to iterate the three equations (5), (6) and (9) or (13). The method of calculation will be further explained below.

Conditionally Strong Acids

In a conditional titration a sample acid, HB, is added to an excess of a salt, NaA, of a weaker acid, whereby an amount of acid HA is liberated equivalent to the amount of the sample acid added. The mixture is titrated with a standardised solution of a strong base.

In the deduction of the equations for conditionally strong acids in Part V some approximations were made. The complete equation for V_e is

$$V_\mathrm{e} = V_j + \frac{V_j - V_i}{\dfrac{K_\mathrm{A}[\mathrm{H}]_i\left[C_{\mathrm{A}o}V_\mathrm{o} - C_\mathrm{B}(V_\mathrm{e} - V_i)\right] + (K_\mathrm{A}[\mathrm{H}]_i + 1)(V_\mathrm{o} + V_i)\left([\mathrm{H}]_i - \dfrac{K_\mathrm{W}}{[\mathrm{H}]_i}\right)}{K_\mathrm{A}[\mathrm{H}]_j\left[C_{\mathrm{A}o}V_\mathrm{o} - C_\mathrm{B}(V_\mathrm{e} - V_j)\right] + (K_\mathrm{A}[\mathrm{H}]_j + 1)(V_\mathrm{o} + V_j)\left([\mathrm{H}]_j - \dfrac{K_\mathrm{W}}{[\mathrm{H}]_j}\right)} - 1} \quad (14)$$

where

$$K_\mathrm{A} = \frac{[\mathrm{HA}]}{[\mathrm{H}][\mathrm{A}]} = \frac{C_\mathrm{B}(V_\mathrm{e} - V) - (V_\mathrm{o} + V)\left([\mathrm{H}] - \dfrac{K_\mathrm{W}}{[\mathrm{H}]}\right)}{[\mathrm{H}]\left\{C_{\mathrm{A}o}V_\mathrm{o} - C_\mathrm{B}(V_\mathrm{e} - V) + (V_\mathrm{o} + V)\left([\mathrm{H}] - \dfrac{K_\mathrm{W}}{[\mathrm{H}]}\right)\right\}} \quad (15)$$

In order to obtain V_e it is necessary to iterate the three equations (14), (15) and (9) or (13).

Di- and Triprotic Acids

In Part V[1] it was shown that V_e for monoprotic acids with $4.2 < \log K < 7$ can be calculated from

$$V_e = V_j + \frac{V_j - V_i}{\frac{V_i}{V_j} \cdot \frac{[H]_i}{[H]_j} - 1} \quad .. \quad .. \quad .. \quad .. \quad (16)$$

It seems logical that for a diprotic acid the equivalence volume, V_{e2}, to the second equivalence point can be calculated from a similar equation:

$$V_{e2} = V_j + \frac{V_j - V_i}{\frac{V_i - 0.5V_{e2}}{V_j - 0.5V_{e2}} \cdot \frac{[H]_i}{[H]_j} - 1} \quad .. \quad .. \quad .. \quad (17)$$

where $0.5V_{e2} < V_i$ and $V_j < V_{e2}$.

With $d = [H]_i/[H]_j$ equation (17) can be rearranged to read

$$(d - 1)V_{e2}^2 - \left[V_i(d - 1) + V_j(d - 2)\right]V_{e2} + 2(d - 1)V_iV_j = 0 \quad .. \quad (18)$$

This equation can easily be solved for V_{e2}.

Some diprotic acids, *e.g.*, succinic acid, have stability constants that lie close together. For such acids the V_{e2} calculated from equation (17) will deviate from the true V_{e2} value. It may even happen that equation (17) has no real root for V close to $0.5V_{e2}$.

By regression analysis it is possible, however, to arrive at a very accurate value for V_{e2} as will be further explained below.

By analogy with equation (17) for diprotic acids a similar equation can be set up for triprotic acids:

$$V_e = V_j + \frac{V_j - V_i}{\frac{3V_i - 2V_{e3}}{3V_j - 2V_{e3}} \cdot \frac{[H]_i}{[H]_j} - 1} \quad .. \quad .. \quad .. \quad (19)$$

where $2V_{e3}/3 < V_i$ and $V_j < V_{e3}$.

The analogous equation to equation (18) is

$$2(d - 1)V_{e3}^2 - \left[V_i(3d - 2) + V_j(2d - 3)\right]V_{e3} + 3(d - 1)V_iV_j = 0 \quad .. \quad (20)$$

An advantage of equations (17), (18), (19) and (20) is that only relative values for the hydrogen ion concentrations $[H]_i/[H]_j$ are needed, which means that very accurate calibration of the electrode couple is not necessary.

Calculation Procedures

To handle the equations presented in this paper a well equipped pocket calaculator is needed. It should preferably have test functions and allow for conditional transfers, as well as have sufficient program and data storage space. A further advantage is a built-in program for multiple linear regression. (The authors used Texas Instruments, TI Programmable 59.) The titrations should preferably be carried out by adding the titrant with an accurate automatic pipette. It should be noted that the electrode couple should be calibrated by means of solutions with known hydrogen ion concentrations and not activities, *i.e.*, ph is defined as ph = $-\log [H]$. The ph or the e.m.f. value is registered after each addition. If the calculations require a value of K_w the titration is carried out until the equivalence volume has been passed by about 50% [examples (*b*) and (*c*)].

The equivalence volume V_e is calculated from combinations of V_i and V_j, where it is normally best to use $i = 1, 2, \ldots, (j - 1)$ and $V_e - V_p < V_j < V_e$, V_p being the volume of the automatic pipette. In those instances where the constant K_w has to be calculated, it is suitable to use $V_N \approx 1.5\ V_e$, *i.e.*, an addition well beyond the equivalence volume. In order to compensate for random errors 2–3 values near to $1.5V_e$ may be used.

The values V_e thus obtained for each combination of V_i and V_j are in practice not exactly equal. The V_e values are therefore in principle plotted *versus* V_i and a graph is fitted to the

points and extrapolated until it intersects the line $V_e = V_i$. This can be carried out either graphically or numerically. In most instances sufficient accuracy can be obtained by fitting a straight line to the points:

$$V_e = a + b\,V_i$$

which, together with $V_e = V_i$, gives

$$V_e = \frac{a}{1 - b} \qquad (21)$$

In some instances (di- and triprotic acids) the graph indicates a curved line that can be closely approximated by a hyperbola of the type

$$(V_i - a)\,(V_e - b) = c \qquad (22)$$

or

$$V_e = b + \frac{c}{V_i - a} \qquad (23)$$

The intersection with the line $V_e = V_i$ gives

$$V_e^2 - (a + b)V_e + ab - c = 0 \qquad (24)$$

which can be solved for V_e. One of the roots to the quadratic equation (24) is the real solution for V_e. The hyperbola, equation (22), can be fitted to the points by solving the equation

$$A(1, i) = A(2, i)a + A(3, i)b + A(4, i)\,(c - ab) \qquad (25)$$

where $A(1, i) = V_i\,V_e$; $A(2, i) = V_e$; $A(3, i) = V_i$; and $A(4, i) = 1$.

At least three pairs of values V_i, V_e are needed to solve a set of equations (25). With more than three pairs of values, the set of equations is over-determined and is solved by a least-squares procedure. This compensates for random errors in the calculations of a, b and c. These values are then used in equation (24) to calculate the final value of V_e.

Evaluation of Calculation Procedures

Synthetic Titration Data

The calculation procedures have been evaluated by testing them on titration data obtained by calculating ph or e.m.f. values to six and three decimal places, respectively, from the appropriate titration equations. These values correspond to titrations producing perfect data, uninfluenced by systematic and random errors. In this instance excellent values of V_e were always obtained.

Data of such accuracy cannot be obtained in practice. To evaluate the effect of systematic errors due to unsatisfactory calibration, the calculated ph values were altered by between +0.1 and −0.1 ph units (corresponding to ± 6 mV in the e.m.f.). Further, the decimal places were reduced to three for ph values and one for e.m.f. values (in millivolts). The results may be summarised as follows:

Monoprotic acids of intermediate strength

If $\log K = 3$ the error in ph must not exceed 0.03 if the error in V_e should be less than 0.2% when linear regression is used. The error in V_e diminishes for acids on both sides of $\log K = 3$. So, for example, the error in ph may be 0.1 unit for an acid with $\log K = 4$.

Very weak monoprotic acids

An error in ph does not affect the V_e value, only the K and the K_w values. Linear regression is sufficient.

Conditionally strong acids

The same comments as for very weak monoprotic acids.

Di- and triprotic acids

The equations are unaffected by a constant error in the ph or e.m.f. values.

Experimental Data

The calculation of V_e will be demonstrated below for some titrations performed by stepwise additions of standardised sodium hydroxide solution to various types of acids. The titrations were carried out as described in Part IV[5] of this series.

Example 1

Titration of a monoprotic acid of intermediate strength. Given that: 1.0018 mmol of benzoic acid are dissolved in an aqueous solution, which was 0.1 M with respect to sodium chloride and 0.033 M with respect to barium chloride; V_0, 100.0 ml; temperature, 25.0 °C; titrant pipette volume, 1.0154 ml; and titrant, 0.09991 M sodium hydroxide solution, which was also 0.033 M with respect to barium chloride. The E'_0 value was not determined, but should, from earlier experience, be in the range 388–403 mV.

The data for the titration are given in Table I. With $E'_0 = 388$ mV, $i = 0, 1, 2, \ldots, 8$ and $j = 9$ and using equations (2) and (3) the V_e values in column 5 were obtained.

These V_e values are plotted against V_i in Fig. 1. The solid graph, fitted to the points, is extrapolated until it intersects the line $V_e = V_i$. This gives V_e the value of 10.00 ml. With numerical evaluation the following values are obtained: arithmetic mean $V_e = 9.9377$ ml, yield=99.11%; linear regression $V_e = 10.085$ ml, yield=100.57%; and hyperbolic regression $V_e = 9.999$ ml, yield = 99.72%. In this example a rather extreme value of E'_0 was used and the graph in Fig. 1 became curved. However, by using a hyperbolic regression a correct V_e was obtained. [Evaluation using equation (9) in reference 4 gave $V_e = 9.994$ ml and yield = 99.68%.] If instead an E'_0 value in the middle of the range 388–403 mV, *e.g.*, 395.5 mV, is chosen, V_e values shown as circles in Fig. 1 are obtained. These values all lie very close to a straight horizontal line.

Example 2

Titration of a very weak monoprotic acid. As an example of a very weak acid glycine has been chosen. Glycine has log $K \approx 9.5$ and is, furthermore, an ampholyte. Given that: 87.70 mg of glycine are dissolved in 49.93 ml of 0.333 M barium chloride solution and 49.95 ml

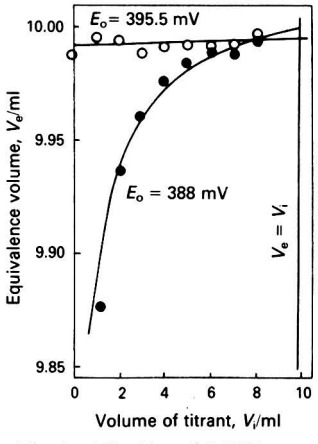

Fig. 1. Titration of 1.0018 mmol of benzoic acid with 0.0999 M sodium hydroxide solution. V_0 = 100 ml; ionic strength, 0.2 M; temperature, 25 °C. Hyperbolic regression analysis for $E_0' = 388$ mV gives $V_e = 9.999$ ml; yield, 99.72%. Linear regression analysis for $E_0' = 395.5$ mV gives $V_e = 9.994$ ml; yield, 99.68%. Note that the scale of the ordinate is 100 times that of the abscissa.

TABLE I

TITRATION OF A MONOPROTIC ACID OF INTERMEDIATE STRENGTH

Titration of 1.0018 mmol of benzoic acid with 0.09991 M sodium hydroxide solution. V_0 = 100 ml; ionic strength 0.2 M; temperature 25 °C.

Value No.	V/ml	E/mV	E'_0 = 388 mV* Log K	E'_0 = 388 mV* V_e/ml	E'_0 = 395.5 mV† Log K	E'_0 = 395.5 mV† V_e/ml
0	0	218.3	3.6602	9.7387	3.9435	9.9881
1	1.0154	204.4	3.7520	9.8763	3.9469	9.9948
2	2.0308	191.7	3.7867	9.9363	3.9458	9.9927
3	3.0462	180.5	3.7994	9.9597	3.9440	9.9892
4	4.0616	170.1	3.8079	9.9755	3.9457	9.9924
5	5.0770	160.1	3.8118	9.9829	3.9460	9.9930
6	6.0924	149.8	3.8138	9.9868	3.9459	9.9929
7	7.1078	138.3	3.8150	9.9891	3.9458	9.9927
8	8.1232	123.8	3.8176	9.9944	3.9477	9.9963
9	9.1386	100.8				

* For E'_0 = 388 mV hyperbolic regression analysis gives V_e = 9.999 ml; yield = 99.72%.
† For E'_0 = 395.5 mV linear regression analysis gives V_e = 9.994 ml; yield = 99.68%.

of water, *i.e.*, V_0 = 99.88 ml; temperature, 25.0 °C; titrant pipette volume, 1.0206 ml; titrant, 0.09898 M sodium hydroxide solution and 0.13 M barium chloride solution. The E_0 value was not determined, but was assumed to be 399 mV, based on earlier experience with the electrode couple that was used. The data for the titration are given in Table II. A preliminary equivalence volume was first calculated from values 4 and 6 using the equation for a weak acid, equation (26) in reference 1. A value of about 12 ml was obtained.

Thus, the values i = 1, 2, 3, . . ., 10 and j = 11 were chosen for the calculations. In this example the value of K_w has to be considered and an excess of titrant was added. Equations (5), (6) and (13) were used for the evaluation. The following results were obtained: arithmetic mean, V_e = 11.831 ml, yield = 100.2%; linear regression V_e = 11.812 ml, yield 100.08%; hyperbolic regression V_e = 11.818 ml, yield = 100.13%. Thus, all three calculation methods gave acceptable results. The values obtained for log K = 9.46 and $-\log K_w$ = 13.56 may be subject to a systematic error due to the fact that E'_0 was not determined. As glycine is an ampholyte the first value is very uncertain and has to be discarded.

In a duplicate titration on 84.00 mg of glycine the following values were obtained for i = 1, 2, . . ., 9 and j = 10 and using hyperbolic regression: V_e = 11.297 ml and yield = 99.9%. Arithmetic mean and linear regression gave essentially the same results.

Example 3

Conditional titration of p-*nitrophenol.* Sodium acetate cannot be used as the added salt in the conditional titration of *p*-nitrophenol. This acid is far too weak (log $K \approx 6.5$) to liberate an equivalent amount of acetic acid from the acetate. In such instances sodium sulphite has been suggested[3] as the salt. The equilibrium constant for the reaction $SO_3^{2-} + H^+ = HSO_3^-$ is $\sim 10^7$.

The sodium hydroxide solution to be used for the titration of the *p*-nitrophenol was standardised against a known amount of hydrochloric acid in a solution of a composition similar to that used in the main titration. The conditions were: 49.93 ml of 0.02000 M hydrochloric acid and 49.95 ml of 0.5 M sodium sulphite solutions mixed; V_0 = 99.88 ml; temperature, 25.0 °C; pipette volume, 1.0206 ml; titrant about 0.1 M sodium hydroxide in 0.4 M sodium chloride solution. The E'_0 value was not determined but was assumed to be 398 mV. Three titrations were performed and the following results were obtained: V_e = 10.333, 10.332 and 10.348, average 10.338, which gives C_b = 0.09660. Linear regression was used in the calculations.

Next 49.93 ml of a 0.02000 M solution of *p*-nitrophenol were substituted for the hydrochloric acid solution. The data for such a titration are given in Table III. Two titrations of *p*-nitrophenol were performed giving (using linear regression analysis) 10.345 and 10.337 ml,

average 10.341 ml. Thus, on average the same equivalence volume was obtained in the standardisation of the sodium hydroxide solution against hydrochloric acid as with the titration of the *p*-nitrophenol solution of the same strength.

TABLE II

TITRATION OF A VERY WEAK MONOPROTIC ACID

Titration of 87.7 mg of glycine dissolved in 49.93 ml of 0.333 M barium chloride solution + 49.95 ml of water. Titrant 0.09898 M sodium hydroxide solution; $V_0 = 99.88$ ml; ionic strength 0.5 M; $E'_0 = 399$ mV; temperature 25 °C. Linear regression analysis gives $V_e = 11.812$ ml; yield = 100.08%. Hyperbolic regression analysis gives $V_e = 11.818$ ml; yield = 100.13%.

Value No.	V/ml	E/mV	Log K	$-$Log K_w	V_e/ml
0	0	47.0	10.1430*	13.9153*	17.4748*
1	1.0206	−99.8	9.4597	13.5575	11.8186
2	2.0412	−120.5	9.4675	13.5586	11.8445
3	3.0618	−133.8	9.4701	13.5590	11.8531
4	4.0824	−144.1	9.4665	13.5585	11.8413
5	5.1030	−153.3	9.4662	13.5585	11.8401
6	6.1236	−161.9	9.4642	13.5582	11.8334
7	7.1442	−170.5	9.4629	13.5580	11.8290
8	8.1648	−179.5	9.4610	13.5577	11.8230
9	9.1854	−189.5	9.4618	13.5578	11.8256
10	10.2060	−200.8	9.4531	13.5565	11.7972
11	11.2266	−214.1			
20	20.4120	−276.2†			

* Values not used in regression analysis.
† Value used in the calculation of K_w.

The calculations were performed in the following way. A preliminary V_e value was first calculated using the simple equation for a strong acid [equation (23) in Part V of this series.[1]] A value of 10.5 was obtained. This value was then used in equations (15), (14) and (13) and the calculations were iterated three times. The values of log K and $-$log K_w in Table III may be wrong because E'_0 was not determined.

Example 4

Titration of a diprotic acid. The sample used was 99.60 mg of succinic acid dissolved in 50.0 ml of 0.333 M barium chloride solution and 50.0 ml of water. Temperature, 25.0 °C; titrant pipette volume, 1.0011 ml; titrant, 0.0989 M sodium hydroxide and 0.1 M barium chloride solution. No value of E'_0 was determined.

The data for the titration are given in Table IV. The e.m.f. values show that V_{e2} is about 17 ml. With $i = 9, 10, \ldots, 16$ and $j = 17$ the following values were obtained using equation (17): arithmetic mean $V_e = 17.067$ ml, yield = 100.06%; linear regression $V_e = 17.008$ ml, yield = 99.72%; and hyperbolic regression $V_e = 17.042$ ml, yield = 99.92%. In a duplicate titration the yields were 100.48, 99.27 and 99.87%, respectively.

It is clear that the highest precision is obtained by using the hyperbolic regression analysis.

Discussion

In Parts V[1] and VI of this series the Gran I method has been used for the calculation of the equivalence volume in acid - base titrations. Earlier, two other programs, EKVOL[4] and TITRA,[5] were published in this series. TITRA is a general program for the evaluation of potentiometric acid - base titrations, where the sample may consist of a mixture of several monoprotic or polyprotic acids. It utilises non-linear regression techniques and requires the availability of a good desk-top calculator.

Simpler titration problems are more advantageously solved by using the EKVOL program based on extended Gran II equations or by using this extension of the Gran I method. These two methods can solve the same types of problems, *i.e.*, the determination of one equivalence volume. The sample may consist of a strong or weak monoprotic acid, an ampholyte or certain types of di- or triprotic acids. Hence, a comparison between the two methods may be of interest.

TABLE III

TITRATION OF *p*-NITROPHENOL AS A CONDITIONALLY STRONG ACID IN SODIUM SULPHITE SOLUTION

49.93 ml of 0.020 00 M *p*-nitrophenol was mixed with 49.95 ml of 0.5 M sodium sulphite solution and titrated with 0.096 60 M sodium hydroxide solution. V_0 = 99.88 ml; ionic strength about 0.75 M; E'_0 = 398 mV (assumed value); temperature 25 °C. Linear regression analysis gives V_e = 10.345 ml; yield = 100.08%.

Value No.	V/ml	E/ml	Log K	$-$Log K_w	V_e/ml
0	0	−70.2	6.5348	13.6408	10.3386
1	1.0206	−73.0	6.5352	13.6408	10.3389
2	2.0412	−76.2	6.5372	13.6409	10.3400
3	3.0618	−79.6	6.5359	13.6408	10.3393
4	4.0824	−83.7	6.5380	13.6409	10.3404
5	5.1030	−88.4	6.5385	13.6410	10.3407
6	6.1236	−94.1	6.5392	13.6410	10.3411
7	7.1442	−101.4	6.5411	13.6411	10.3421
8	8.1648	−111.4	6.5428	13.6412	10.3431
9	9.1854	−127.8	6.5507	13.6416	10.3475
10	10.2060	−168.8			
15	15.3090	−268.2*			

* Value used in the calculation of K_w.

The EKVOL program is based on the solution of a set of linear equations with a maximum of three unknown variables and thus requires that the computer can solve such problems. The calculations in the Gran I method are simpler still and in most instances a pocket calculator is sufficient.

Both the EKVOL and the Gran I method use different equations for the calculation of titrations on strong acids, relatively strong acids, weak acids and very weak acids. The EKVOL program automatically chooses a suitable equation without any information about the stability constant of the acid. In the Gran I method one has to choose a suitable equation, *i.e.*, one has to have at least a rough idea of the size of the stability constant. In practice this is also normally so. (It is only in instruction laboratories that samples are completely unknown.)

TABLE IV

TITRATION OF A DIPROTIC ACID

Titration of 99.60 mg of succinic acid dissolved in 50.0 ml of 0.333 M barium chloride solution + 50.0 ml of water. Titrant 0.098 9 M sodium hydroxide and 0.1 M barium chloride solution. V_0 = 100 ml; ionic strength about 0.5 M; E'_0 = 397 mV (assumed value); temperature 25 °C. Linear regression analysis gives V_e = 17.008 ml; yield = 99.72%. Hyperbolic regression analysis gives V_e = 17.042 ml; yield = 99.92%.

Value No.	V/ml	E/ml	V_e/ml
9	9.0099	135.1	17.1761
10	10.0110	126.9	17.0726
11	11.0121	118.6	17.0566
12	12.0132	109.8	17.0504
13	13.0143	100.4	17.0471
14	14.0154	89.6	17.0453
15	15.0165	76.1	17.0441
16	16.0176	55.7	17.0434
17	17.0187	−43.3	

No significant difference in the results is obtained if the EKVOL program or the Gran I method is used. The latter has the advantage that it allows the easy calibration of a glass electrode through titration of a known amount of hydrochloric acid. Both the conditional normal potential E'_0 and the constant j_H in the expression for the liquid junction potential can be determined at the same time as the strong base titrant is standardised.

Errors from the ph Determination

In the calculations of all three methods we have presumed that the volume errors can be made small in comparison with the errors in the determinations of ph or e.m.f. In order to achieve this the titrations have been performed with accurate automatic pipettes. They were pneumatically operated, which results in a continuous stream of reagent, with no drops remaining on the tip of the pipette.

Hence, the titration error originating from the uncertainty in the determination of ph or e.m.f. will be discussed first. As an example we have chosen a Gran I titration of a strong acid, but the results will also be very similar for other types of acids.

Discussion of the errors in potentiometric titrations has been given by, for example, Hansson and Jagner,[6] Ingman and Still[7] and Meites *et al.*[8,9]

For a strong acid the following relationship applies:

$$V_e = V_j + \frac{V_j - V_i}{\left(\frac{V_0 + V_i}{V_0 + V_j} \cdot \frac{[H]_i}{[H]_j}\right) - 1} \quad .. \quad .. \quad .. \quad (26)$$

where it is assumed that $V_j > V_i$.

Differentiation of V_e with respect to $[H]_i$ gives

$$\partial V_e = -\frac{\frac{V_0 + V_i}{V_0 + V_j} \cdot \frac{V_j - V_i}{[H]_j}}{\left\{\left(\frac{V_0 + V_i}{V_0 + V_j} \cdot \frac{[H]_i}{[H]_j}\right) - 1\right\}^2} \cdot \partial \mathrm{ph}_i \quad .. \quad .. \quad .. \quad (27)$$

If $-\mathrm{d}[H] = (\ln 10)[H]\mathrm{dph}$ is inserted and the equation is rearranged one obtains

$$\partial V_e = \frac{(V_e - V_i)(V_e - V_j)}{V_j - V_i} (\ln 10)\, \partial \mathrm{ph}_i \quad .. \quad .. \quad .. \quad (28)$$

In the same way differentiation with respect to $[H]_j$ leads to

$$\partial V_e = -\frac{(V_e - V_i)(V_e - V_j)}{V_j - V_i} (\ln 10)\, \partial \mathrm{ph}_j \quad .. \quad .. \quad .. \quad (29)$$

In general, differentiation of all equations of the type

$$V_e = V_j + \frac{V_j - V_i}{\left(\frac{a_i}{a_j} \cdot \frac{[H]_i}{[H]_j}\right) - 1} \quad .. \quad .. \quad .. \quad .. \quad (30)$$

leads to equations (28) and (29).

On the presumption that ∂ph is constant during the whole titration, equation (29) shows that the error in V_e decreases rapidly as V_j approaches V_e. It is thus important to choose a value for V_j as close to V_e as possible. If only one value for V_j is used in the calculation of V_e the error in V_e caused by the error in V_j will be of a systematic character. Thus, it will be suitable to calculate V_e for two values of V_j just before the equivalence volume, if this, for experimental reasons, is not inconvenient or prohibited. A conceivable reason may be the slow approach of the equilibrium ph value near the equivalence point. In the neighbourhood of V_e the presence of impurities may also strongly influence the ph determination.

Errors in the Volume Determination

When an automatic pipette is not used the error in V_e caused by errors in V_i and V_j may be of importance. In such instances the error in V_i and V_j may amount to 0.01–0.02 ml. If V_e in equation (26) for a strong acid is differentiated with respect to V_i or V_j the following two expressions can be deduced:

$$\partial V_e = -\frac{V_o + V_e}{V_o + V_i} \cdot \frac{V_e - V_i}{V_j - V_i} \cdot \partial V_i \quad .. \quad .. \quad .. \quad (31)$$

$$\partial V_e = \left(1 + \frac{V_o + V_e}{V_o + V_j} \cdot \frac{V_e - V_i}{V_j - V_i}\right) \cdot \partial V_j \quad .. \quad .. \quad .. \quad (32)$$

In general, differentiation of equations of the type

$$V_e = V_j + \frac{V_j - V_i}{\left(\frac{P + V_i}{P + V_j} \cdot \frac{[H]_i}{[H]_j}\right) - 1} \quad .. \quad .. \quad .. \quad (33)$$

where $P = V_o$ for strong acids,

$P = 0$ for weak acids ($4.2 < \log K < 7$) and

$P = \frac{C_{AO}\, V_o}{C_B} - V_e$ for conditionally strong acids

leads to expressions of the type

$$\partial V_e = -\frac{P + V_e}{P + V_i} \cdot \frac{V_e - V_j}{V_j - V_i} \cdot \partial V_i \quad .. \quad .. \quad .. \quad (34)$$

$$\partial V_e = \left(1 + \frac{P + V_e}{P + V_j} \cdot \frac{V_e - V_i}{V_j - V_i}\right) \cdot \partial V_j \quad .. \quad .. \quad .. \quad (35)$$

where P has the same meaning as in equation (33).

It is obvious that an error in V_e caused by an error in V_i decreases rapidly as V_j approaches V_e and that for strong acids and conditionally strong acids, where $P > 5V_e$, $\partial V_e/\partial V_j$ is relatively uninfluenced by the size of V_i when V_j is close to V_e.

For weak acids $P = 0$ and

$$\partial V_e = -\frac{V_e}{V_i} \cdot \frac{V_e - V_j}{V_j - V_i} \cdot \partial V_i \quad .. \quad .. \quad .. \quad .. \quad (36)$$

$$\partial V_e = \left(1 + \frac{V_e}{V_j} \cdot \frac{V_e - V_i}{V_j - V_i}\right) \cdot \partial V_j \quad .. \quad .. \quad .. \quad (37)$$

It is easily found that $\partial V_e/\partial V_i$ has a minimum for $V_i = V_j/2$. Again $\partial V_e/\partial V_j$ is relatively uninfluenced by the value of V_i, if V_j is close to V_e.

It may also be of interest to see how V_e would be influenced by an error in the determination of V_o.

Differentiation of equation (26) with respect to V_o gives

$$\partial V_e \approx -\frac{V_e - V_i}{V_o + V_i} \cdot \frac{V_e - V_j}{V_o + V_j} \cdot \partial V_o \quad .. \quad .. \quad .. \quad (38)$$

In a similar way differentiation of equation (33) with $P = (C_{AO}\, V_o/C_B) - V_e$ with respect to V_o gives

$$\partial V_e \approx -\frac{C_{AO}}{C_B} \cdot \frac{V_e - V_i}{P + V_i} \cdot \frac{V_e - V_j}{P + V_j} \cdot \partial V_o \quad .. \quad .. \quad .. \quad (39)$$

Finally, differentiation of equation (33) with respect to C_{AO} leads to

$$\partial V_e \approx + \frac{V_e}{C_B} \cdot \frac{V_e - V_i}{P + V_i} \cdot \frac{V_e - V_j}{P + V_j} \cdot \partial C_{AO} \quad .. \quad .. \quad .. \quad (40)$$

Conclusions

The errors in V_e caused by the various parameters will be different for strong, conditionally strong and weak acids, but the total error will be of about the same size, 0.02 ml, as shown in Table V.

TABLE V

ERRORS IN V_e CAUSED BY VARIOUS PARAMETERS

	Acid type		
Parameter causing error	Strong	Conditionally strong	Weak
Δph = 2 × 0.002	0.0184	0.0184	0.0184
ΔV_i = 0.0002 ml	0.0002	0.0002	0.0003
ΔV_j = 0.0002 ml	0.0006	0.0006	0.0006
ΔV_o = 1 ml	0.0002	0.0001	—
ΔC_{AO} = 0.0025 M	—	0.0001	—
Sum of errors	0.0194	0.0194	0.0193

All values in Table V have been calculated for the following conditions: V_o = 100 ml, V_e = 10 ml, V_j = 9 ml, V_i = 8 ml, C_{AO} = 0.25 M and C_B = 0.1 M.

It is obvious that the greatest errors in V_e are caused by errors in the measurement of ph, at least when accurate pneumatically operated pipettes are used for adding the titrant. The error caused by an error in ph will be smaller if low values of V_i are chosen. This error also decreases rapidly, if V_j approaches V_e, *e.g.*, if V_j = 9.5 ml and V_i = 8.5 ml, ΔV_e caused by errors in ph_i and ph_j would be only = 0.007 ml.

In most instances only the errors due to inaccuracy in the ph measurement have to be taken into account. If a data pair (V_j — ph_j) is used together with all possible data pairs (V_i — ph_i) and the graph $V_{e(i)} = f(V_i)$ is plotted against V_i, the errors in ph_i should tend to cancel each other, as should the errors in V_i. Other errors, however, will be of a more systematic type. This might be at least partly eliminated by making the calculations using two data pairs (V_j — ph_j).

The error considerations given above have been made by differentiating the simple equations in Part V. It will be tedious to differentiate the corresponding equations in Part VI. However, for relatively strong acids the conditions should be somewhere between strong and weak acid and as shown above no great differences would be expected.

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NOTE—References 4, 5 and 1 are to Parts III, IV and V of this series, respectively.

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SHORT PAPERS

Spectrophotometric Determination of Nitrite in Waters

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Keywords: Nitrite determination; water; 4-aminosalicylic acid reagent; diazotisation; spectrophotometry

Because of the role of nitrite ion as an important precursor in the formation of *N*-nitrosamines, many of which have been shown to be potent carcinogens,[1–3] and as nitrite, when correlated with other forms of nitrogen in water, can provide an index of organic pollution,[4] a sensitive and rapid method for the determination of nitrite is desirable. A method based on the formation of an azo dye (anion) is proposed here for the micro-determination of nitrite.

Experimental

Apparatus

Spectral and absorbance measurements were made with a Shimadzu UV-210A double-beam spectrophotometer using 10-mm glass cells.

Reagents

All chemicals used were of analytical-reagent grade.

Standard nitrite solution. Dissolve 1.4990 g of sodium nitrite in distilled water, add 1 ml of spectroscopic grade chloroform and a pellet of sodium hydroxide[5] and dilute the solution to 1 l with distilled water. This solution contains 1000 p.p.m. of nitrite ion. Prepare less concentrated solutions by dilution with distilled water.

4-Aminosalicylic acid solution. Dissolve 0.25 g of the sodium salt of 4-aminosalicylic acid monohydrate in 0.14 M hydrochloric acid and dilute to 100 ml with 0.14 M hydrochloric acid.

Naphth-1-ol solution. Add 0.35 g of naphth-1-ol to distilled water containing 1.5 g of sodium hydroxide, dissolve by stirring and dilute to 100 ml with distilled water.

Interfering ions solution. Prepare a 1000 μg ml^{-1} solution of each ion to be tested.

Procedure

Transfer an aliquot of the sample solution containing 1–30 μg of nitrite into a series of 10-ml calibrated flasks, add 1 ml of 0.25% 4-aminosalicylic acid solution and 1 ml of 0.35% naphth-1-ol solution (in 1.5% sodium hydroxide solution), dilute to the mark with distilled water and mix thoroughly. Measure the absorbances against a reagent blank prepared in the same manner but containing no nitrite ion, at 520 nm using 10-mm glass cells.

The colour develops immediately and is stable for up to 36 h. The nitrite concentration can be determined from a calibration graph constructed by plotting known concentrations of nitrite against the corresponding absorbance values, which gives a straight line passing through the origin at nitrite concentrations in the range 0–3 μg ml^{-1}.

Results and Discussion

Spectral Studies

Fig. 1 shows the absorption spectrum of the azo dye formed in alkaline medium, the maximum absorption being centred at 520 nm. The slight absorption of the reagent blank at 520 nm emphasised the need for measurements to be performed against the reagent blank.

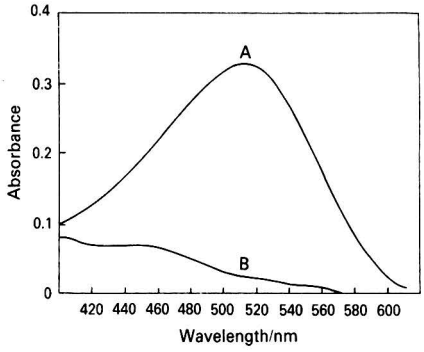


Fig. 1. Absorption spectra of: A, sample solution, treated as in the procedure, measured against reagent blank; and B, reagent blank measured against distilled water.

The calculated apparent molar absorptivity, in the region of least photometric error, at 520 nm was 14.7×10^3 l mol^{-1} cm^{-1} and the sensitivity of the method (expressed as the amount of nitrite corresponding to an absorbance of 0.001) in a 10-mm cell at 520 nm was 0.0031 μg cm^{-2}.

In subsequent experiments, 10 μg of nitrite were taken and the final volume was made up to 10 ml.

Choice of Diazo Component

For the determination of nitrite with naphth-1-ol in alkaline medium, many primary aromatic amines, including orthanilic acid, 2-aminophenetole, 4-aminophenetole, 2-nitroaniline and 4-aminosalicylic acid, were tested for optimum conditions. Of these primary amines, only 4-aminosalicylic acid gave useful results. Moreover, 4-aminosalicylic acid is easily soluble in mineral acids, giving a colourless solution, is easily diazotised and gives a water-soluble azo dye under the conditions of the determination, thus eliminating the extraction process, which is a time-consuming procedure. Therefore, this compound was chosen for further investigation.

Effects of 4-Aminosalicylic Acid and Hydrochloric Acid Concentrations

The effect of 4-aminosalicylic acid concentration on the absorbance of the coloured product, using the described procedure, was tested. To a series of nitrite solutions were added 1 ml of a solution of 4-aminosalicylic acid in 0.1 M hydrochloric acid in the concentration range 0.05–0.35%. The results showed that 0.25% of 4-aminosalicylic acid gave the most pronounced effect; higher concentrations decreased the absorbance significantly (which can be attributed to self-coupling of the acid).

The effect of the concentration of hydrochloric acid, which is essential for the diazotisation process, on absorbance was examined in the range 0.03–0.23 M. A concentration of 0.14 M gave the maximum colour intensity; higher concentrations decreased the absorbance to low values. Other acids, when tested, did not give results as good as those obtained with hydrochloric acid; for example, acetic acid at the same concentration changed the pinkish red colour to yellow (which is much less sensitive).

The effect of the volume of the composite reagent (4-aminosalicylic acid + hydrochloric acid) was studied. It was found that 1 ml of the composite reagent gave maximum absorption.

Choice of Coupling Agent

Tiron (disodium salt), quinolin-8-ol, naphth-2-ol and napth-1-ol were tested as coupling agents for the determination of nitrite with 4-aminosalicylic acid. Only naphth-1-ol gave

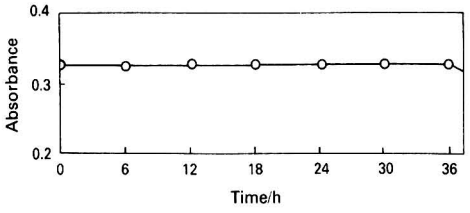


Fig. 2. Stability of the azo dye.

satisfactory results. Tiron, for example, although water soluble, gave a pale yellow colour, and naphth-2-ol coupled but showed a hypsochromic effect in comparison with naphth-1-ol and is toxic. Therefore, only naphth-1-ol was studied further.

Effects of Naphth-1-ol and Sodium Hydroxide Concentrations

The effect of the concentration of naphth-1-ol on the colour intensity was studied. To a series of nitrite solutions were added 1 ml of solutions of naphth-1-ol in 1% sodium hydroxide solution in the concentration range 0.1–0.35%. The 0.35% naphth-1-ol solution gave the maximum absorbance; larger volumes of the reagent decreased the absorbance slightly, and lower concentrations caused decrease in absorbance due to multiple coupling.[6]

Sodium hydroxide solution was used to dissolve the naphth-1-ol because of the low solubility of the latter in water. Organic solvents in which the compound is soluble gave adverse effects. For example, *NN*-dimethylformamide slowed the reaction and gave a net decrease in the absorbance.

The concentration of sodium hydroxide necessary for colour development was examined in the range 1–2.5%. The results obtained showed that 1.5% sodium hydroxide solution was the optimum; a 2% solution decreased the absorbance by 15%. The volume of this second composite reagent was investigated and 1.0 ml gave the optimum absorbance; 1.5 ml caused a decrease in absorbance of 16%.

Colour Stability

The pinkish red colour of the azo dye formed develops instantaneously and remains stable for 36 h, after which there was a gradual deterioration (Fig. 2).

Accuracy and Precision of the Method

Under the above optimum conditions, the accuracy and precision (five replicate determinations) of the method were checked. The results are given in Table I, and indicate high accuracy and precision.

TABLE I

ACCURACY AND PRECISION OF THE METHOD

Nitrite taken/μg	Error, %	Relative standard deviation, %
1	+0.63	0.75
15	+0.67	0.49
25	−0.34	0.32
30	−0.003	0.24

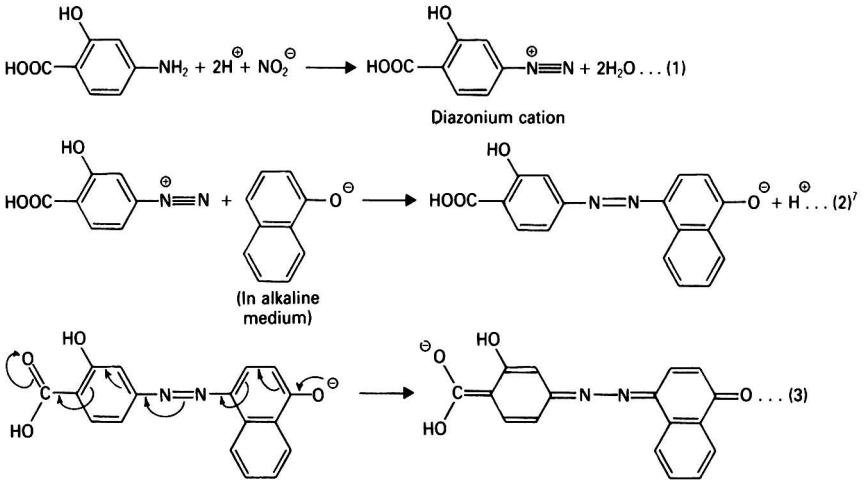
Study of Diverse Ions

In order to assess the possible analytical applications of this colour reaction, the effects of foreign ions that often accompany nitrite were examined by carrying out determinations on 10 μg of nitrite in the presence of each of these ions. The results are given in Table II.

The method is more selective towards anions than cations; this is not unexpected because these azo dyes are commonly used chromogens for metal ions. However, an ion-exchange procedure can be used to remove the seriously interfering ions.

Proposed Reaction Mechanism

The suggested reaction sequence in the proposed method is the following:



Comparison With Other Methods

The method is superior to most of the Griess modifications owing to its simplicity, rapidity, availability of reagents, non-toxicity and its elimination of time-consuming extraction. Further, it is more accurate, precise and has a wider range than the quinolin-8-ol method.[8]

TABLE II

EFFECT OF DIVERSE IONS ON THE DETERMINATION OF NITRITE

Interferent	Permissible amount in the presence of 10 μg of nitrite/μg*
Ammonia	150
Carbonate	50
Chloride	180
Cyanide	75
Fluoride	120
Iodide	140
Hydrogen carbonate	110
Nitrate	80
Phosphate	110
Sulphide	4
Sulphite	25
Calcium	60
Cadmium	9
Cobalt(II)	8
Copper(II)	10
Iron(III)	7
Magnesium	15
Mercury(II)	30
Lead(II)	30
Tin(II)	20
Phenol	170

* Amount of diverse ion causing an error of less than 2% in the determination.

Conclusion

A sensitive, selective, accurate and precise method has been developed for the determination of nitrite in the range 0.1–3 μg ml^{-1}. Because of its advantageous characteristics, the method will be useful in routine analysis of nitrite. However, many ions commonly present in waters can interfere in the determination at concentrations at which they are normally present (see Table II). A suitable preliminary ion-exchange clean-up may therefore frequently be required.

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Determination of Water in *NN*-Dimethylformamide by the Kinetic Method of Tangents Using the Oxidation of Catechol as the Indicator Reaction

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Keywords: NN-*Dimethylformamide*; *sodium metaperiodate*; *catechol*; *spectrophotometry*; *kinetics*

The oxidation of catechol in water and other solvents has been shown to be first order in catechol and in oxidising agents and second order for the over-all reaction.[1–5] In aprotic solvents the rate of reaction is dependent on the water content in the solvent[6–8] and is several orders of magnitude slower than in aqueous media.

Although water probably enters into the reaction by changing the oxidising species,[9,10] analytically it functions as a catalyst and the method of tangents can be used to determine the amount of water in *NN*-dimethylformamide (DMF).

Experimental

Reagents

All reagents were of analytical-reagent grade. All oxidising agents were dried at 150 °C and stored over magnesium perchlorate before being used.

Sodium chlorate, $NaClO_3$.
Sodium perchlorate, $NaClO_4$.
Sodium metaperiodate, $NaIO_4$.
Catechol. $ClH_4(OH)_2$.
NN-*Dimethylformamide* (*DMF*). Dried over molecular sieves and vacuum distilled before use.

Apparatus

A Bausch and Lomb Spectronic 70 instrument and a 10 × 10 mm matched quartz cuvette were used.

Procedure

Standard solutions of catechol (1.02×10^{-3} M) and oxidising agent (2.00×10^{-2} M) were separately prepared in DMF and deoxygenated with argon for approximately 30 min. A blank was prepared by mixing equal amounts of the above. For the standards, the water was weighed directly into calibrated flasks. Catechol (5 ml) and oxidising agent (5 ml) were added and the samples were diluted to volume with DMF.

The solutions were placed in a constant-temperature bath. The sodium metaperiodate solutions were thermostatically maintained at 24 $\pm$ 1 °C and the sodium chlorate and perchlorate solutions at 85 $\pm$ 1 °C. Aliquots were withdrawn at timed intervals and the absorbance of the solution was determined at 514 nm (the $\lambda_{max.}$ for *o*-benzoquinone) using DMF in the reference beam.

The spectrophotometric results were processed on an IBM 370–75 computer by the method of least squares. Only results with a correlation coefficient of 0.94 or better were retained for evaluation. An aliquot of the unknown DMF solution was placed in a calibrated flask and determined by the same technique as was used for the standard.

Results

The initial graph of log[*o*-benzoquinone] *versus* time does not yield a linear relationship (Fig. 1). This is consistent with previous observations,[1,11] which have shown that the initial rate-determining step is the formation of a cyclic diester. A linear relationship is obtained after a steady-state system is achieved and the rate-determining step becomes the decomposition of the cyclic diester to *o*-benzoquinone. A graph was prepared for each set of results. The rate, as expressed by the second-order rate equation for the steady-state system *versus* time, was used for the analysis.

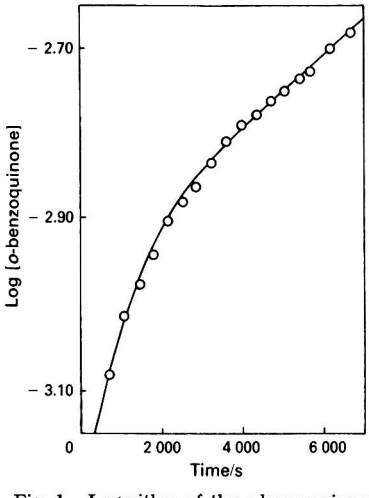

Fig. 1. Logarithm of the *o*-benzoquinone concentration in DMF as a function of time. 4.95×10^{-2} M $NaIO_4$, 4.76×10^{-3} M catechol and 2.21 M water.

A series of straight lines were obtained. The slope increased with increase in the water concentration. The zero intercepts were 6.3×10^{-4} l mol^{-1} s^{-1} for sodium metaperiodate and 1.6×10^{-4} l mol^{-1} s^{-1} for sodium chlorate and perchlorate. A direct application of the equation for the method of tangents[12]

$$C = \frac{\tan \alpha - \tan \alpha_0}{K_a}$$

cannot be used because a graph of C *versus* tan α–tan α_0 yields a curve, but a graph of $[H_2O]^2$ *versus* rate (Figs. 2 and 3) yields a straight line.

The amount of water in DMF can be determined at levels from 100 p.p.m. to 50%. At the higher concentrations (above 20%), the reaction rate is too rapid to obtain accurate results using sodium periodate and either sodium chlorate or perchlorate should be used. At the higher concentrations the method is accurate to within $\pm$ 4%. At lower concentrations (parts per million range) errors of $\pm$ 10% occur unless the samples are prepared in a dry box.

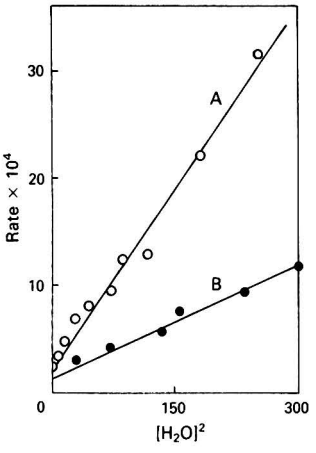

Fig. 2. Rate constant for the oxidation of catechol in DMF as a function of the molarity of water squared. Oxidising agent: A, $NaClO_3$; and B, $NaClO_3$.

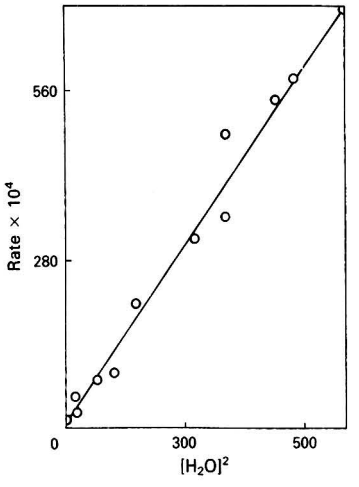

Fig. 3. Rate constant for the oxidation of catechol in DMF as a function of the molarity of water squared. Oxidising agent: $NaIO_4$.

Discussion

In water periodate is only 80% hydrated[9] and the equilibrium can be expressed by the equation

$$IO_4^- + 2H_2O \rightarrow H_4IO_6^- \quad .. \quad .. \quad .. \quad .. \quad (1)$$

As the $H_4IO_6^-$ species is probably the oxidising agent in both aqueous and non-aqueous media, this equation would explain the catalytic-type effect that water imparts to the oxidation of catechol.

As a similar relationship exists in the graphs of rate *versus* $[H_2O]^2$ for sodium chlorate and perchlorate, the same explanation [equations (2) and (3)] can be put forth for the kinetic effect of water on these oxidising agents:

$$ClO_3^- + 2H_2O \rightarrow H_4ClO_5^- \quad .. \quad .. \quad .. \quad .. \quad (2)$$

$$ClO_4^- + 2H_2O \rightarrow H_4ClO_6^- \quad .. \quad .. \quad .. \quad .. \quad (3)$$

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Spectrophotometric Determination of Vanadium(V) Using 4-Benzoyl-3-methyl-1-phenyl-5-pyrazolone

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Keywords: *Vanadium determination*; *spectrophotometry*; *4-benzoyl-3-methyl-1-phenyl-5-pyrazolone*; *solvent extraction*

4-Benzoyl-3-methyl-1-phenyl-5-pyrazolone(BMPP) is a chelating agent that has been used as an efficient extractive reagent for the isolation and separation[1] of several elements. Jensen[2,3] was the first to describe its synthesis and properties, and it has been investigated in more detail by many workers.[4–6]

In previous papers,[7,8] we described an atomic-absorption spectrophotometric method using BMPP as a chelating agent, and found that it is very useful for the extraction of many cations. Also, BMPP has been used for the spectrophotometric determination of vanadium(IV) and vanadium(V) in a vanadium catalyst having a silica matrix.[9] However, there have been few studies of the use of BMPP in the practical situations.

BMPP reacts with vanadium(V) to give a red complex, which is extractable into chloroform - butan-1-ol (4 + 1) solvent mixture in the pH range 2.5–4.5. This paper describes the use of BMPP as a reagent for the selective spectrophotometric determination of vanadium(V). The method has been applied to the determination of vanadium in several steels.

Experimental

Apparatus

A Shimadzu QV50 spectrophotometer with a 1-cm silica cell was used for absorbance measurements. A Toa Denpa HM-5BS pH meter was used for pH measurements.

Reagents

The reagents used were of analytical-reagent grade.

BMPP reagent solution. BMPP was synthesised according to an established method.[2] Elemental analysis confirmed the synthesis (calculated for $C_{17}H_{14}N_2O_2$, C = 73.37, H = 5.07, N = 10.07 and O = 11.50%; found, C = 73.27, H = 4.91, N = 9.98 and O = 11.83%). A 0.1% *m*/*V* solution of BMPP was prepared by dissolving 0.1 g of BMPP in 100 cm^3 of chloroform - butan-1-ol (4 + 1).

Vanadium(V) standard solution, 1 mg cm^{-3}. This solution was prepared by dissolving 1.1489 g of ammonium metavanadate in 1 mol dm^{-3} sulphuric acid and diluting to 500 cm^3 with distilled water.

Buffer solution, pH 3.0. Acetic acid (0.1 M) was adjusted to pH 3.0 with 0.1 M sodium acetate solution.

Procedure

To 10 cm^3 of sample solution in a separating funnel containing up to 100 μg of vanadium(V), add 10 cm^3 of buffer solution (pH 3.0) and dilute to 30 cm^3 with distilled water. Shake vigorously for 5 min with 5 cm^3 of chloroform - butan-1-ol (4 + 1) containing 0.1% *m/V* BMPP solution. Allow the phases to separate, transfer the organic layer into a 10-cm^3 centrifuge tube and centrifuge it for 5 min at 2500 rev min^{-1} to give an organic layer free from water. Measure the absorbance, at 490 nm, of the organic layer against a reagent blank prepared in the same way.

Beer's law was obeyed up to at least 100 μg of vanadium(V) in 5 cm^3 of the solution and the molar absorptivity of the complex, ϵ, was 2.5×10^3 dm^3 mol^{-1} cm^{-1}.

Results and Discussion

The absorption spectra of BMPP and its vanadium(V) complex extracted with chloroform - butan-1-ol (4 + 1) at pH 3 and 1 are shown in Fig. 1. The complex exhibits maximum absorbance at 490 nm. BMPP itself does not absorb at this wavelength. All subsequent studies were carried out at 490 nm.

The effect of pH on the extraction of the vanadium(V) was studied by varying the pH of the aqueous solution from 1 to 6 (Fig. 2). The optimum pH range was 2.5–5.0. The pH of the aqueous solution was therefore adjusted to 3 with acetate buffer solution in all subsequent work. The complex is stable for 2.5 h, but the absorbance decreases slightly with passage of time.

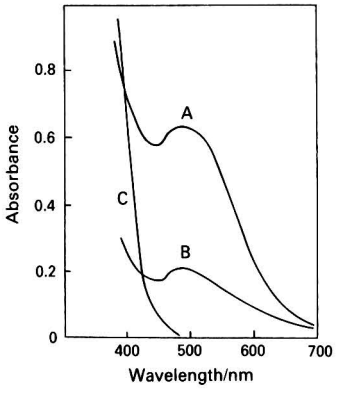

Fig. 1. Absorption spectra of BMPP and vanadium(V) - BMPP complex extracted into 5 cm^3 of chloroform - butan-1-ol (4+1) solvent mixture. Concentration of vanadium(V), 2.4×10^{-4} mol dm^{-3}. A, Vanadium(V) - BMPP complex extracted at pH 3; B, vanadium(V) - BMPP complex extracted at pH 1; and C, reagent blank.

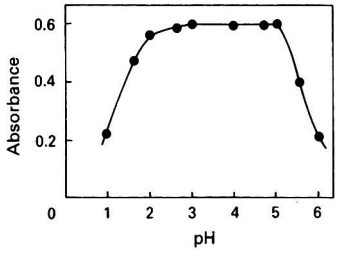

Fig. 2. Effect of pH on the extraction of 50 μg of vanadium(V).

The concentration of BMPP in chloroform - butan-1-ol (4 + 1) was varied from 0.025 to 0.3% *m/V* using 50 μg of vanadium(V). It was found that 5 cm^3 of 0.1% *m/V* BMPP solution is sufficient for the quantitative extraction of 5–100 μg of vanadium(V).

The use of different organic solvents was studied. The vanadium complex is partially extractable by benzene, chloroform, xylene and carbon tetrachloride and the sensitivity of the colour system in these solvents is therefore poor. Also, the complex is extracted with isobutyl methyl ketone but it is colourless at low concentrations of vanadium. When butan-1-ol is used as the organic solvent the colour system shows high sensitivity, but it is less stable than in

chloroform. The mixed solvent chloroform - butan-1-ol (4 + 1) gave a higher sensitivity than the other solvents examined. Moreover, this solvent renders the vanadium complex more stable than butan-1-ol. The precision of the method was studied by measuring the absorbance of ten solutions, each containing 5 and 50 μg of vanadium(V), in the absence of other metals. The coefficients of variation were 5.7 and 1.5% for 5 and 50 μg of vanadium(V), respectively.

Various ions were examined for their effect on the determination of vanadium(V). For this study different amounts of ionic species were added to the solution containing 50 μg of vanadium(V). The results are shown in Table I.

TABLE I

DETERMINATIONS OF 50 μg OF VANADIUM(V) IN THE PRESENCE OF DIVERSE IONS

Ion	Amount of ion added/mg	Vanadium (V) found/μg	Recovery, %
Ag(I)	5	49.9	99.8
Al(III)	5	49.4	98.8
Au(IV)	5	51.2	102.4
Ba(II)	5	48.8	97.6
Bi(III)	5	16.7	33.4
	1	48.3	96.6
Ca(II)	5	50.7	101.4
Cd(II)	5	49.3	98.6
Co(II)	5	49.0	98.0
Cr(VI)	5	48.8	97.6
Cu(II)	1	44.7	89.4
	0.5	50.5	101.0
Fe(III)	0.01	61.7	123.4
	0.005	54.3	108.6
K(I)	5	50.0	100.0
Mg(II)	5	50.3	100.6
Mn(II)	5	49.7	99.4
Mo(VI)	5	7.9	15.8
	0.5	38.4	76.8
	0.25	45.9	91.8
Ni(II)	5	49.4	98.8
Pb(II)	5	50.1	100.2
Pd(II)	5	49.8	99.6
Pt(IV)	5	49.5	99.0
Rh(III)	5	23.7	47.4
Sb(III)	1	44.5	89.0
Ti(IV)	2	20.9	41.8
	0.5	35.6	71.2
W(VI)	0.5	11.1	22.2
	0.05	44.4	88.8
Zn(II)	5	49.7	99.4

Three samples of Japanese Iron and Steel Standards (JSS) were subjected to the method. A sample of about 0.3 g was digested on a thermostatic hot-plate with 20 cm^3 of concentrated hydrochloric acid - nitric acid (3 + 1), and then evaporated almost to dryness. After cooling, 2 cm^3 of perchloric acid were added and the mixture was heated until digestion was complete. The residue was dissolved in 30 cm^3 of 6 mol dm^{-3} hydrochloric acid and then the solution was transferred into a separating funnel and most of the iron was removed by extraction with isobutyl methyl ketone (extract 3). The colour of the organic layer was used as the criterion for the completeness of the extraction.

The aqueous phase remaining from the extraction of iron was evaporated almost to dryness. It was then transferred into a 100-cm^3 calibrated flask and diluted to the mark with water. A 10-cm^3 aliquot of this solution was pipetted into a 50-cm^3 separating funnel. The solution was adjusted to pH 3 with acetate buffer solution and two drops of 0.1% potassium permanganate solution were added. The determination was carried out five times and the results are shown in Table II. The results for the vanadium content obtained with the proposed method agree

TABLE II

ANALYSIS OF STANDARD STEEL SAMPLES

Sample	Mass of sample/g	Vanadium content, % Certified	Found	Elements other than vanadium and iron, %
JSS 102–3	0.3236	0.035	0.0362 ± 0.0005	C, 4.65; Si, 0.26; Mn, 0.54; P, 0.089; S, 0.024; Ni, 0.019; Cr, 0.075; Cu, 0.011; Ti, 0.054; As, 0.002; Sn, 0.002; N, 0.006
JSS 151–7	0.3265	0.053	0.0585 ± 0.0017	C, 0.4; Si, 0.077; Mn, 1.48; P, 0.033; S, 0.017; Ni, 2.99; Cr, 0.10; Cu, 0.11; Mo, 0.046; Al, 0.006; N, 0.0102
JSS 175–3	0.3162	0.093	0.0911 ± 0.0023	C, 0.038; Co, 0.011; Al, 0.054; B, 0.0091; Nb, 0.011; Zr, 0.031; Sb, 0.0196

satisfactorily with certified values for the standard materials. This procedure is suitable for the determination of vanadium in the approximate range 0.01–0.3%.

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Book Reviews

FACTOR ANALYSIS IN CHEMISTRY. By EDMUND R. MALINOWSKI and DARRYL G. HOWERY. Pp. xii + 251. John Wiley. 1980. Price £14.30. ISBN 0 471 05881 5.

Owing to the great advances in analytical instrumentation and digital computers, analytical results are being generated on an unprecedented scale and extremely complex analytical problems are being conceived and tackled. It follows that, in order to make maximum use of the data, advanced mathematical methods have to be adapted to routine analytical use. Already a discipline of chemometrics has been defined and workers within it have demonstrated the power and applicability of mathematical methods to chemical problems.

One of the methods used by social scientists for nearly 60 years, but only recently applied to chemistry, is factor analysis, and this is the one treated by Malinowski and Howery in the most admirable and exemplary manner. They have faced up to the major problems of communication in the field of chemometrics. First, the cynical remark that "chemists are failed mathematicians" has a high degree of truth. Secondly, the need to present the mathematics in an honest way, showing the basis of the method and its limitations as well as its strengths.

The authors have followed the adage of the old preacher, "First I tell them what I'm going to say, then I say it and then I tell them what I've said." The message is clear. Factor analysis can be used to establish the number and relative importance of variables used to model or interpret complex sets of data, it can be used to determine systematic and random errors in the data and it can perform cluster analysis. It has a number of practical advantages over standard analysis of variance procedures, and in particular allows one parameter or a source of error to be tested independently.

The exposition throughout the book is admirably clear. A qualitative account of the method is given in the opening chapters and applications are reviewed at the end of the book. Many of the applications, such as the comparison of instruments and analytical procedures, and the resolution of closely overlapping chromatographic peaks or spectral bands, are of great interest to analysts. The core of the book is a detailed account of the method, the procedures for its application and the way in which its results may be interpreted. A knowledge of matrix algebra is assumed, and available computer programs are referenced but not reproduced. Worked examples ensure that the approach is practical whilst elucidating the principles upon which it is based.

Chemometrics must be a growth area in analytical science, so it is to be hoped that this excellent text is taken as a model by future authors.

D. BETTERIDGE

DYNAMICS, EXPOSURE AND HAZARD ASSESSMENT OF TOXIC CHEMICALS. Edited by RIZWANUL HAQUE. Pp. viii + 496. Ann Arbor Science Publishers. 1980. Price £18.50. ISBN 0 250 40301 3.

This is a compilation of the papers presented at a symposium held at Miami Beach, Florida, in September 1978 and sponsored by the Division of Environmental Chemistry, American Chemical Society. Undoubtedly the prime reason for such a meeting in the USA at that time was the need, following the enactment of that country's Toxic Substances Control Act (TOSCA) of 1976, for the exchange of information and the promotion of better communications on the evaluation of exposure, hazard and risk presented by toxic substances to man and his environment. Thus, a main theme of the book is the development and use of transport and fate studies in predicting the likely effects of toxic chemicals on the environment.

To the uninformed the use of the term "dynamics" in the title may be puzzling. However, this is defined immediately in the first chapter as "what happens to a chemical, where it goes and when it degrades" once it is in the environment. The coverage of the book is wide and the first seven of the total of 31 chapters set the scene by discussing the background to the need for transport and fate studies of toxic chemicals and summarise the part played by industry, regulatory agencies and universities in meeting the requirements of TOSCA.

The key chapter is that by Haque *et al.*, in which the basic concepts underlying transport and fate studies on toxic chemicals are reviewed. This is done with regard to the different parts of the environment, either through water, air or soil, with all routes eventually leading to the possibility of exposure of a variety of living organisms by these chemicals or their degradation products. The ten chapters following are each concerned with an examination of the different physical and chemical mechanisms and processes by which chemicals, mainly organic, can be transported through and/or degraded in the environment. By way of example, the contributions of several of these mechanisms on the fate of 3,3′-dichlorobenzidine in the aquatic environment are described.

The use of predictive tests or of mathematical models for hazard assessment are discussed at some length in the next three chapters; of particular topical interest is a well referenced paper on the ecological effects of the breakdown products of chlorinated hydrocarbon pesticides, many of which have been shown to be more toxic than the parent compounds. The special problems associated with carcinogenic compounds are covered in two papers. Of the later contributions, that on the identification of the complexities of predicting the harmful effects of chemicals to an ecosystem is of particular note. Two contributions on tests for the hazard evaluation of chemicals and another on the pathways for environmental human exposure and the consequent health risk assessment complete the volume.

Of considerable merit, this volume will have interest for those in a variety of scientific disciplines in addition to that of chemistry. Although primarily for an American readership, most of the material is generally applicable. The only minor criticism the reviewer has of an otherwise well balanced volume is the measure of duplication of material within certain chapters. In such a compilation, of papers presented at a symposium, this is probably unavoidable. However, this apart, this volume at £18.50, cheap by present day standards, can be readily recommended to both the general reader seeking an overview or to the specialist seeking more detailed information on the effects of toxic chemicals on the environment.

R. Wood

ADDITIVE MIGRATION FROM PLASTICS INTO FOOD. By T. R. CROMPTON. Pp. xii + 234. Pergamon Press. 1979. Price $47. ISBN 0 08 022465 2.

This book describes the analysis of plastic packaging materials for certain constituents that may be extracted into foodstuffs. The first two chapters review the types of polymer materials used for food packaging together with non-plastic additives and contaminants that may also be present. In Chapter 3, a discussion of the problems encountered in the use of simulants for extractability testing is presented. In the main this is based on the recommendations of the British Plastics Federation (1962) and the Food and Drug Administration of the USA (1962 onwards). This chapter also contains a description of the theory of migration from plastics into food based on the work of Garlanda and Masoero. Other theories of the migration phenomenon have, however, been ignored, as has the concept and determination of total migration. The following four chapters contain detailed analytical methods for the determination of selected compounds and degradation products in aqueous, alcoholic and hydrocarbon solvents, as well as in edible oils and fatty food extractants. There is a brief review of the determination of antioxidants in foods, along with a detailed consideration of legislative aspects, although more recently enacted UK legislation is excluded.

Whilst there is no doubt that there is great concern and interest amongst the food and packaging industry in the subject matter covered by this book, there is as yet no evidence of any hazard. However, the usefulness of this book to the analyst will depend on the choice of detailed analytical procedures presented. The book has been reproduced directly from the typescript. Unfortunately there are a number of typographical errors and the layout and presentation are poor. In Chapter 6, results are quoted so excessively that text and associated figures become widely separated. Newer analytical techniques, such as HPLC, have been largely ignored and there are no references later than 1975. Many methods are quoted without reference to the original work so that the book is rather unbalanced. The first four chapters contain only 10 citations, whilst there are 90 in Chapter 6, out of a total of 150. A fairly comprehensive ten-page index completes the book.

N. T. Crosby

QUANTITATIVE ANALYSIS OF ORGANIC MIXTURES. PART ONE: GENERAL PRINCIPLES. By T. S. MA and ROBERT E. LANG. Pp. xviii + 366. John Wiley. 1979. Price £15.85. ISBN 0 471 55800 1.

This publication is the first part of a two-volume work that describes methods for the analysis of mixtures of organic compounds, this first volume being concerned with general principles. Presumably working details of the methods will be presented in the second volume.

The first chapter describes the various problems affecting composition such as environment, the effect of impurities and other extraneous matter. In later chapters sampling techniques under various conditions are discussed and guides to selection of the most suitable analytical method are included. There is an extensive discussion on methods for the determination of several components in a mixture with and without separation. The final chapters deal with the separation of acidic, basic and neutral substances.

Conventional separation methods are described, *i.e.*, liquid chromatography, ion-exchange, ion-pair and complexation chromatography, thin-layer and paper chromatography, electrophoresis, extraction and distillation. Examples are given of other methods of separation that may have to be used in special instances, such as precipitation, crystallisation and sublimation. The various methods recommended for separation and determination are tabulated and well referenced.

A proper assessment of the worth of this book can only be made when the second volume is published and working details of the recommended methods are made available. However, it can be said after reading the first volume that the work as a whole promises well to supplement an area that has tended to be badly neglected.

R. BELCHER

AN ATLAS OF SPECTRAL INTERFERENCES IN ICP SPECTROSCOPY. By M. L. PARSONS, ALAN FORSTER and DONN ANDERSON. Pp. x + 644. Plenum. 1980. Price $59.50. ISBN 0 306 40334 X.

Inductively coupled plasma (ICP) spectroscopy, like any other form of emission spectroscopy, is subject to spectral interferences. These are of the usual kind, such as instrument broadening of the spectral lines. In addition to these, the high temperature of the plasma causes the introduction of a few extra interferences.

Although spectral interferences in no way invalidate the technique, it is important that the spectroscopist is aware of these effects and by proper selection of lines and operating conditions and the use, where necessary, of background corrections is able to nullify them. This atlas gives the spectroscopist the information he needs to make this proper selection of lines.

As well as a compilation of experimentally used analysis lines and a list of all currently known transitions between 1850 and 2000 Å, a list of all known argon transitions is given. However, the most important part of the book is the listing of all transitions within about 1 Å and 5 Å of each important analytical line.

The use of computer printout in the compilation of this book means that chemical symbols are given in capitals, which this reviewer found irritating. Nevertheless, this volume will be useful to the practising spectroscopist who can afford it or can find someone else to buy it.

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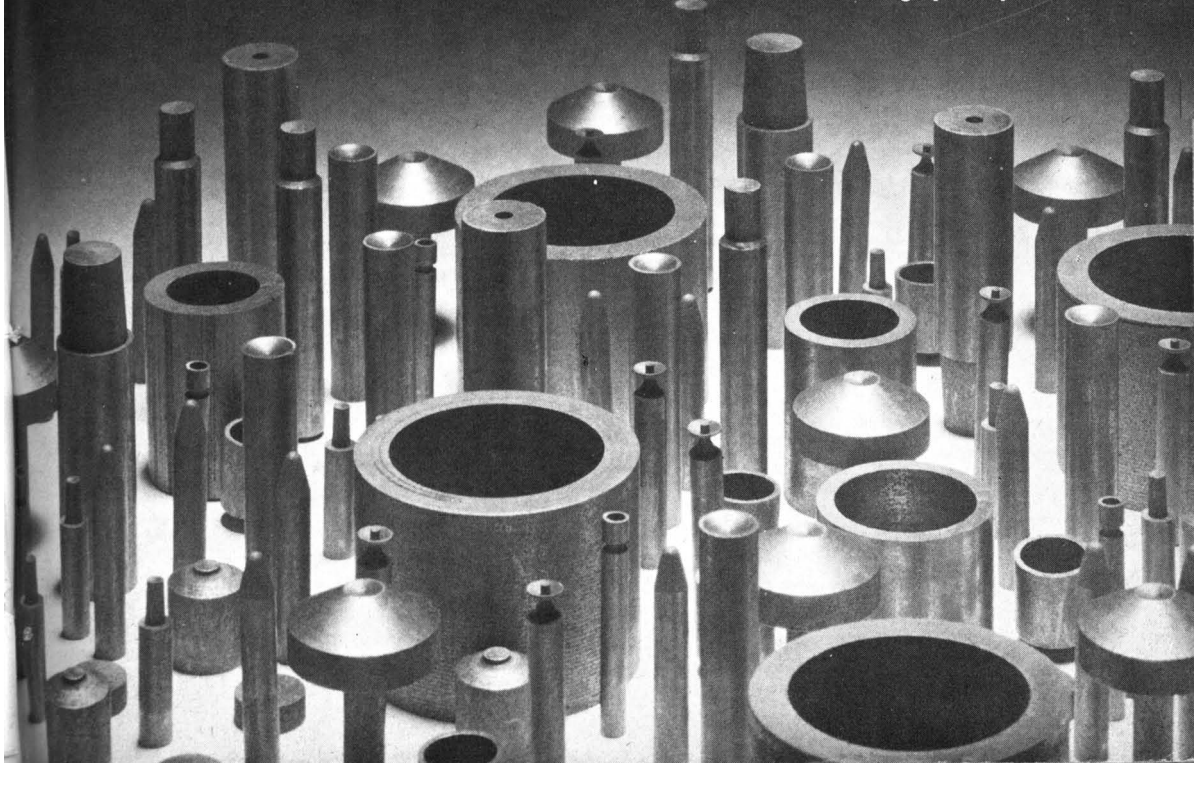

Application of an Automatically Triggered Digital Integrator to Flame Atomic-absorption Spectrometry of Copper Using a Discrete Nebulisation Technique

A signal integrator with an automatic trigger and a voltage to frequency (V/F) counter was applied to the flame atomic-absorption spectrometry of copper using a discrete nebulisation technique. When an injection volume larger than 50 μl was used, there was a good linear relationship between the absolute amount of copper and the integrated value, irrespective of the copper concentration and the injection volume. There was also a linear relationship between the ratio of the integrated value to the aspiration time and the copper concentration, irrespective of the injection volume. The relative standard deviation of the measurement was less than 4%, even with an injection volume of 20 μl. This method was applied to the determination of copper in standard biological samples (NBS-SRM 1577 Bovine Liver and 1566 Oyster Tissue). The results agreed well with the certified or reported values.

Keywords: *Automatic integration*; *atomic-absorption spectrometry*; *copper determination*; *biological standards*; *discrete nebulisation*

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Analyst, 1981, **106**, 206–212.

Determination of "Inorganic" Arsenic(III) and Arsenic(V), "Methylarsenic" and "Dimethylarsenic" Species by Selective Hydride Evolution Atomic-absorption Spectroscopy

A technique for the determination of "inorganic" arsenic(III) and arsenic(V), "methylarsenic" and "dimethylarsenic" species is described which is based on the trapping of arsines and selective volatilisation into a heated quartz atomiser tube situated in the optical path of an atomic-absorption spectrometer. Improved reproducibility is obtained by the use of a continuous flow reduction stage and detection limits are approximately 0.25 ng (based on twice the standard deviation of 10 blank measurements). For a typical sample volume of 10 ml this corresponds to a detection limit of 0.025 ng ml^{-1} of arsenic. Interferences were investigated and depression of results was observed in the presence of silver(I), gold(III), chromium(VI), iron(II), iron(III), germanium(IV), molybdenum(VI), antimony(III), antimony(V), tin(II), manganese(VII) and nitrite. Various approaches to overcoming such interferences were investigated, and for general use masking with EDTA is advocated. The choice of extraction procedures for speciation analysis is discussed.

Keywords: *Arsenic determination*; *"methylarsenic" species*; *atomic-absorption spectroscopy*; *hydride generation*; *environmental analysis*

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Analyst, 1981, **106**, 213–220.

Studies in Chemical Phase Analysis. Part II. Determination of the Solubilities of Carbides, Nitrides, Oxides and Sulphides in Certain Organic Solvent - Bromine Mixtures

As part of a study on the quantitative separation of carbide, nitride, oxide and sulphide inclusions from metals, the solubilities of five carbides, seven nitrides, sixteen oxides and eleven sulphides have been determined at 25 °C in methyl acetate - and acetonitrile - bromine mixtures (10 + 1 *V/V*) both after shaking at room temperature and after refluxing. Aluminium nitride and the carbides and nitrides of chromium, niobium, titanium and vanadium have low or very low solubilities, particularly in methyl acetate - bromine at room temperature, but iron carbide and iron and manganese nitrides are extensively decomposed, with the iron and manganese passing into solution. None of the oxides is more than sparingly soluble but most of the sulphides are appreciably soluble. Appropriate conditions are suggested for achieving a clean separation of oxides and stable carbides and nitrides from metals.

Keywords: *Solubilities of carbides, nitrides, oxides and sulphides*; *methyl acetate - bromine*; *acetonitrile - bromine*

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Analyst, 1981, **106**, 221–226.

Determination of Microgram Amounts of Calcium in Small Biological Samples by EDTA Titration Using Patton and Reeder's Indicator

An EDTA titration method has been developed for the determination of calcium in small biological samples. Fresh or dried material is extracted with 1 N nitric acid and the extract is alkalinised to pH 13.4 and titrated with EDTA in the presence of Patton and Reeder's indicator and sodium tartrate. Barium, magnesium, manganese, iron, lead, zinc, oxalate, phosphate and sulphate do not interfere owing to the high pH and the presence of tartrate. Copper interferes, but up to 5 μg of copper can be masked with cyanide if the copper concentration does not exceed one tenth the calcium concentration. This method permits the quantification of calcium in the range 0.5–40 μg and with a reproducibility of $\pm 2.5\%$ or better.

Keywords: *Calcium microdetermination*; *EDTA titration*; *biological samples*

POUL PRENTØ and ANNETTE PRENTØ

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Analyst, 1981, **106**, 227–230.

Automatic Titration by Stepwise Addition of Equal Volumes of Titrant

Part VI. Further Extension of the Gran I Method for Calculation of the Equivalence Volume in Acid - Base Titrations

In Part V of this series a very simple method for the calculation of the equivalence volume (V_e) from titration data was presented. It was based on the method of stepwise addition of equal volumes of titrant combined with an extended version of the Gran I method. The method presented had some limitations, however, as it could not be used for acids with log K between 2 and 4.2 or for very weak acids (log $K > 7$).

In this paper equations of a more complicated nature are given, which can be used for monoprotic acids with log $K \leqslant 10$. The equations contain, besides titrant volumes and hydrogen ion concentration, values for K and K_w, which, however, need not be known but are calculated from the titration data at the same time as V_e. The same type of calculation can be used in the calculation of V_e for most di- and triprotic acids.

A discussion is given of possible causes for errors in the equivalence volume, calculated using the equations presented. When accurate automatic pipettes are used for adding the titrant the uncertainty in the determination of the hydrogen ion concentration is the main source of error.

Keywords: *Gran I method*; *acid - base titration*; *automatic titration*; *equivalence volume calculation*; *potentiometric titration*

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Analyst, 1981, **106**, 231–242.

Spectrophotometric Determination of Nitrite in Waters

Short Paper

Keywords: *Nitrite determination*; *water*; *4-aminosalicylic acid reagent*; *diazotisation*; *spectrophotometry*

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Department of Chemistry, College of Education and College of Science, University of Mosul, Mosul, Iraq.

Analyst, 1981, **106**, 243–247.

Determination of Water in *NN*-Dimethylformamide by the Kinetic Method of Tangents Using the Oxidation of Catechol as the Indicator Reaction

Short Paper

Keywords: NN-*Dimethylformamide*; *sodium metaperiodate*; *catechol*; *spectrophotometry*; *kinetics*

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Analyst, 1981, **106**, 247–250.

Spectrophotometric Determination of Vanadium(V) Using 4-Benzoyl-3-methyl-1-phenyl-5-pyrazolone

Short Paper

Keywords: *Vanadium determination*; *spectrophotometry*; *4-benzoyl-3-methyl-1-phenyl-5-pyrazolone*; *solvent extraction*

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