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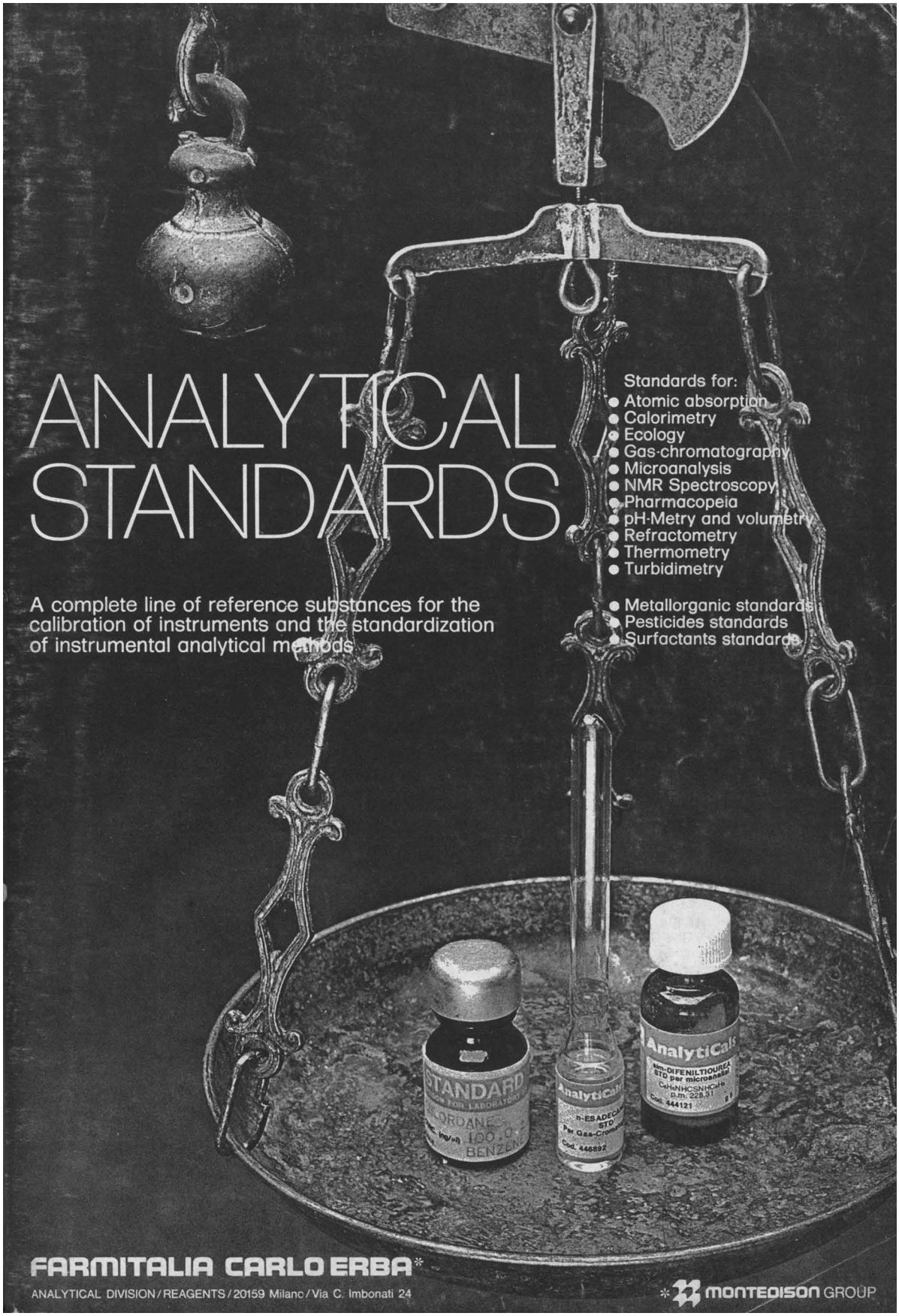
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Summaries of Papers in this Issue

Spectroelectrochemistry of Morphine and Related Alkaloids and their Investigation by Fluorescence in a Gold Micromesh Cell

A standard microflow cell containing a transparent gold micromesh electrode has been designed for *in situ* fluorescence monitoring of electrogenerated species by frontal illumination. Oxidative dimerisation of morphine to the fluorescent pseudomorphine proved to be a model fluorogenic reaction for study. The fluorescence calibration graph was linear over the concentration range 1×10^{-3}–1×10^{-6} M and the limit of detection was 5×10^{-7} M. The procedure, which is selective and free from interference from most of the opium alkaloids, enabled morphine to be assayed directly in papaveretum.

Keywords: *Spectroelectrochemistry*; *morphine*; *pseudomorphine*; *electrogeneration*; *fluorogenic reaction*

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Analyst, 1982, **107**, 1–11.

Physico-chemical Study of Mixed-ligand Selenium(IV) Complexes: Ternary Complex of Selenium(IV) with Alizarin Maroon and Eosin

The reaction of selenium(IV) with alizarin maroon (AZM) as a primary ligand and eosin as a secondary ligand was examined spectrophotometrically and potentiometrically at 20 ± 0.1 °C and an ionic strength of 0.1 M (perchloric acid). The solution spectra of the mixed-ligand complex formed is characterised by an absorption band with $\lambda_{max.}$ at 560 nm within the pH range 6.5–7.2. The pink selenium - $(AZM)_2$ - $(eosin)_2$ association complex conformed to Beer's law over the concentration range 0.16–2.0 μg ml^{-1} of selenium with a molar absorptivity of 2.5×10^4 l mol^{-1} cm^{-1}.

Keywords: *Selenium(IV) mixed-ligand complexes*; *alizarin maroon*; *eosin*; *spectrophotometry*; *potentiometry*

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Analyst, 1982, **107**, 12–16.

Automatic Determination of Sulphur Dioxide by a Coulometric Method: Interferences and Reliability of Measurements

The selectivity of filters used in coulometric sulphur dioxide analysers was evaluated for various volatile organic substances that can be present in the atmosphere of an industrial area with different chemical plants. The poor selectivity of chemical filters, mainly with respect to vinyl acetate and styrene, was ascertained by several tests at various concentrations of the interfering substance. It is possible that the interferences arise from reactions with the bromine present in the analysis cell. The magnitude of the interference appeared to be related to the kinetics of addition of the bromine to unsaturated organic interferents. Pre-heating the air sample to 800 °C resulted in complete elimination of the vinyl acetate interference; less promising results were obtained with styrene. Practical modifications of air pre-treatment on the analysers were consequently adopted.

Keywords: *Sulphur dioxide determination*; *coulometry*; *air pollution control*; *interferences*

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Analyst, 1982, **107**, 17–24.

Comparative Study of the Determination of Nitrates in Calcareous Soils by the Ion-selective Electrode, Chromotropic Acid and Phenoldisulphonic Acid Methods

A comparative study of the determination of nitrates in calcareous soils by the chromotropic acid, phenoldisulphonic acid and nitrate ion-selective electrode methods was investigated, using 0.02 N copper(II) sulphate solution as extractant, which, in addition to being a preservative for nitrates, helps filtration and eliminates interference by hydrogen carbonates in the ion-selective electrode method. Silver sulphate, which had no effect on either procedure, can be added to the extractant for the precipitation of chlorides.

Nitrate in soil suspensions was determined by the ion-selective electrode method. Nitrites if present were eliminated by acidifying the extract with 1 N sulphuric acid containing sulphamic acid.

To avoid reaction between soluble organic matter and sulphuric acid in the chromotropic acid method, reagents were added in two steps with continuous cooling. Nitrites were eliminated as in the ion-selective electrode method.

Both methods were faster than the phenoldisulphonic acid method and gave identical results; the correlation coefficient was 0.9998.

Keywords: *Nitrate determination*; *ion-selective electrode*; *chromotropic acid method*; *phenoldisulphonic acid method*; *calcareous soils*

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Analyst, 1982, **107**, 25–29.

Determination of Formaldehyde Vapour in the Atmospheres of Clinical Laboratories Using Chromotropic Acid

Ethanol and xylene vapours were found to interfere with the determination of formaldehyde vapour by the chromotropic acid method, whether water or a mixture of chromotropic and sulphuric acids were used as the scrubbing solution. The interference can be removed by a porous polymer sorbent, Tenax GC. Thus, with a Tenax GC pre-trap, the chromotropic acid method can be applied to the determination of formaldehyde vapour in clinical laboratories where alcohol and xylene vapours are often encountered. The advantages of selecting chromotropic - sulphuric acid instead of water as the scrubbing solution are discussed.

Keywords: *Formaldehyde determination*; *chromotropic acid*; *spectrophotometry*; *ethanol and xylene interference*; *Tenax-GC pre-trap*

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Analyst, 1982, **107**, 30–34.

Spectrophotometric Determination of Cobalt in Paints and Environmental Paint Samples

A method for the spectrophotometric determination of cobalt, based on the formation of a coloured chelate ($\lambda_{max.}$ 480 nm, $\epsilon_{max.}$ 2.83 $\times$ 10^4 l mol^{-1} cm^{-1} and colour contrast 134 nm) with benzyl 2-pyridyl ketone 2-pyridylhydrazone, is described. Under the optimum conditions, colour development is instantaneous and the colour is stable for several weeks. Under appropriate working conditions, the method is applicable to the determination of cobalt in manufactured paints and environmental samples of paint. The fundamental solution chemistry of the reagent and a brief description of its more interesting colour reactions are also reported.

Keywords: *Benzyl 2-pyridyl ketone 2-pyridylhydrazone reagent*; *cobalt determination*; *paint analysis*; *spectrophotometry*

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Analyst, 1982, **107**, 35–40.

Spectrophotometric Determination of Trace Amounts of Molybdenum with 1,4-Dihydroxyphthalimide Dithiosemicarbazone

1,4-Dihydroxyphthalimide dithiosemicarbazone reacts with molybdenum(VI) to produce a yellow 1:1 complex in acidic dimethylformamide - water solution. The yellow complex can be extracted into isopentyl alcohol ($\lambda_{max.}$ = 435 nm; ϵ = 9.4 $\times$ 10^3 l mol^{-1} cm^{-1}) and used for the spectrophotometric determination of trace amounts of molybdenum in the range 10–95 μg. The interferences of many metallic ions have been examined and a sensitive and selective method for the determination of molybdenum is proposed.

Keywords: *Molybdenum determination*; *spectrophotometry*; *1,4-dihydroxyphthalimide dithiosemicarbazone*

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Analyst, 1982, **107**, 41–46.

Determination of Iron(II) in Silicates by Gravimetric Titration

A rapid method, suitable for batch working, is described for determining iron(II) in silicates. Hot 1 + 1 hydrofluoric - sulphuric acid decomposes the subsample in a polypropylene bottle previously filled with carbon dioxide. After dilution with a boric - phosphoric - sulphuric acid mixture, iron(II) is titrated gravimetrically with potassium dichromate solution, in the bottle used for sample decomposition. A sample-preparation procedure that minimises oxidation of iron(II) is described. Results obtained for iron(II) in various international reference rocks by the proposed method agree well with recommended values.

Keywords: *Iron(II) determination*; *silicates*; *gravimetric titration*

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Analyst, 1982, **107**, 47–52.

Substitution in Cellulose Ethers. Part I. Determination of Glucose Units According to Number and Type of Ether Substituents Using Quantitative Thin-layer Chromatography

A procedure for the quantitative characterisation of the distribution of substituents in cellulose ethers is presented. After total hydrolysis in dilute sulphuric acid the sample is separated by thin-layer chromatography on silica gel according to number and type of substituents attached to the glucose unit. Quantitative evaluation of the chromatograms is performed by scanning photometry in the visible range. The procedure allows the determination of the percentages of tri-, di-, mono- and unsubstituted monomeric units in alkyl and alkylhydroxyalkyl celluloses. Data on the distribution of the hydroxyalkyl substituents in mixed ethers were also obtained. The reproducibility of the thin-layer chromatographic separation and that of the entire procedure are discussed.

Keywords: *Cellulose ethers*; *distribution of substituents*; *determination of glucose units*; *thin-layer chromatography*; *scanning photometry*

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Analyst, 1982, **107**, 53–60.

Stability Indicating Assay for Dipyrone. Part I. Separation and Quantitative Determination of Dipyrone and Its Degradation Products by Thin-layer Chromatography

A thin-layer chromatographic procedure is described for the separation and quantification of dipyrone *in situ* in the presence of three of the major degradation products. The repeatability and detection limits are given and the applicability of the assay in checking the stability of tablets and injections is shown.

Keywords: *Dipyrone determination*; *degradation products determination*; *thin-layer chromatography*; *tablet stability*; *injection stability*

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Analyst, 1982, **107**, 61–66.

Stability Indicating Assay for Dipyrone. Part II. Separation and Quantitative Determination of Dipyrone and Its Degradation Products by High-performance Liquid Chromatography

A rapid, sensitive and reproducible procedure for separation and quantification of dipyrone and three of the degradation products is proposed, using high-performance liquid chromatography on a reversed-phase column and ultraviolet detection. The method is about ten times more sensitive (detection limit 3–5 ng) than thin-layer chromatography and requires only a 12-min elution time. This method has been applied to the analysis of tablets and injections.

Keywords: *Dipyrone determination*; *degradation products determination*; *high-performance liquid chromatography*; *reversed-phase chromatography*

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Analyst, 1982, **107**, 67–70.

Determination of the Active Ingredient Content of Technical and Formulated Dinobuton, Dinoseb, Dinoterb and DNOC by High-performance Liquid Chromatography, Spectrophotometry and Gas - Liquid Chromatography

A reversed-phase high-performance liquid chromatographic (HPLC) method is described for the determination of the active ingredient content of technical and formulated dinobuton, dinoseb, dinoterb and DNOC. The results obtained by HPLC are compared with those obtained using gas - liquid chromatography for dinoterb and spectrophotometry for the other compounds.

Keywords: *Dinobuton, dinoseb, dinoterb, DNOC analysis*; *formulation analysis*; *high-performance liquid chromatography*

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Analyst, 1982, **107**, 71–75.

Determination of Acid and Hydroxybenzonitrile Herbicide Residues in Soil by Gas - Liquid Chromatography after Ion-pair Alkylation

The herbicides are extracted from soil with saturated calcium hydroxide solution. After clean-up the residues are ethylated using iodoethane and tetrabutylammonium hydrogen sulphate as counter ion. Liquid - liquid partition and the use of a macroreticular resin column were compared as clean-up steps and the reaction conditions for optimum yield of ethyl ester or ether were evaluated. Recoveries in excess of 80% were achieved for 2,4-D, dicamba, 3,6-dichloropicolinic acid, dichlorprop, picloram, 2,4,5-T, fenoprop, 2,3,6-TBA, bromoxynil and ioxynil.

Keywords: *Herbicide determination*; *soil*; *gas - liquid chromatography*; *alkylation*

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Analyst, 1982, **107**, 76–81.

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Spectroelectrochemistry of Morphine and Related Alkaloids and their Investigation by Fluorescence in a Gold Micromesh Cell

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A standard microflow cell containing a transparent gold micromesh electrode has been designed for *in situ* fluorescence monitoring of electrogenerated species by frontal illumination. Oxidative dimerisation of morphine to the fluorescent pseudomorphine proved to be a model fluorogenic reaction for study. The fluorescence calibration graph was linear over the concentration range 1×10^{-3}–1×10^{-6} M and the limit of detection was 5×10^{-7} M. The procedure, which is selective and free from interference from most of the opium alkaloids, enabled morphine to be assayed directly in papaveretum.

Keywords: *Spectroelectrochemistry*; *morphine*; *pseudomorphine*; *electrogeneration*; *fluorogenic reaction*

Spectroscopic techniques have been used extensively over the last decade for the *in situ* monitoring of electrogenerated species. Such studies have been mainly concerned with mechanistic aspects of electrode reactions and valuable information has been obtained by coupling electrochemical experiments with optical and electron spin resonance methods.[1–3] Little attention has, however, been devoted to analytical applications of the spectroelectrochemical technique although the possibility was suggested some time ago.[4]

Recently, absorptiometric monitoring at electrode surfaces has been shown to have potential for the trace determination of metal ions and certain organic compounds.[5–8]

A fluorescence-coupled electrochemical experiment may offer the additional analytical advantages of increased sensitivity and selectivity. To investigate this, a microvolume fluorescence cell incorporating gold micromesh as the working electrode was designed for the *in situ* fluorescence measurement of electrogenerated species. Gold micromesh was chosen as it exhibits favourable electrochemical characteristics[9] and was convenient for cell fabrication. The electrode material has been employed by a number of workers, notably Murray and co-workers[10–12] and others,[13,14] for spectroelectrochemical thin-layer studies. For application the possibility of electrochemically initiating and concurrently monitoring the course of fluorogenic reactions was considered. The fluorogenic reaction, whereby a weakly or non-fluorescent compound is converted by an appropriate chemical reaction (for example, a redox reaction) into a highly fluorescent species, is employed for trace determinations in the biological and medical fields. The texts by Guilbault[15] and White and Argauer[16] provide comprehensive lists of such reactions. Morphine, a powerful but addictive analgesic, is a good example of a potential fluorogen, undergoing oxidation to the highly fluorescent dimer, pseudomorphine, when treated with alkaline potassium hexacyanoferrate(III) solution. The reaction has attracted the attention of analysts in the pharmaceutical,[17] clinical[18,19] and forensic fields.[20]

Oxidative dimerisation of morphine, initiated by electrolysis at 0.25 V *versus* S.C.E., was performed successfully in the gold micromesh cell and preliminary experiments were concerned with establishing the optimum conditions for the formation and stability of pseudomorphine and determining the performance characteristics of the cell. Once interference studies had been evaluated, the potential of the cell for semi-automatic assay was demonstrated by determining morphine in the presence of other opium alkaloids.

Experimental

Construction of Cell

A piece of transparent (45% transmission) gold micromesh (1000 lines per inch; Buckbee Mears, Minneapolis, USA), the working electrode, was inserted between the two halves of a standard microflow cell (Hellma Ltd., Southend; Cell No. 136 in Suprasil; path length 0.5 mm) and the unit was made leakproof by sealing with silicone rubber sealant. The cell volume was approximately 120 μl. Electrical connection to the gold micromesh was made with a thin brass strip that was permanently attached to the cell. The mesh is extremely fragile and care must be exercised when handling. A platinum tube (internal diameter 1 mm) sealed to the cell outlet served as the counter electrode. Sample was delivered to the cell with a single-channel peristaltic pump (Perpex 10200) and tubing (Technicon 116–0522–11). A saturated calomel electrode (S.C.E.) placed in the downstream reservoir completed the three-electrode cell. A schematic diagram of the cell and associated equipment is shown in Fig. 1.

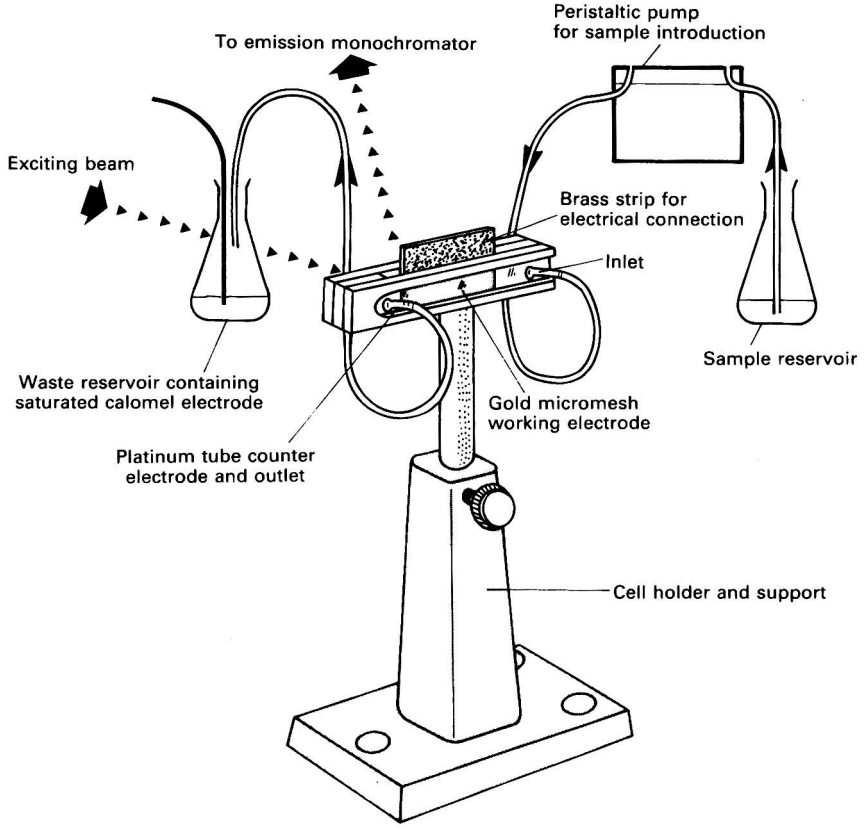


Fig. 1. Gold micromesh cell and associated equipment.

Equipment

Fluorescence measurements, which were uncorrected for grating/detector response characteristics, were obtained with a Farrand spectrofluorimeter, which utilised a 150-W xenon arc source, two modified Czerny–Turner-type monochromators (relative aperture *f*/3.5; dispersion 10 nm mm^{-1}), an RCA IP 28 photomultiplier and a Honeywell Electronik 15

recorder. Slits providing a band pass of 20 nm were used on both monochromators unless stated otherwise. The method of frontal illumination was used, with the gold micromesh cell being positioned at an angle approximately 30° to the excitation beam. Corning filters, 7–54 (maximum transmission 330 nm) and 3–73 (maximum transmission 550 nm), were fitted to the excitation and emission monochromators, respectively, to reduce scattered exciting light. Stray light was minimised by enclosing the gold micromesh cell within a light-tight box constructed from expanded polystyrene and black cotton cloth.

A PAR 174A polarographic analyser was employed for controlled potential electrolysis during fluorescence measurements. The instrument was also used in conjunction with a Metrohm cell (EA 875–20) and a Servoscribe two-channel recorder for obtaining the voltammetric curves of alkaloid solutions. A gold micro-wire and a platinum wire were used as the working and counter electrodes, respectively, and a saturated calomel electrode served as the reference.

Materials

Morphine hydrochloride, heroin hydrochloride, codeine phosphate, Papaveretum Tablet (BPC) and Omnopon injection ampoule (Roche) were of pharmaceutical grade. Pseudomorphine, narcotine hydrochloride, cotarnine hydrochloride, sinomenine hydrochloride, narceine and laudanosine were of normal reagent quality.

Reagent

Sodium pyrophosphate solution, 0.1 M, *pH* 8.5. An 11.15-g amount of sodium pyrophosphate (AnalaR grade) was dissolved in distilled water (250 cm^3) and adjusted to pH 8.5 (or the appropriate pH) with a small volume of sulphuric acid (1 M). This solution, freshly prepared each day, was used throughout unless stated otherwise.

Preparation of Alkaloid Solutions

Stock alkaloid solutions (typically 1×10^{-3} M) were prepared by dissolving the required amount of alkaloid in dilute sulphuric acid. Aliquots of the appropriate stock solution were then diluted to 25 cm^3 with sodium pyrophosphate background electrolyte.

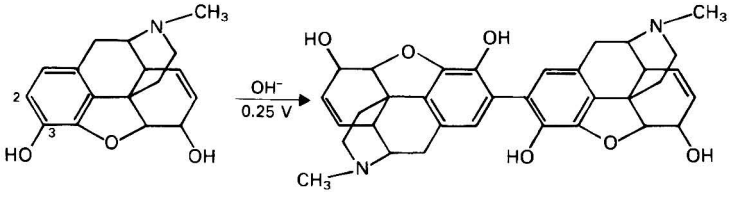
Operating Procedure

The sample solution was delivered to the cell by a peristaltic pump. With a stationary solution, a potential, sufficient to effect oxidation, was applied to the gold micromesh electrode and the resulting fluorescence with respect to time was monitored at the appropriate wavelength combination. For pseudomorphine, the excitation and emission maxima were 320 and 442 nm, respectively, and the fluorescence response curve went through a maximum and decayed to the background level. The procedure could be repeated by returning the electrode potential to 0 V and introducing fresh sample. Blank solution was generally passed through the cell for 10 min between different sample runs. This cleaning cycle was required for consistent results and was particularly important when examining solutions of differing pH.

Results and Discussion

Development of Method

Morphine undergoes oxidative dimerisation in alkaline solution to the highly fluorescent pseudomorphine (2,2′-bimorphine).[21]



The electrode reaction involves oxidation of the ionised phenolic hydroxy group and subsequent coupling of phenolate radicals at the C-2 position. As pseudomorphine also contains phenolic hydroxy groups, further oxidation is likely. Chemical oxidation studies by Burkhalter *et al.*[18] indicated that the optimum pH for fluorescence measurement of pseudomorphine is 8.5. To determine the optimum conditions in this study, the effects of the working electrode potential and pH on the fluorescence of electro-generated pseudomorphine were investigated. Prior to this the fluorescence spectra of pseudomorphine were obtained.

Fluorescence spectra

The excitation and emission spectra of the fluorescence signal were recorded *in situ* during the controlled potential oxidation (0.2 V *versus* S.C.E.) of morphine hydrochloride (2×10^{-4} M) in sodium pyrophosphate solution. The spectra, which were obtained with a band pass of 5 nm on both monochromators and without filters (7–54, 3–72), are shown in Fig. 2. They reveal the characteristic excitation (three peaks at 250, 280 and 320 nm) and emission pattern (one peak at 422 nm) for pseudomorphine previously reported by Burkhalter *et al.*[18] The spectra recorded in the presence of the complementary filters were distorted but the excitation and emission maxima remained the same. Subsequent fluorescence measurement was performed at the wavelength combination $\lambda_{ex.}$ 320 nm and $\lambda_{em.}$ 442 nm.

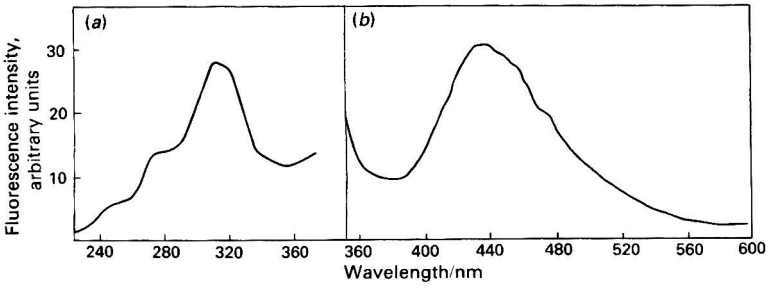


Fig. 2. Fluorescence excitation (*a*) and emission (*b*) spectra of electrogenerated pseudomorphine. Morphine hydrochloride (2×10^{-4} M) in pH 8.5 sodium pyrophosphate solution (0.1 M); electrode potential, 0.20 V *vs.* S.C.E.; excitation wavelength, 320 nm; emission wavelength, 442 nm; and band pass, 5 nm for both monochromators.

Effect of electrode potential

Morphine hydrochloride (8×10^{-5} M) in sodium pyrophosphate solution (0.1 M, pH 8.5) was oxidised in the gold micromesh cell at various electrode potentials. The results, summarised in Table I, indicate the effect of the electrode potential on the formation and stability

TABLE I

EFFECT OF ELECTRODE POTENTIAL ON THE FLUORESCENCE OF ELECTROGENERATED PSEUDOMORPHINE

Morphine hydrochloride (8×10^{-5} M) in pH 8.5 sodium pyrophosphate solution (0.1 M); excitation wavelength 320 nm; emission wavelength 442 nm.

Electrode potential/ V *vs.* S.C.E.	Maximum fluorescence intensity, arbitrary units	$Time_{MF}$*/min
0.15	—	—
0.20	—	—
0.25	40	9.8
0.30	35	6.2
0.40	22	3.2
0.50	18	2.4
0.60	16	2.2

* MF = maximum fluorescence.

of pseudomorphine. For potentials less than that required for oxidation, for example 0.15 V *versus* S.C.E., pseudomorphine was not formed and consequently fluorescence was not generated. At potentials up to 0.2 V *versus* S.C.E., the fluorescence developed slowly and did not reach a maximum in an acceptable time. For increasingly positive potentials, the rate of formation and removal of pseudomorphine increased, with the result that the fluorescence maxima progressively decreased. The fluorescence behaviour indicated the increasing rate of oxidation of the phenolic hydroxy groups of morphine and pseudomorphine with increasing electrode potential, the oxidation product of the latter compound being non-fluorescent. Maximum fluorescence (at pH 8.5) was obtained with an applied potential of 0.25 V *versus* S.C.E.

Effect of pH

Morphine hydrochloride solutions (8×10^{-5} M) containing sodium pyrophosphate (0.1 M) and covering the pH range 6.0–10.2 were individually oxidised at 0.3 V *versus* S.C.E. and the fluorescence response was recorded. The rate of fluorescence development and decay increased as the solution became more alkaline. The results are summarised in Table II. Maximum fluorescence was obtained for the pH 7.7 solution. Fluorescence was not recorded for solutions of pH 6.0 and 6.4 and, for the solution of pH 7.2, the time to reach maximum fluorescence was considerable. These results indicate that the oxidation of morphine and pseudomorphine was pH dependent and a subsequent study of the voltammetric behaviour of the two compounds with respect to pH confirmed this. For solutions in the pH range 7.5–8.5, and with oxidation at 0.25 V *versus* S.C.E., differences in the fluorescence maxima were less pronounced. Maximum fluorescence was still recorded for the pH 7.7 solution, but the time taken to reach the maximum was considerable.

Summarising, to obtain maximum fluorescence for electrogenerated pseudomorphine an oxidation potential of 0.25 V *versus* S.C.E. with a solution pH of about 7.7 should be chosen. If the development time is unacceptable then a faster fluorescence response can be obtained at the expense of sensitivity by increasing either (or both) parameter(s). In subsequent experiments solutions of pH 8.5 were used.

TABLE II

EFFECT OF pH ON THE FLUORESCENCE OF ELECTROGENERATED PSEUDOMORPHINE

Morphine hydrochloride (8×10^{-5} M) in sodium pyrophosphate solution (0.1 M); electrode potential 0.3 V *vs.* S.C.E.; excitation wavelength 320 nm; emission wavelength 442 nm.

pH	Maximum fluorescence intensity, arbitrary units	$Time_{MF}$/min
6.0	—	—
6.4	—	—
7.2	40	17.7
7.7	53	7.5
8.0	41	3.7
8.6	28	2.6
9.1	20	2.3
9.6	16	1.9
10.2	12	1.4

Efficiency of Electrochemical Conversion and Comparison with Potassium Hexacyanoferrate(III) Oxidation

The efficiency of electrochemical dimerisation was determined by comparing the maximum fluorescence of electrogenerated pseudomorphine with the fluorescence of standard pseudomorphine solutions. The molar concentrations of the morphine and pseudomorphine solutions were in the ratio 2:1, as two molecules of morphine are required for dimerisation. The efficiency was also compared with the chemical oxidation method of Burkhalter *et al.*[18]

Procedure

The maximum fluorescence of pseudomorphine was recorded in the gold micromesh cell for three situations. First, morphine (2×10^{-5} M) in pH 8.5 sodium pyrophosphate solution

(0.1 M) was oxidised at 0.25 V *versus* S.C.E. and the fluorescence response was recorded. Next, morphine (2×10^{-5} M) in pH 8.5 sodium pyrophosphate solution (0.1 M) was oxidised by potassium hexacyanoferrate(III) (6×10^{-5} M) - potassium hexacyanoferrate(II) (3.7×10^{-6} M) solution according to the procedure of Burkhalter *et al.*[18] and the fluorescence at zero applied potential was recorded. Finally, the fluorescence of standard pseudomorphine solution (10^{-5} M) in pH 8.5 sodium pyrophosphate solution (0.1 M) was recorded for zero applied potential. The fluorescence results and the appropriate percentage conversion values are presented in Table III. It can be seen that electrochemical dimerisation is comparable to the chemical oxidation method of Burkhalter *et al.*[18]

In both instances the efficiency of morphine dimerisation was about 55%. Dimerisation efficiency via chemical oxidation has, however, been improved by Takemori.[19]

TABLE III

EFFICIENCY OF ELECTROCHEMICAL AND CHEMICAL OXIDATION

Alkaloids in pH 8.5 sodium pyrophosphate solution (0.1 M); excitation wavelength 320 nm; emission wavelength 442 nm.

Parameter	Pseudomorphine (10^{-5} M)	Morphine (2×10^{-5} M)	
		Chemical	Electrochemical
Maximum fluorescence intensity of pseudomorphine, arbitrary units	27.5	15.5	15.2
Conversion, %	—	56.4	55.3

Calibration and Limit of Detection

The fluorescence calibration graph was obtained for morphine hydrochloride solutions covering the concentration range 1×10^{-2}–1×10^{-6} M. Measurements recorded for oxidation at 0.25 V *versus* S.C.E. in pH 8.5 solutions were generally repeated at least once and an average value taken. The calibration graph showed an extremely good fit of the experimental data, linearity being observed over a 1000-fold range. Fluorescence measurements were not extended beyond 1×10^{-3} M because precipitation of morphine occurred in the pH 8.5 media. Fluorescence was, however, measured for morphine concentrations up to 3×10^{-3} M in pH 11 media. The calibration graph still exhibited a wide linear analytical range, but the onset of self-absorption, which became noticeable at concentrations above 1×10^{-3} M, brought a departure from linearity. Decreased sensitivity in the pH 11 media was observed, as expected. The limit of detection, based on a limiting signal twice the standard deviation of the background noise level, was calculated to be 5×10^{-7} M morphine (as hydrochloride). The detection limit for morphine (free alkaloid) based on the cell volume of 120 μl was 19 ng.

Interference Studies

It might be expected that the morphine dimerisation reaction could offer a degree of specificity and provide an interference-free route for morphine. Unfortunately, a large number of phenolic compounds undergo this reaction. Burkhalter *et al.*[18] reported that the phenolic alkaloids nor-morphine, allylmorphine, dihydromorphine and monoacetylmorphine yielded fluorescence characteristics identical with those of pseudomorphine and that they would interfere in the determination of morphine. Non-phenolic compounds including codeine and its derivatives and heroin (diacetylmorphine) did not yield fluorescence products similar to pseudomorphine and it was stated that such compounds would not cause interference. Essentially the same results were reported by Deys[22] for the anodic voltammetric determination of morphine in acidic solution: those alkaloids bearing a phenolic hydroxy group (pseudomorphine, dihydromorphine, nalorphine, apomorphine) yielded oxidative waves similar to morphine, causing interference, whereas compounds containing alkylated hydroxy groups (codeine, narcotine, narceine, thebaine, papaverine) were not electroactive and did not interfere. With the results of Burkhalter *et al.* and Deys providing the basis for interference studies, a selection of opium alkaloids that were readily available—codeine, narceine, narcotine, papaverine, cotarnine, laudanosine and sinomenine (phenolic alkaloid)—were examined for possible interference (papaverine, apomorphine and thebaine

were considered, but were insoluble at pH 8.5). Heroin, a synthetic derivative of morphine, was also investigated. Experiments were first concerned with establishing the electroactivity of each alkaloid and whether it exhibited oxidative fluorescence before conducting interference studies. Possible interference mechanisms considered were spectral, from either natural or oxidative fluorescence (not necessarily phenolic coupling), and chemical, whereby the morphine dimerisation reaction is inhibited.

Voltammetric behaviour

The current - potential graphs for each alkaloid (5×10^{-4} M) in pH 8.5 sodium pyrophosphate solution (0.1 M) were recorded in a Metrohm cell during the cyclic sweep (0 to 0.75 V *versus* S.C.E. at 20 mV s^{-1}) of the gold wire working electrode. Oxidative waves were recorded for morphine, sinomenine and heroin solutions but codeine, narceine, cotarnine were electroinactive. The scan for laudanosine indicated possible electroactivity and a further sweep from 0.5 to 1.25 V *versus* S.C.E. confirmed this. The voltammograms for sinomenine, morphine, codeine and heroin are presented in Fig. 3 and a summary of the electrochemical behaviour of the alkaloids investigated is given in Table IV. Of the compounds investigated, sinomenine is the only one to possess a phenolic hydroxy group and, as shown in Fig. 3(*b*), an oxidative wave similar to that for morphine was recorded. The oxidative wave for heroin [solid line, Fig. 3(*d*)] was indicative of phenolic oxidation. Heroin, however, undergoes alkaline hydrolysis[23] to monoacetylmorphine and thus the characteristic phenolic oxidative wave was observed.

On the basis of this study it would be expected that sinomenine and heroin would cause interference either by scavenging morphine free radicals and lowering the dimerisation yield of pseudomorphine and/or by undergoing self-coupling to give a fluorescent product. It

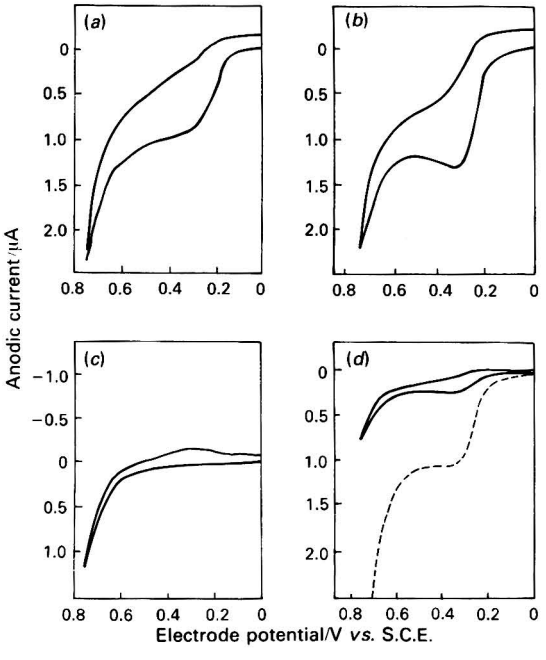


Fig. 3. Voltammetric curves for (*a*) sinomenine, (*b*) morphine, (*c*) codeine and (*d*) heroin. Alkaloid (5×10^{-4} M) in pH 8.5 sodium pyrophosphate solution (0.1 M); working electrode, gold wire; and scan rate 20 mV s^{-1}. For broken line [in (*d*)] response sensitivity was increased by a factor of 5.

TABLE IV

VOLTAMMETRIC CHARACTERISTICS OF ALKALOIDS

Alkaloids (5×10^{-4} M) in pH 8.5 sodium pyrophosphate solution (0.1 M). Cyclic sweep, 0 to 0.75 to 0 V *vs.* S.C.E. at 20 mV s^{-1}, except for laudanosine (0.5 to 1.25 to 0.5 V *vs.* S.C.E.); working electrode, gold wire.

Alkaloid	Oxidative wave	$E_{\frac{1}{2}}$/V *vs.* S.C.E.
Morphine	Yes	0.24
Sinomenine	Yes	0.21
Heroin*	Yes	0.27
Laudanosine	Yes	—†
Codeine	No	—
Narceine	No	—
Cotarnine	No	—
Narcotine	No	—

* After hydrolysis to monoacetylmorphine.
† Difficult to estimate.

cannot be predicted at this stage whether oxidation of laudanosine would interfere either chemically or spectrally. The remaining electroinactive alkaloids should not interfere (neglecting the natural fluorescence of the alkaloid).

Fluorescence studies

Solutions of each alkaloid (3×10^{-4} M) in pH 8.5 sodium pyrophosphate solution (0.1 M) were examined individually in the gold micromesh cell during oxidation at various positive applied potentials. Only the heroin and laudanosine solutions exhibited fluorescence (excitation, 320 nm; emission, 442 nm) during electrolysis. The emission spectrum for the heroin product was identical with that of pseudomorphine, confirming the findings of Burkhalter *et al.*[18] When the heroin solution was prepared immediately before examination only a small signal was recorded on electrolysis, indicating that hydrolysis to monoacetylmorphine was not significant. The laudanosine product exhibited sensitive fluorescence, the excitation and emission maxima being 361 and 470 nm, respectively. The oxidation product was not identified. The remaining alkaloid solutions did not exhibit fluorescence.

The possible interference of each alkaloid on the fluorescence of electrogenerated pseudomorphine was next investigated in a systematic manner. Morphine solutions (3×10^{-5} M) containing the particular alkaloid (1) at the same concentration (3×10^{-5} M), (2) in a 10-fold excess (3×10^{-4} M) and (3) in a 100-fold excess (3×10^{-3} M), all in pH 8.5 sodium pyrophosphate solution (0.1 M), were oxidised at 0.25 V *versus* S.C.E. and the maximum fluorescence was recorded. This procedure was carried out for sinomenine, heroin, laudanosine, narceine, cotarnine and narcotine; for codeine, concentrations 1000-fold greater (3×10^{-2} M) were examined. The fluorescence results are summarised in Table V.

The electroinactive alkaloids, codeine narceine and narcotine, caused no interference at any of the concentration levels investigated but cotarnine interfered at the 3×10^{-3} M level when its natural fluorescence became significant. The remaining electroactive alkaloids, sinomenine heroin and laudanosine caused interference. For sinomenine at the 3×10^{-5} M level, the fluorescence signal was reduced to 33% of the original value whereas morphine solutions containing 3×10^{-4} and 3×10^{-3} M sinomenine did not exhibit fluorescence on electrolysis. Laudanosine and heroin solutions individually caused serious positive interference (increased fluorescence) at concentrations 3×10^{-4} M and higher. Interference was not observed at the 3×10^{-5} M level.

The results of this study are in good agreement with the predictions made on the basis of the voltammetric behaviour of the alkaloids. Alkaloids containing a phenolic hydroxy group undergo phenolic oxidation and decrease the efficiency of pseudomorphine formation by scavenging the morphine free radical species. This interference mechanism was exhibited by sinomenine. Spectral interference from non-phenolic alkaloids which become fluorescent on oxidation is also possible; this was demonstrated for laudanosine. The high specificity of the dimerisation reaction in the presence of electroinactive alkaloids (codeine, narcotine, cotarnine and narceine) was evident, however, and the result for codeine was particularly important.

TABLE V

INTERFERENCE OF ALKALOIDS ON THE FLUORESCENCE OF ELECTROGENERATED PSEUDOMORPHINE

Morphine hydrochloride (3×10^{-5} M) and alkaloid in pH 8.5 sodium pyrophosphate solution (0.1 M); electrode potential 0.25 V *vs.* S.C.E.; excitation wavelength 320 nm; emission wavelength 442 nm.

Alkaloid	Oxidative fluorescence	Interference results: alkaloid concentration/M*			
		3×10^{-5}	3×10^{-4}	3×10^{-3}	3×10^{-2}
Morphine	Yes				
Sinomenine	No	×	×	×	
Heroin†	Yes	—	×	×	
Laudanosine	Yes	—	×	×	
Codeine	No	—	—	—	—
Narceine	No	—	—	—	
Cotarnine	No	—	—	—	
Narcotine	No	—	—	—	

* × represents interference; — represents no interference.
† After hydrolysis to monoacetylmorphine.

Assay of Papaveretum Tablet and Omnopon Injection Ampoule

The official assay method for morphine in pharmaceutical preparations based on opium requires a preliminary extraction procedure prior to titrimetry.[24] An extraction procedure is also required in the recently recommended gas - liquid chromatographic methods.[25] To test the suitability of the gold micromesh technique for the direct determination of morphine, papaveretum, an important preparation based on the principal opium alkaloids, was selected. Papaveretum consists of the hydrochloride salts of morphine (47.5–52.5% *m/m*), naroctine (16–22% *m/m*), codeine (2.5–5% *m/m*) and papaverine (2.5–7% *m/m*) and is obtained in the form of a tablet [10 mg of papaveretum (BPC)] or injection ampoule (Omnopon, 20 mg ml^{-1} of papaveretum; Roche).

Procedure

One papaveretum tablet weighing 63.4 mg was powdered in a mortar. An accurately weighed amount (58.6 mg) was transferred into a calibrated flask (10 cm^3) containing dilute sulphuric acid (0.1 M) and left overnight to allow extraction of the alkaloids. A few drops of sodium hydroxide solution (1 M) were added to precipitate papaverine and also some narcotine. The cloudy solution was filtered (Whatman No. 52 paper) and diluted to 10 cm^3, giving stock solution P. Omnopon injection solution (1 cm^3) was similarly treated, giving stock solution O.

Stock morphine solution (1500 $\mu g\ cm^{-3}$) was prepared and suitable aliquots were diluted with pH 8.5 sodium pyrophosphate solution (0.1 M) to 25 cm^3, giving standard solutions with concentrations ranging from 3 to 30 $\mu g\ cm^{-3}$. Aliquots (500 μl) of the stock P and O solutions were treated likewise. The fluorescence of the standard and unknown solutions was measured in the usual manner at 0.25 V *versus* S.C.E. and the results are presented in Table VI. The unknown concentrations, 9.6 $\mu g\ cm^{-3}$ (solution P) and 19.8 $\mu g\ cm^{-3}$ (solution O) are also included in the table and were read from the calibration graph defined by the equation $y = 3.75x + 6.25$. The result of the assay is shown in Table VII and confirms that the procedure is accurate and interference free.

In a separate experiment, the relative standard deviations (ten determinations on the same solution) at the 6, 30 and 60 $\mu g\ cm^{-3}$ levels were 2.56, 1.20 and 0.74%, respectively.

Conclusions

The gold micromesh fluorescence cell has been demonstrated to be an attractive analytical tool, providing a novel approach for the semi-automatic determination of morphine at trace levels. Electrogeneration of pseudomorphine was reproducible and permitted the direct assay of morphine in pharmaceutical preparations based on papaveretum. The sensitivity

TABLE VI

ASSAY OF PAPAVERETUM TABLET BPC AND OMNOPON INJECTION AMPOULE: FLUORESCENCE RESULTS

Solutions in pH 8.5 sodium pyrophosphate solution (0.1 M); electrode potential 0.25 V *vs.* S.C.E.; excitation wavelength 320 nm; emission wavelength 442 nm.

Morphine concentration/ $\mu g\ cm^{-3}$	Maximum fluorescence intensity, arbitrary units
3.0	10.2,10.3,10.2
6.0	26.8,26.6,26.7
12.0	51.0,50.6,50.8
18.0	75.9,74.3,75.1
24.0	95.4,93.6,94.5
30.0	117.0,115.8,116.4
Unknown solution P—	
9.6	42.6,41.9,42.2
Unknown solution O—	
19.8	81.0,80.4,80.6

of the procedure was comparable to that of the chemical oxidation method of Burkhalter *et al.*[18] A sensitive fluorescence response was obtained for hydrolysed heroin solutions and the above conclusions would apply for heroin. Initial studies have been promising and future work should be directed at assessing the suitability of the cell for routine determinations of morphine (and heroin) in biological samples. The possibility of utilising the gold micromesh cell as a general-purpose fluorogenic unit should also be considered. Compounds examined successfully so far in addition to morphine, monoacetylmorphine and laudanosine include reserpine, thioguanine and homovanillic acid.

TABLE VII

MORPHINE ASSAY IN PAPAVERETUM TABLET BPC AND OMNOPON INJECTION AMPOULE

Sample	Morphine concentration: Specified	Found
Papaveretum Tablet BPC (10 mg)	47.5–52.5% *m/m*	52.0% *m/m*
Omnopon injection ampoule (20 mg cm^{-3}) ..	10 mg cm^{-3}	9.9 mg cm^{-3}

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Physico-chemical Study of Mixed-ligand Selenium(IV) Complexes: Ternary Complex of Selenium(IV) with Alizarin Maroon and Eosin

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The reaction of selenium(IV) with alizarin maroon (AZM) as a primary ligand and eosin as a secondary ligand was examined spectrophotometrically and potentiometrically at 20 ± 0.1 °C and an ionic strength of 0.1 M (perchloric acid). The solution spectra of the mixed-ligand complex formed is characterised by an absorption band with $\lambda_{max.}$ at 560 nm within the pH range 6.5–7.2. The pink selenium - $(AZM)_2$ - $(eosin)_2$ association complex conformed to Beer's law over the concentration range 0.16–2.0 μg ml^{-1} of selenium with a molar absorptivity of 2.5×10^4 l mol^{-1} cm^{-1}.

Keywords: *Selenium(IV) mixed-ligand complexes*; *alizarin maroon*; *eosin*; *spectrophotometry*; *potentiometry*

Considerable work has been done on the formation of association complexes using fluorescein compounds as counter anions.[1–11] The analytical applications of the ternary complexes of some of the transition and rare earth elements have been investigated,[1,2,4,7,9] but little is known about the ternary complexes of selenium(IV).[11]

In continuation of our work on the binary complexes of amino-substituted anthraquinones,[12–14] we have studied the ternary complexes of alizarin maroon (3-amino-1,2-dihydroxyanthraquinone) (AZM) with selenium(IV) using eosin as a secondary ligand.

Selenium is usually determined by fluorimetry after complexation with diaminonaphthalene or diaminobenzidine,[15] but neutron-activation analysis,[16] gas chromatography,[17] colorimetric,[18,19] atomic-absorption spectroscopic[20,21] and electrochemical methods[22] have also been used.

This paper describes spectrophotometric and potentiometric studies of the mixed ligand complex of selenium(IV) with alizarin maroon and eosin. The use of the coloured complex formed for the micro-determination of selenium(IV) has been investigated. The stability constant of the selenium(IV) - AZM - eosin complex has been determined.

Experimental

Reagents

All chemicals were of analytical-reagent grade and doubly distilled water was used for the preparations of all solutions. The solutions were diluted as necessary to prepare working standard solutions.

AZM stock solution, 5×10^{-3} M. Prepared from Merck reagent.

Eosin stock solution, 5×10^{-3} M. Prepared from Merck reagent.

Selenium(IV) stock solution, 10^{-2} M. Prepared using AnalaR sodium selenite and standardised as recommended.[23] The solution was diluted as necessary to prepare standard working solutions.

EDTA stock solution, 10^{-1} M. Prepared from BDH Chemicals reagent.

Buffer solutions. Buffer solutions of pH 3–9 consisting of boric acid, borax, succinic acid and sodium sulphate were prepared as described by Britton.[24]

Sodium hydroxide standard solution 0.1 M.

Perchloric acid standard solution 0.028 M.

Sodium perchlorate standard solution 1.0 M.

Solutions of ions. Solutions of the diverse ions used for the interference studies were prepared by dissolving the calculated amount of each compound in doubly distilled water in order to give 1–10 mg ml^{-1} of the particular ion.

Apparatus

The absorption spectra of solutions to be tested were recorded on a Unicam SP 8000 spectrophotometer in the range 350–650 nm using 1-cm matched silica cells. An aqueous buffer was used as a blank solution.

pH measurements were carried out using a Radiometer pH-meter, Model 28b, with a saturated calomel - glass electrode system. All measurements were performed at 20 $\pm$ 0.1 °C and the ionic strength of all solutions measured was adjusted to $I = 0.1$ (sodium perchlorate).

Procedure

An aliquot of a standard solution of selenium(IV) containing 3.95–79.0 μg of selenium was introduced into a 25-ml calibrated flask then 1.0 ml of 10^{-1} M EDTA solution and 0.5 ml of 10^{-3} M AZM solution were added. The pH was adjusted to pH 7, 5 ml of 10^{-4} M eosin were added and the solution was diluted to volume with doubly distilled water. After thoroughly mixing the reaction mixture, the absorbance was measured at 560 nm against a reagent blank similarly prepared but containing no selenium. For examining the effect of interfering ions, solutions of such ions were pipetted first, followed by the selenium(IV), EDTA, AZM, buffer and eosin solutions. The above procedure was followed.

Results and Discussion

Absorption Spectra

The visible spectra of AZM exhibit an absorption band at about 480–500 nm within the pH range 6.5–7.5. Eosin solution (1×10^{-4} M) shows an absorption band at about 510 nm (Fig. 1) and there are no significant changes in colour or in the absorption spectrum in the presence of selenium(IV) or AZM (10^{-4} M). However, the solution containing AZM and eosin undergoes a change in colour, from orange - yellow to pink, when mixed with 2.5 ml of 10^{-4} M selenium(IV). The spectrum of the reaction mixture against a blank solution containing the same concentration of the two ligands shows an apparent decrease in the absorption at 510 nm and exhibits a new band at 560 nm. The latter is presumably due to the formation of a mixed-ligand complex of selenium(IV). The maximum colour development for the ternary system was attained at pH 6.5–7.2.

It is worth mentioning that the visible spectrum of a mixture containing eosin and selenium(IV) exhibited no absorption when the solution was scanned *versus* eosin.

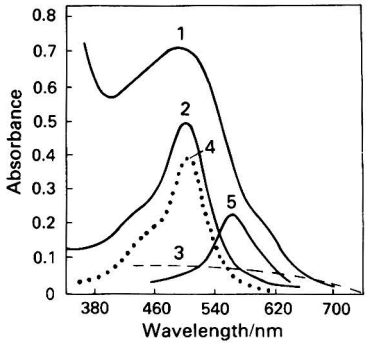

Fig. 1. Absorption spectra of Se(IV) - AZM - eosin ternary complex in aqueous solution. (1) AZM; (2) eosin, Se - eosin, AZM - eosin (all give essentially the same spectrum); (3) Se - eosin *versus* eosin; (4) Se - AZM - eosin *versus* Se; (5) Se - AZM - eosin *versus* AZM - eosin.

Effect of Masking Agents and Diverse Ions

The addition of EDTA as a masking agent in up to a 2500-fold molar excess over selenium(IV) had no effect on the sensitivity of the method. The effect of diverse ions at levels of 1.0–

14 mg per 25 ml on the determination of selenium was studied for a sample containing 27.63 μg of selenium(IV) by the recommended procedure. Each ion was tested individually. The solutions were also 4×10^{-3} M in EDTA. There was no interference from 14 mg (about a 500-fold excess) of Li^+, Na^+, Ba^{2+}, Pb^{2+}, Th^{4+}, UO_2^{3+}, Cl^-, I^-, NO_3^-, SO_4^{2-} and HPO_4^{2-}, 8 mg (about a 300-fold excess) of Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, As^{3+}, Br^-, CO_3^{2-}, $Cr_2O_4^{2-}$, NO_2^- and SO_3^{2-} or 4.0 mg (about a 100-fold excess) of Cr^{3+}, Ni^{2+}, F^- and S^{2-}.

The presence of about 1 mg of aluminium(III) caused a positive error corresponding to about 10 μg of selenium(IV). The interference due to aluminium(III) was eliminated by increasing the concentration of EDTA to 1×10^{-2} M. The maximum amount that can be tolerated is about 2.5 mg. Of the anions investigated, cyanide caused a serious negative error even when present in only a 10-fold excess. The presence of about 1 mg of cyanide seems to prevent any reaction between selenium(IV) and AZM. Experiments to determine the stoicheiometry of the reaction of cyanide with a solution containing AZM and eosin were inconclusive.

Calibration Graphs and Reproducibility

The system followed Beer's law over the range 2×10^{-6}–2.5×10^{-5} M of selenium(IV). The molar absorptivity was 2.5×10^4 l mol^{-1} cm^{-1}. Ten identical samples, each with a final selenium(IV) concentration of 1.4×10^{-5} M, were treated according to the recommended procedure and their absorbances were measured. The mean absorbance was 0.35, with a standard deviation of 0.003 absorbance unit.

Stoicheiometry of the Complex

Job's method of continuous variation[25,26] was applied to establish the composition of the ternary complexes under investigation. The molar fractions of two of the components were varied continuously, keeping their combined concentration constant, and keeping the third component in a large constant excess for all solutions in the series. Under these conditions the ternary system was modified to a quasi-binary system. The results shown in Fig. 2 indicate that the over-all selenium(IV) - AZM - eosin composition is 1:2:2.

The stoicheiometry of the ternary system was also determined by applying the molar ratio method[27] (*cf.*, Fig. 3). In the formation of this complex, the first ligand (AZM), on entering

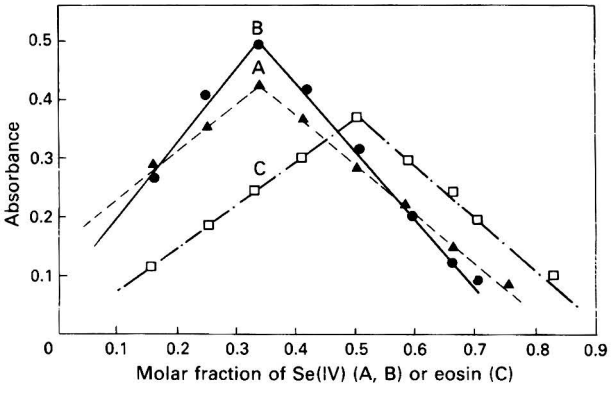

Fig. 2. Job plots: (A) eosin - Se(IV) (in excess of AZM), varying ratios of 1×10^{-4} M eosin and Se(IV) plus 5 ml of buffer and 1 ml of 10^{-3} M AZM per 25 ml; (B) AZM - Se(IV) (in excess of eosin), varying ratios of 5×10^{-4} M AZM and Se(IV) plus 5 ml of buffer and 5 ml of 5×10^{-4} M eosin per 25 ml; and (C) eosin - AZM [in excess of Se(IV)], varying ratios of 5×10^{-4} M eosin and AZM plus 5 ml of buffer and 5 ml of 5×10^{-4} M Se(IV) per 25 ml.

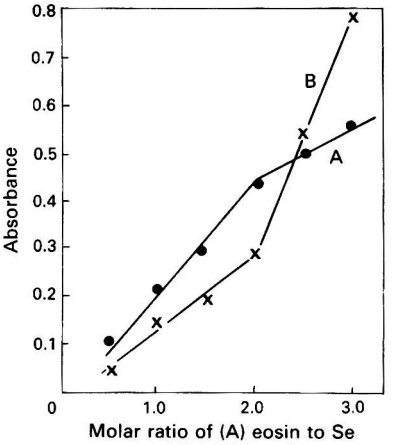

Fig. 3. Molar ratio plots: (A) variation of eosin against constant [Se(IV)], 0.5–12 ml of 10^{-4} M eosin added to 4.0 ml of 10^{-4} M Se(IV), 1.0 ml of 10^{-3} M AZM, 5 ml of buffer per 25 ml; (B) variation of AZM against Se(IV), 0.5–6 ml of 5×10^{-4} M Se(IV), 7.0 ml of 5×10^{-4} M eosin and 5 ml of buffer per 25 ml.

the coordination sphere of selenium(IV), fully satisfies it, but does so in a purely dative fashion, so that the complex ion still bears the over-all positive charge of the original central ion, $(O{=}Se)^{2+}$, and is free to ion associate with a second ligand (eosin) of suitable anionic charge to form a ternary complex.

Potentiometric Measurements

Potentiometric titration of the following mixtures (total volume made up to 50 ml) was studied: (A) 5 ml of 0.028 M perchloric acid + 5 ml of 1 M sodium perchlorate solution, (B) mixture A + 5 ml of 5×10^{-3} M AZM solution, (C) mixture B + 2.5 ml of 5×10^{-3} M selenium-(IV) solution, (D) mixture B + 5 ml of 5×10^{-3} M eosin solution and (E) mixture D + 2.5 ml of 5×10^{-3} M selenium(IV) solution, all against carbon dioxide-free sodium hydroxide solution (10^{-1} M). The concentrations were $N^0 = 0.1$ M, $E^0 = 0.0028$ M, $TC_{L^0} = 0.0005$ and $TC_{M^0} = 0.00025$ M (symbols have their usual meanings). The titration curves obtained are shown in Fig. 4.

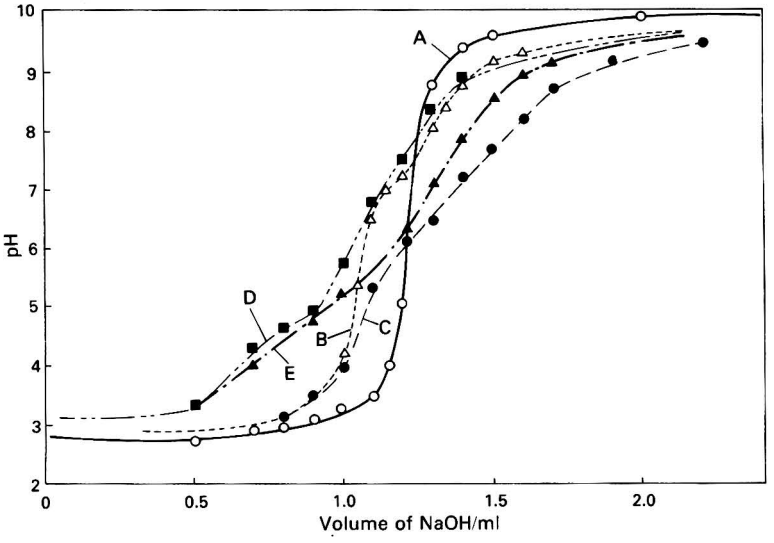

Fig. 4. pH - titration graphs for Se(IV) - AZM - eosin. For identification of graphs A–E, see text.

The titration curves were used to evaluate $\bar{n}_A$ (average number of protons associated with the ligand AZM), $\bar{n}$ (average number of ligand AZM molecules attached per metal ion) and pL (free ligand exponent). From these data, the values of the proton - ligand and metal - ligand stability constants were obtained (Table I).

From curves A, B and C in Fig. 4, it can be seen that complete formation of Se - AZM species occurs at about pH 5.3, and they remain stable at higher pH values. In the mixture

TABLE I

IONISATION CONSTANTS OF AZM AND STABILITIES OF ITS BINARY AND TERNARY COMPLEXES WITH SELENIUM(IV)

AZM	pK_1	= 9.1
	pK_2	= 7.1
	pB_H	= 16.2
Se(IV) - AZM	Log K_1	= 6.9
	Log K_2	= 6.0
	Log B	= 12.9
Se - AZM - eosin	Log K_m	= 11.35

containing selenium(IV) and the two ligands (curve E), the selenium(IV) - AZM complex is formed first (at low pH), then subsequently associates with the eosinate anion to form the mixed-ligand complex in a stepwise manner. The composite curve (curve D) is not superimposable with the mixed-ligand titration curve (curve E), thereby confirming the formation of a selenium(IV) - AZM - eosin complex. The horizontal distances between curves D and E were measured and used for the calculation of $\bar{n}_{mix.}$ {average number of secondary ligand molecules attached per $[Se(IV) - (AZM)_2]^{2+}$ ion} using the equation

$$\bar{n}_{mix.} = \frac{(V_E - V_D)\,(N^0 + E^0)}{(V^0 + V_A)\,\bar{n}_A\,TC_{Se-AZM^0}} \qquad (1)$$

where V_D and V_E are the volumes of alkali consumed to reach the same pH value in curves D and E of the mixed-ligand system and $TC_{Se\text{-}AZM^0}$ is the total initial concentration of the selenium - AZM complex, which is equivalent to the initial selenium(IV) concentration taken in mixture C; all other symbols have their usual meanings.[28] Values of $\bar{n}_A$ at different pH values were available from the data for the binary complexing system. From the values obtained for $\bar{n}_{mix.}$, the free ligand exponent, $pL_{mix.}$, was calculated using the equation

$$pL_{mix.} = \log \left[\frac{\sum_{n=0}^{i} B_n^H \left(\frac{1}{\text{antilog } B}\right)^n}{TC_{L^0} - \bar{n}_{mix.}\,TC_{Se-AZM}} \times \frac{V^0 + V_E}{V^0} \right] \qquad (2)$$

where B_n^H is the proton–ligand stability constant, B is the metal–ligand stability constant, TC_{L^0} is the total concentration of the ligand and n is the number of protons released from the ligand molecule.

By plotting $\bar{n}_{mix.}$ against $pL_{mix.}$, the formation curve was obtained and the formation constant of the ternary system was evaluated.

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Automatic Determination of Sulphur Dioxide by a Coulometric Method: Interferences and Reliability of Measurements

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The selectivity of filters used in coulometric sulphur dioxide analysers was evaluated for various volatile organic substances that can be present in the atmosphere of an industrial area with different chemical plants. The poor selectivity of chemical filters, mainly with respect to vinyl acetate and styrene, was ascertained by several tests at various concentrations of the interfering substance. It is possible that the interferences arise from reactions with the bromine present in the analysis cell. The magnitude of the interference appeared to be related to the kinetics of addition of the bromine to unsaturated organic interferents. Pre-heating the air sample to 800 °C resulted in complete elimination of the vinyl acetate interference; less promising results were obtained with styrene. Practical modifications of air pre-treatment on the analysers were consequently adopted.

Keywords: *Sulphur dioxide determination*; *coulometry*; *air pollution control*; *interferences*

On the basis of an evaluation[1] of the various systems for the automatic determination of sulphur dioxide reported in the literature, the spectrophotometric method proved to be the most sensitive and interference free. The coulometric method appeared sensitive to oxidising and reducing substances, which therefore needed removal before introducing the air sample into the measuring cell.

In spite of this, the need for automatic analysers for incorporation in complex monitoring networks (requiring low maintenance and giving signals processable by a computer) shifted interest again to monitors based on the coulometric method,[2–4] defined as equivalent to the US Environmental Protection Agency reference method.[5] They have therefore found widespread use in the last 10 years.[6]

Though the use of analysers to control the air quality in urban areas ensures a satisfactory reliability of the measurements,[7] the situation could be very different in large industrial areas owing to the qualitative and quantitative composition of the atmospheric pollutants. The simultaneous presence in the atmosphere of various substances (such as nitrogen oxides, ozone, chlorine, hydrogen sulphide, thiols, saturated and unsaturated hydrocarbons and their derivatives) required a deeper investigation of the role they play in the redox process used in the coulometric method and a critical evaluation of the filters used for interfering substances, developed by the producers of the monitors.

Exceptionally high sulphur dioxide concentrations in some places and in particular examples of chemical pollution recorded by the monitoring stations of a network installed in a wide industrial area, could not in fact be correlated either with significant changes in sulphur dioxide emissions from the industries or with the meteorological conditions.

There was evidence for the presence of interferences in the sulphur dioxide determination during the analytical runs carried out by a mobile laboratory placed near the monitoring stations; in some instances sulphur dioxide values higher than the total airborne sulphur concentrations value ($S_{tot.}$) were recorded.

We have therefore carried out a systematic investigation on the interferences on sulphur dioxide measurements in the ambient air in order to find the most adequate systems to eliminate them.

Experimental

Apparatus

The monitors used in this work are parts of a monitoring network installed in an industrial area and in the surrounding urban area.[8,9]

A mobile laboratory is equipped with NO_x, particulates, total hydrocarbons, hydrogen sulphide and total sulphur analysers and integrates the fixed monitoring system.[10]

The sulphur dioxide monitors of the network are connected by telephone cables to a Philips minicomputer, Model P 855 M, located at the network Control Centre, which converts the electrical signals into instantaneous concentration values and processes them, following suitable routine processes, into average values for 30-min periods.

The total sulphur monitor, as well as the other analysers installed in the mobile laboratory, is linked to a Philips microcomputer, Model ECO IV, which processes the instantaneous electrical signals giving average concentration values over 30-min periods.

A simple map, showing the location of the network monitors, is given in Fig. 1.

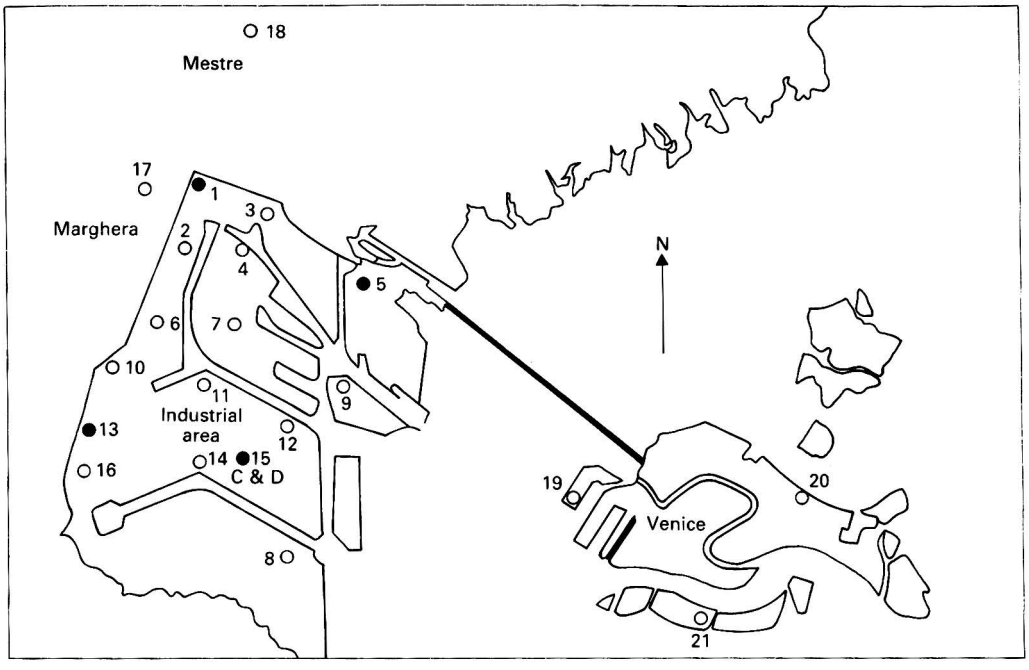

Fig. 1. Simple map of the air-pollution control monitoring network of the Industrial Board of Porto Marghera (Venice).[8,9] ○, SO_2 monitors; and ●, SO_2 plus meteorological monitors. Distance between monitors 1 and 2 = 1 km.

Four coulometric monitors, Philips, Model PW 9700, equipped with selective filters, either heated silver gauze or "chemical" type, were used for sulphur dioxide measurements. The "chemical" filter would appear to consist, on the basis of X-ray diffractometric analyses conducted by us, of activated carbon impregnated with potassium sulphate and natural zeolites and, according to the manufacturer, should be replaced every 3 months.

A coulometric monitor, Philips, Model PW 9755, supplied with a pre-heating furnace, was used for measuring total sulphur content ($S_{tot.}$), determined as sulphur dioxide.

The concentration range selected for both sulphur dioxide and $S_{tot.}$ was 1.15 p.p.m. full scale. A measurement accuracy of better than 15% of the signal, a reproducibility of better than 1% and a detection level of lower than 4 p.p.b., for both sulphur dioxide and $S_{tot.}$, were quoted by the manufacturer.

The calibrations of the monitors were made automatically daily by zeroing with air purified on activated charcoal and measuring the sulphur dioxide concentration resulting from a given amount of sulphur dioxide emitted in a purified air flow by a standard sulphur dioxide source.[11]

In an attempt to reduce the effect of some interfering substances, the PW 9700 monitor was equipped with a pre-heating furnace, consisting of a quartz tube, i.d. 4 mm, wound in 14 coils, each having a mean diameter of about 12 mm, and placed inside a thermostatically regulated heating unit. A selective filter was placed down-stream of the furnace. An air flow-rate of 150 ml min^{-1} was used.

The PW 9700 monitor with the calibration apparatus and the pre-heating furnace is shown in Fig. 2.

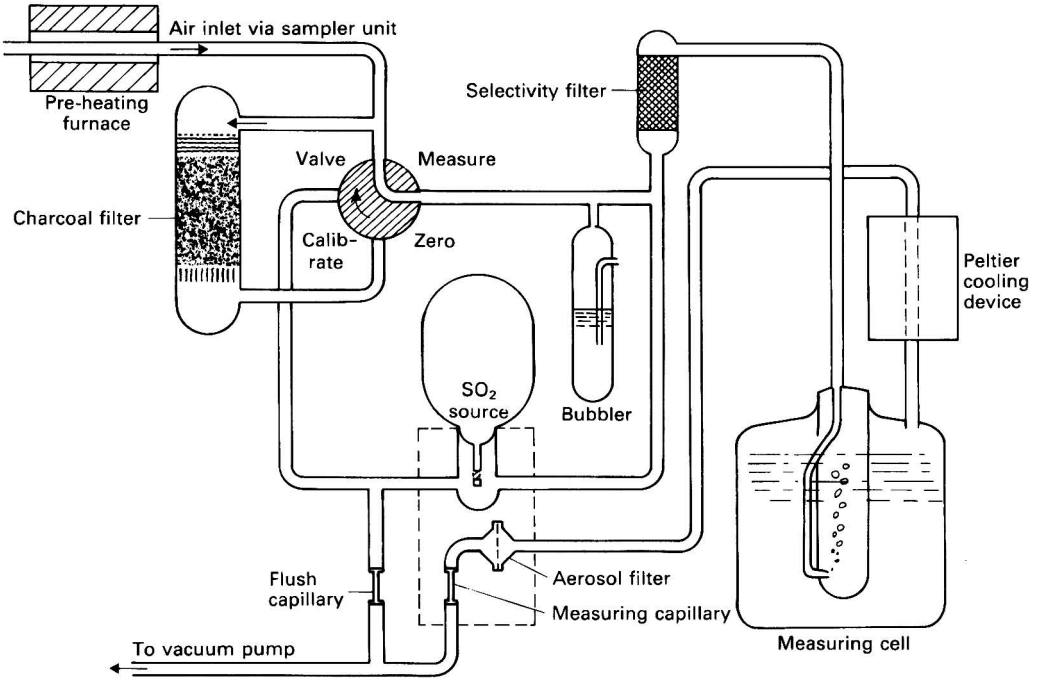

Fig. 2. Diagram of the monitor PW 9700 with the calibration apparatus[11] and the pre-heating furnace.

Procedure

The average measurements over 30-min periods of sulphur dioxide and $S_{tot.}$ were compared at two positions in the industrial area, using sulphur dioxide monitors of the monitoring network, supplied with "chemical" filters, and the $S_{tot.}$ monitor available in the mobile laboratory placed, for this purpose, near the measuring stations.

In order to evaluate the efficiency of the various selective filters, two sulphur dioxide monitors were placed in a given position of the network in the middle of the industrial area, and the average values over 30-min periods were processed.

The degree of selectivity of the filters for some substances was evaluated by means of sulphur dioxide monitors located at the network Control Centre. Calibration mixtures of the substances under investigation were prepared by a static method[12]: the actual concentrations of the mixtures were controlled by gas chromatography or infrared spectrometry.

The calibrated mixtures were sucked in by the analyser pump, simultaneously balancing the gas container by introducing purified air. The maximum value of the recorded signal was measured and the concentration of the substance fed in was calculated, taking into account the container volume, the time interval from feed start-up and the amount of air used for dilution.

Results and Discussion

Our investigation was originated, as already stated, by the high sulphur dioxide values not attributable to appreciable variations in the sulphur dioxide emissions in the industrial area or to particular meteorological conditions. It was developed in stages as a result of the data reported and discussed in the tables.

The $S_{tot.}$ values recorded by the mobile laboratory during analysis runs carried out near two sulphur dioxide monitoring stations of the network have been compared over three different time periods. The results obtained are reported in Table I. The mean value of the ratio $[SO_2]/[S_{tot.}]$ is lower than unity when all the data collected are considered, but it was found that the ratio becomes higher than unity when the data lower than a pre-fixed concentration [40 p.p.b. (10^9)] is rejected. In this last instance, which is less affected by errors in measurements and differences in instrument calibration, a decrease in the scattering of the ratio around the mean value was obtained.

TABLE I

COMPARISON OF THE $[SO_2]/[S_{tot.}]$ RATIOS AT GIVEN POSITIONS OF THE NETWORK

Station number (see Fig. 1)	Measuring period	$[SO_2]/[S_{tot.}]$ ratio calculated from all data collected			$[SO_2]/[S_{tot.}]$ ratio calculated with values greater than 40 p.p.b.		
		Average	Standard deviation	Number of tests	Average	Standard deviation	Number of tests
4	April–May	0.85	0.50	2210	1.20	0.35	818
11	July–August	0.80	0.45	1824	1.25	0.35	319
11	November	0.76	0.46	1083	0.97	0.24	316

These anomalies could, in some way, be connected with the fact that the sulphur dioxide monitor is equipped only with a selective filter, while the $S_{tot.}$ monitor is also fitted with a pre-heating furnace suitable for oxidising all volatile sulphur compounds to sulphur dioxide.

In order to verify if the anomalies mentioned above arise from the differences in the air pre-treatment, the average data for 30-min periods from two monitors placed at a given position of the network and equipped with different filters, were collected. The experiment was conducted for 10–15 d, a time period that is long enough to cover different conditions of atmospheric pollution.

The results are given in Table II.

TABLE II

FREQUENCIES OF OCCURRENCE FOR DIFFERENT RANGES OF THE VARIATIONS IN THE RATIO BETWEEN THE DATA RECORDED BY TWO MONITORS PLACED AT A GIVEN POSITION OF THE NETWORK

Range of data recorded for ratios of A/B	Frequency for A and B monitors equipped with "chemical" filters already in use, %	Frequency for A/B,* %	Frequency for A/B,† %
0–0.3	23.7	30.5	74.6
0.3–0.6	16.3	22.0	7.9
0.6–1.0	35.6	38.6	6.2
1.0–1.5	5.5	0.6	0.8
1.5–2.0	2.0	0.4	0.7
2.0–∞	17.0	7.9	9.8
Geometric average	0.66	0.50	<0.10

* Monitor A with "chemical" filter already in use and monitor B with a new "chemical" filter.

† Monitor A with "chemical" filter already in use and monitor B with a heated silver gauze filter.

For the "chemical" filters already in use (column 2), the geometric average of the ratios was 0.66, *i.e.*, the values recorded by monitor B tended to surpass those recorded by monitor A, with a frequency of 75.6%.

After replacing the "chemical" filter with a new one in monitor B, this frequency was expected to decrease, owing to the better efficiency of a new filter in comparison with one already in use and probably exhausted; in contrast, B values, higher than A values (column 3), increased up to 91.1%. This would be, partially at least, imputable to a general decrease in the pollutant concentrations, as it also appeared that a higher frequency of zero values were obtained from monitor A, and mostly to differences in the monitor calibration, which become more important in the low concentration ranges at which the geometric average of the ratios decreases to 0.5. When replacing the "chemical" filter in monitor B with the silver gauze type, which was not supposed to be as effective (according to the indications of the manufacturer), the absolute value of B data with respect to A data was expected to increase; instead, monitor B went on recording a comparable number of values at concentrations lower than A (column 4), equal to 88.7% compared with 91.1% as found previously. This could also have been due to the greater number of zero values recorded by monitor A.

Therefore, if there is a difference between the "chemical" filter and the silver gauze filter, it did not emerge clearly during the tests.

Table III reports the selectivity of the Philips, Model PW 9700, monitor including a heated silver gauze filter, as indicated by the manufacturer.[11] No further information could be found in the literature.

TABLE III

SELECTIVITY OF THE PHILIPS MONITOR PW 9700[11]

Interferent	S,* %
Nitrogen oxide	<1
Nitrogen dioxide	<5
Ozone	<1
Chlorine	<2
Hydrogen sulphide	<1
Ethylene	<2
Aldehydes	<1
Benzene	<1
Chloroform	<1
Carbon disulphide	<1
Methane thiols	About 180

* S = signal from 0.5 p.p.m. of interferent/signal from 0.5 p.p.m. of sulphur dioxide.

Owing to the peculiar situation of the industrial area investigated, an attempt has been made to widen the knowledge on other possible interferences.

In the subsequent tables the interference from a chemical substance will be generally expressed by the ratio

$$\frac{\text{signal measured for a given concentration of interferent}}{\text{signal measured for the same concentration of } SO_2} \times 100$$

The pollutant concentrations adopted in the tests were often at high levels, which are likely to occur under particular conditions and close to some production plants. The results obtained are given in Table IV. As can be seen, the most surprising fact is the lack of selectivity differences using the "chemical" filter and the silver gauze one, and, above all, the lack of selectivity with respect to the monitor without any filter. Another interesting aspect of the results is the poor selectivity of both filters for vinyl acetate and styrene.

The same results were obtained for vinyl acetate and styrene using a wider concentration range and different analysers equipped with filters at various degrees of exhaustion. In Table V the mean values of the percentage interference, using monitors equipped with a silver filter, a "chemical" filter and without a filter, are reported. Mixtures of interferents in air were fed at different concentration values in the ranges indicated in column 1; the

TABLE IV

EVALUATION OF FILTER SELECTIVITY FOR VARIOUS SUBSTANCES

Interfering substance	Mean fed concentration, p.p.m. *V/V*	Interference, % Ag filter	Interference, % "Chemical" filter	Interference, % No filter
Ethylene	60	0.9	0.6	0.7
Toluene	6.5	0.9	0.8	0.7
Vinyl acetate	0.87	145	130	130
	0.16	105	105	105
Vinyl chloride	24.9	0		
	10	0.07		
	8.4		0.2	0
Acrylonitrile	24.3	0.1		
	8.6	0.16		
	6.2		0.3	0
Hexane	19.4	0	0	0
Dimethylacetamide	7	0	0	0
Nitrogen oxide + nitrogen dioxide (2 + 3)	2.3	0	0	0
Gasoline	11	1	0.9	ND*
Dichloroethane	VST†	0	0	ND*
Trichloroethylene	VST†	0	0	0
Acetylene	100	0	0	0
Ammonia	VST†	0	0	0
Cyclohexanone	VST†	0	0	0
Acetaldehyde	114		0.34	
	93	0.42		
	76			0.35
Styrene	0.84	50		32
	1.09		33	

* ND = not determined.
† VST = vapour-saturated air.

number of mixtures tested is reported in column 2. As can be seen, the selectivity of the two filters is still low, although they show a wider degree of variability. The results, moreover, did not appear to be correlated with the concentration of interferent fed in.

A theoretical and experimental investigation on the reactivity of the analysed substances with the bromine contained in the analysis cell in the presence of sulphuric acid was beyond the aims of our work. However, on the basis of kinetic data found in the literature[13] on addition of bromine to alkenes, mainly ethylene, halosubstituted alkenes, styrene and its derivatives, it could be reasonably stated that vinyl acetate and styrene interferences are to be attributed to oxidation reactions in the analysis cell. In contrast the absence of interferences, for instance for ethylene and vinyl chloride, could be attributed to the much lower values of the coefficients of the rates of reaction of the bromine addition under the analytical conditions.

TABLE V

SELECTIVITY EVALUATION USING DIFFERENT INSTRUMENTS AND FILTERS AT VARIOUS DEGREES OF EXHAUSTION

Fed concentration range, p.p.m. *V/V*	Number of tests	Mean value of interference and standard deviation, % Ag filter	"Chemical" filter	No filter
A. Vinyl acetate—				
0.42–1.38	3	32.7 ± 1.18		
0.17–1.05	6		61.0 ± 11.8	
0.34–0.84	3			47.7 ± 17.8
B. Styrene—				
0.43–2.10	5	33.6 ± 13.3		
0.17–1.34	6		36.7 ± 5.16	
0.88–1.00	3			26.0 ± 7.13

We consequently tried to eliminate or reduce the interferences of volatile organic substances by submitting the gas sample to a thermo-oxidation treatment, before its inlet to the analysis cell. When using the thermo-oxidation treatment, possible sulphur-containing substances, not retained by the selective filters, are likely to give rise to apparent sulphur dioxide concentrations higher than those actually present in the atmosphere. On the basis of the knowledge of the chemical plants in the industrial area investigated, such occurrence, however, appears very unlikely.

A set of tests at different temperatures were thus carried out by placing a pre-heating furnace up-stream of the selective filter and considering the main interferents investigated, that is, vinyl acetate and styrene.

The air samples containing the interferents were fed to the monitor for 6 min; after this period, the maximum response of the analyser was obtained. As can be seen in Table VI, the treatment at 800 °C succeeds in eliminating vinyl acetate interference. For styrene, at the same furnace temperature, it appears that the interference could not be eliminated completely.

TABLE VI

EFFECT OF THERMO-OXIDATION ON VINYL ACETATE AND STYRENE INTERFERENCE ON DETERMINATION OF SULPHUR DIOXIDE

	Furnace temperature/°C	Concentration of interfering substance, p.p.b. *V/V*	Concentration recorded as SO_2, p.p.b. *V/V*	Interference, %
A.	*Vinyl acetate*—			
	25	657	990	151
	580	792	888	112
	800	955	14	1.5
B.	*Styrene*—			
	800	760	114	15
	800	1180	306	26

Conclusion

The influence of chemical interferences on the coulometric determination of sulphur dioxide, even in a complex industrial area, can be considered in most instances as being negligible and not affecting the reliability of the data collected. In particular positions and for very short periods, this statement, however, does not hold true.

As a consequence of our findings, although on all analysers of the network the "chemical" filters have been replaced with the heated silver gauze filters, which show comparable performance and require lower operating costs, in some positions, where interfering substances are involved, the analysers have been equipped with a pre-heating furnace. In these instances the total or partial elimination of organic interferents was preferred to the possible higher sulphur dioxide values resulting from non-retained sulphur compounds.

The first data obtained from the new arrangement of the analysers in the monitoring network agree with our expectations.

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Comparative Study of the Determination of Nitrates in Calcareous Soils by the Ion-selective Electrode, Chromotropic Acid and Phenoldisulphonic Acid Methods

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A comparative study of the determination of nitrates in calcareous soils by the chromotropic acid, phenoldisulphonic acid and nitrate ion-selective electrode methods was investigated, using 0.02 N copper(II) sulphate solution as extractant, which, in addition to being a preservative for nitrates, helps filtration and eliminates interference by hydrogen carbonates in the ion-selective electrode method. Silver sulphate, which had no effect on either procedure, can be added to the extractant for the precipitation of chlorides.

Nitrate in soil suspensions was determined by the ion-selective electrode method. Nitrites if present were eliminated by acidifying the extract with 1 N sulphuric acid containing sulphamic acid.

To avoid reaction between soluble organic matter and sulphuric acid in the chromotropic acid method, reagents were added in two steps with continuous cooling. Nitrites were eliminated as in the ion-selective electrode method.

Both methods were faster than the phenoldisulphonic acid method and gave identical results; the correlation coefficient was 0.9998.

Keywords: *Nitrate determination*; *ion-selective electrode*; *chromotropic acid method*; *phenoldisulphonic acid method*; *calcareous soils*

The various methods[1–18] for the determination of nitrates in soil can be divided into three groups: spectrophotometric,[1–3] distillation[2] and ion-selective electrode.[4–13,15,16,18] More rapid and accurate methods are needed to replace the lengthy classical phenoldisulphonic acid[1,2] and distillation[2] methods which suffer from interferences from other ions.[1,2] A rapid spectrophotometric method is that with chromotropic acid used originally for water[14] and later for soil analysis.[3] The nitrate ion-selective electrode has been extensively used, even though there are interferences from other ions.[5–7,11,13,15,16] The rapidity and the good accuracy achieved using this electrode[4–13,15,18] have made it suitable for use in routine analysis and in soil agrochemical research.[7,10,17]

Various workers[4–13,15,18] have used different extraction solutions in the ion-selective electrode method, depending on the soil being analysed. The most important are water,[5–8,10,18] potassium sulphate,[12] aluminium sulphate,[9] copper(II) sulphate,[6] calcium hydroxide[5] and copper sulphate(II) with aluminium and silver resins.[13]

The main purpose of this work was to investigate the application of the nitrate ion-selective electrode to calcareous soil suspensions. For comparison, the chromotropic acid method was modified for the analysis of soils containing calcium carbonate. These methods were compared with each other and with the phenoldisulphonic acid method. In addition, ways of eliminating interfering ions were studied.

Experimental

Apparatus

An Orion, Model 93–07, nitrate ion-selective electrode with a 1 × 2 sensing module construction, and an Orion, Model 90–02, double-junction reference electrode fitted on a Radiometer, Model PHM62, pH meter were used. The outer chamber was filled with 0.04 M ammonium sulphate solution and the inner chamber with Orion 90–00–02 solution.

A Bausch and Lomb Spectronic 70 spectrophotometer was used in the phenoldisulphonic and chromotropic acid methods.

Reagents

All reagents were of analytical-reagent grade, except for phenoldisulphonic and sulphamic acids, which were of laboratory-reagent quality.

Phenoldisulphonic acid method

The solutions were those used by Jackson.[1]

Chromotropic acid method

Copper(II) sulphate solution, 0.02 N. Dissolve 2.5 g of $CuSO_4.5H_2O$ in water and dilute to 1 l.

Chromotropic acid, 0.1%. Dissolve 0.184 g of chromotropic acid, sodium salt, in 100 ml of concentrated sulphuric acid. This solution was stable for 2 weeks when kept in the dark.

Stock standard nitrate solution, 1000 mg l^{-1} *of* NO_3-*N*. Dissolve 7.2184 g of potassium nitrate, dried for 2 h at 105 °C, in 1 l of water.

Working standard nitrate solutions, 0–3.5 mg l^{-1} *of* NO_3-*N*. Dilute the stock standard solution with 0.02 N copper(II) sulphate solution. Standard solutions were prepared in the range 1 to 100 mg l^{-1} of NO_3-N in 0.02 N copper(II) sulphate solution for the method using the ion-selective electrode.

Procedures

Phenoldisulphonic acid method

The phenoldisulphonic acid method was used as directed by Jackson.[1]

Chromotropic acid method

Weigh 10 g of air-dried soil, add 50 ml of 0.02 N copper(II) sulphate solution, shake the mixture for 15 min and filter it through a double Whatman No. 42 filter-paper. Transfer a 3-ml volume into a 25-ml calibrated flask and cool in ice. Add 1 ml of 0.1% chromotropic acid solution drop by drop, cool for 3–5 min and then swirl the mixture. Add 6 ml of concentrated acid, cool for 2 min, mix and leave at room temperature for 45 min for the colour to develop. Read at 430 nm in a 1-cm cuvette. Standard solutions and blanks were subjected to the same treatment. The colour produced is stable for at least 2 h. If filter-papers give coloured solutions with copper(II) sulphate solution, they must be washed with distilled water and dried before use.

Ion-selective electrode method

Weigh 20 g of air-dried soil, add 50 ml of 0.02 N copper(II) sulphate solution, shake for 15 min and leave the suspension for 30 min at room temperature to reach equilibrium. Then, after continuous stirring for 1 min, read on a Radiometer pH meter. Standard nitrate solutions were measured in the same way. Standard solutions have to be re-read every hour because of drift. The temperature of the soil suspensions and standards must be the same or temperature differences[16] must be taken into consideration.

Results and Discussion

The soils used were representative of the major soil type of Cyprus. Table I shows that they had pH values of more than 7.5, with low chloride values and generally high calcium carbonate and hydrogen carbonate values. In the chromotropic acid method, copper(II) sulphate helped filtration and gave clear, colourless solutions. In the ion-selective electrode method, it precipitated carbonates and hydrogen carbonates and eliminated interferences by these ions in soil suspensions.[6,8,11,16]

Sims and Jackson[3] used other extractants in the chromotropic acid method but, with calcareous soils, filtration was slow and some soils gave coloured extracts. Copper(II) sulphate showed no absorption at 430 nm, gave colourless extracts and standards prepared with this solution gave results identical with those for aqueous solutions. As shown in Table I, the soils used were low in organic matter, but it caused some interference. To

TABLE I

SOIL TEXTURE AND CHEMICAL COMPOSITION OF THEIR AQUEOUS EXTRACTS

Soil : water ratio = 1 : 10.

Soil No.	Soil type	pH (1 : 2.5 H_2O)	Organic matter, % *m/m**	$CaCO_3$, % *m/m*	Composition/mg kg^{-1}						
					Magnesium	Calcium	Sodium	Potassium	Hydrogen carbonate	Sulphate	Chloride
1	Sandy clay loam	7.40	1.76	5.36	58	344	195	188	1135	77	0
2	Sandy loam	8.10	0.42	9.06	39	176	253	20	1281	48	0
3	Clay loam	8.15	1.70	70.4	19	444	30	88	1403	67	0
4	Silty clay	8.15	1.10	22.5	47	260	118	50	1281	29	47
5	Clay	8.15	0.99	18.3	54	304	155	80	1391	29	128
6	Sandy clay loam	7.50	1.34	0	44	44	34	25	464	19	0
7	Clay	7.90	0.77	2.76	49	272	238	240	1269	58	78
8	—†	8.15	0.71	5.86	54	120	136	6	805	0	71
9	Loam	8.10	0.53	29.9	19	176	111	34	903	38	21
10	Clay loam	7.90	0.67	27.8	19	208	43	35	805	29	21
11	Clay	7.90	1.44	19.2	24	200	90	54	964	29	25
12	Clay loam	7.65	2.08	21.8	32	284	101	165	927	403	36
13	Clay	7.40	0.39	18.5	34	156	298	36	549	336	163
14	Sandy clay loam	7.50	1.34	2.47	46	44	48	26	488	19	0

* Determined by the Walkley and Black method.[13]
† Insufficient amount of soil for determination of type.

eliminate any undesirable reaction with soluble organic matter, sulphuric acid and chromotropic acid, it was found necessary to add the reagents to the aliquot in a different sequence than that used by Sims and Jackson[3] and to cool the solution.

Chlorides interfere in the determination of nitrates.[3,14] Addition of hydrochloric acid to the stock solution increased the sensitivity of the chromotropic acid, but the absorbance did not comply with Beer's law. Without hydrochloric acid the calibration graph was linear and followed Beer's law. Although the soils used were low in chlorides, the addition of 50 ml of extraction solution containing 0.06% *m/V* of silver sulphate eliminated the interference caused by 650 mg kg^{-1} of chlorides and clarified the solutions. This is shown in Table II for the addition of 500 mg l^{-1} of chlorides to soils.

TABLE II

EFFECT OF SILVER SULPHATE ON CHLORIDE INTERFERENCE IN THE CHROMOTROPIC ACID METHOD

Soil No.	Addition of chlorides/mg l^{-1}: 0 — Nitrate-nitrogen/mg kg^{-1}: 0.02 N $CuSO_4$	Addition of chlorides/mg l^{-1}: 500 — Nitrate-nitrogen/mg kg^{-1}: 0.02 N $CuSO_4$	Addition of chlorides/mg l^{-1}: 500 — Nitrate-nitrogen/mg kg^{-1}: 0.02 N $CuSO_4$ + 0.06% *m/V* Ag_2SO_4
1	208	205	203
2	19.9	21.3	20.4
3	33.4	39.3	33.9
4	17.2	26.8	17.8
5	12.0	11.7	12.7
6	3.5	2.0	3.2
7	126	122	125
8	6.3	6.4	6.4
9	7.8	10.4	8.9
10	7.2	7.6	8.0
11	6.1	5.3	6.2
12	14.5	14.1	15.5
14	3.6	2.1	3.2

Nitrites interfere strongly in the determination of nitrates by the chromotropic acid method. The addition of 0.1 ml of 0.2% *m/V* sulphamic acid in 0.1 N sulphuric acid to the 3-ml sample solution could eliminate up to 150 mg kg^{-1} of nitrites in 10 g of soil.

Calcareous soils are sometimes difficult to filter. The special 1 × 2 construction of the sensing module of the Orion ion-selective electrode enabled us to make direct readings in the soil suspensions without filtering. The reasons for choosing copper(II) sulphate have been

discussed above, and it also acted as a preservative preventing biological degradation of nitrates. Other concentrations of copper(II) sulphate were investigated by Øien and Selmer-Olsen[6] and it was found that concentrations of copper(II) sulphate higher than 0.02 N increased the ionic strength and decreased the nitrate concentration. With calcareous soils, 0.03 N copper(II) sulphate solution gave the same results as 0.02 N copper(II) sulphate solution, and the addition of silver sulphate (0.09% *m/V*) eliminated chloride interference.

With regard to interference by nitrites several workers[5,8,16] have solved the problem by adding sulphamic acid to the extractant. In this work sulphamic acid, which reacts only in weakly acidic solutions, could not be used with the alkaline extracts. If the soil nitrite is high the suspension is filtered and 3–4 drops of 1 N sulphuric acid containing the necessary concentration of sulphamic acid are added. In order to detect trace amounts of nitrites semi-quantitatively, Merckoquant nitrate test papers can be used.

The influence of interfering ions[3,5–7,11,13–16] has been studied on pure nitrate solutions. However, some ions in soil extracts interfered. The chromotropic acid and ion-selective electrode procedures were compared with the phenoldisulphonic acid method without taking into consideration the presence of low concentrations of anions.

Table III shows the results of the analysis of soils by the three methods. Tables I and III show that both methods under investigation could be used to analyse soils with low and high concentrations of nitrates and with a wide range of calcium carbonate contents. The ion-selective electrode method was the fastest, followed by the chromotropic acid method; the phenoldisulphonic acid method took much longer.

Table III

Soil nitrate-nitrogen determined by phenoldisulphonic acid, chromotropic acid and ion-selective electrode methods

	Nitrate-nitrogen, mg kg^{-1}		
Soil No.	Phenoldisulphonic acid method	Chromotropic acid method	Ion-selective electrode method
1	210	208	204
2	18.3	19.9	21.0
3	29.6	33.4	29.4
4	15.2	17.2	17.1
5	9.6	12.0	12.4
6	3.4	3.5	3.8
7	126	126	122
8	4.8	6.3	6.4
9	6.5	7.8	7.7
10	6.4	7.2	7.5
11	4.8	6.1	6.1
12	11.8	14.5	13.7
13	57.1	60.3	54.2
14	3.0	3.6	3.3

The relationships between the three methods are shown in Table IV. There is a very close relationship between the methods; the correlation coefficients are almost unity, indicating that the phenoldisulphonic acid method could be replaced with the ion-selective electrode or the chromotropic acid method.

Table IV

Regression equations and correlation coefficients between the three methods for nitrate-nitrogen determination

Regression equation	Correlation coefficient
Chromotropic method = 1.92 + 0.99 (phenoldisulphonic acid method)	0.9998
Ion-selective electrode method = 1.58 + 0.96 (phenoldisulphonic acid method) ..	0.9998
Chromotropic method = 0.31 + 1.03 (ion-selective electrode method)	0.9996

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Determination of Formaldehyde Vapour in the Atmospheres of Clinical Laboratories Using Chromotropic Acid

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Ethanol and xylene vapours were found to interfere with the determination of formaldehyde vapour by the chromotropic acid method, whether water or a mixture of chromotropic and sulphuric acids were used as the scrubbing solution. The interference can be removed by a porous polymer sorbent, Tenax GC. Thus, with a Tenax GC pre-trap, the chromotropic acid method can be applied to the determination of formaldehyde vapour in clinical laboratories where alcohol and xylene vapours are often encountered. The advantages of selecting chromotropic - sulphuric acid instead of water as the scrubbing solution are discussed.

Keywords: *Formaldehyde determination*; *chromotropic acid*; *spectrophotometry*; *ethanol and xylene interference*; *Tenax-GC pre-trap*

In clinical laboratories, formalin is commonly used for preserving specimens. Formaldehyde is highly odorous, lachrymatory and physiologically active and its threshold limit value is 2 p.p.m. V/V.[1,2] It is therefore necessary to monitor the formaldehyde vapour in these work places. Among the various existing methods for determining formaldehyde in air [3–5] spectrophotometry with chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulphonic acid) has received most attention because of its simplicity, sensitivity and reproducibility.[6–8]

However, this method cannot be used directly for determining formaldehyde vapour in the atmospheres of clinical laboratories because of negative interferences from ethanol and xylene vapours.[7–9] Ethanol and xylene are used in these laboratories as a disinfectant and a microscope-slide cleaner, respectively, and their concentrations may be much higher than that of formaldehyde. Methods have been suggested for removing these interferents before the spectrophotometric measurement. Evaporation of the scrubbing solution to dryness to expel any retained interferents had been suggested by Bricker and Aubrey,[10] but the procedure is cumbersome and time consuming. Water has been used as an absorbing medium to reduce the extent of the interference.[7] However, the trapping efficiency of water for formaldehyde vapour is low compared with that of 0.1% m/V chromotropic acid in concentrated sulphuric acid (chromotropic - sulphuric acid).[7] Also, when water is used as the scrubbing solution, a large volume of concentrated sulphuric acid has to be added to bring the sulphuric acid strength to above 86% V/V for optimum colour development.[11] This means that there is a greater dilution (often 3–4 fold) and concomitantly a loss in sensitivity, compared with the use of chromotropic - sulphuric acid as the scrubbing solution.[7]

The possibility of using a porous polymer sorbent in a pre-trap to remove ethanol and xylene vapours so that chromotropic - sulphuric acid can still be used as the scrubbing solution was therefore studied. The investigation showed that Tenax-GC is a suitable sorbent as it can cope with more than the maximum levels of interferents commonly encountered in clinical laboratories.

Experimental

Reagents and Apparatus

The water used for preparing the reagents and for dilutions was purified by an ion-exchange purification system (Barnstead D1794).

Formaldehyde stock standard solution, 1000 p.p.m. m/V. Prepared by appropriate dilution with water of the commercially available analytical-reagent grade formaldehyde solution (E. Merck), standardised by a method published elsewhere.[12] The standard formaldehyde

solutions (0–1.6 p.p.m.) used for constructing the calibration graph were freshly prepared by dilution of the stock solution with water.

Chromotropic acid in sulphuric acid, 1% *m/V*. Prepared by dissolving 1.0 g of sodium 1,8-dihydroxynaphthalene-3,6-disulphonate (BDH Chemicals Ltd.) in 100 ml of concentrated sulphuric acid (98% *m/m* analytical-reagent grade) and filtering through a sintered-glass crucible (porosity No. 3). A 0.1% *m/V* chromotropic acid solution was then obtained by diluting the 1% solution with concentrated sulphuric acid.

Porous sorbents. Tenax-GC (Applied Science Laboratory Institute), Porapak Q (Waters Associates Inc.), Chromosorb G (Varian Aerograph), Chromosorb P (Varian Aerograph) and Chromosorb P (AW, DMCS treated, Pierce) were all 80–100 mesh size; 300 mg of each of these porous sorbents were placed in a 1-cm bore × 6-cm long glass tube, which was then attached to the inlet of the scrubber train.

Scrubber. Modified from the one described elsewhere,[13] by adding a Teflon stopcock (3-mm bore) at the bottom of the flask. This small modification was useful as it facilitated the quantitative transfer of solution with minimum rinsing.

Spectrophotometer. Absorbance of the purple mono-cationic dibenzoxanthylium dye formed by the reaction of formaldehyde with chromotropic acid in concentrated sulphuric acid medium was measured at 570 nm in a 1-cm silica cell with a Beckman Acta III spectrophotometer.

Procedure

Constant known concentrations of xylene or ethanol vapour were generated continuously by purging the diffusion tube containing the corresponding compound with a constant flow (200 ml min^{-1}) of purified nitrogen.[14,15] The vapour concentration was calculated from the flow-rate and the mass loss of the diffusion tube.[14,15] A constant concentration of formaldehyde vapour was similarly produced from the formalin solution (37–44% *m/V*, analytical-reagent grade, E. Merck) and its concentration was determined by measuring the absorbance of the resulting scrubbing solution after a known period of time.[7] The vapour generating system was kept at a temperature of 25.0 ± 0.1 °C in a thermostatically controlled water-bath. The amount of vapour produced could be varied easily by altering the dimensions of the diffusion path.

Chromotropic - sulphuric acid was used as the scrubbing solution. The vapour of formaldehyde or formaldehyde plus interferent(s) in purified nitrogen was bubbled through about 15 ml of the scrubbing solution, with or without a pre-trap, for a known period of time. The resulting solution was then transferred into a 25-ml calibrated flask and the scrubber container was rinsed with 3.5 ml of water. The rinsing water was then added to the calibrated flask and the colour of the solution was developed under the liberated heat of dilution. The solution was made up to the mark with concentrated sulphuric acid after cooling to the ambient temperature. The final acid concentration in the solution was about 86% *V/V*. The calibration graph was constructed by plotting the concentration of formaldehyde against the absorbance of solutions prepared by mixing 21.50 ml of 0.1% chromotropic - sulphuric acid solution with 3.50 ml of various formaldehyde standard solutions.

Parallel analyses were also performed with water as the scrubbing solution in order to assess its efficiency to reduce the interferences from xylene and ethanol vapours. The procedure for colour development and calibration when water was used as scrubbing solution has been described elsewhere.[12] The final sulphuric acid concentration in the resulting solution was about 60% *V/V*.

Results and Discussion

Table I shows one advantage of chromotropic - sulphuric acid over water as a scrubber in the absence of interferents. The collection efficiency by a single scrubber was found to be practically 100% for chromotropic - sulphuric acid and varied between 76% and 82% for water in the concentration range 0.1–1.5 p.p.m. of formaldehyde. These results support those obtained by Altshuller *et al.*[7] The third and fourth columns of Table II compare the applicability of chromotropic acid and water in the presence of ethanol and xylene. The negative interfering effect caused by xylene (when the ratio of the concentration of xylene

TABLE I

EFFICIENCY OF TRAPPING OF FORMALDEHYDE BY CHROMOTROPIC - SULPHURIC ACID AND WATER

Formaldehyde vapour generated, p.p.m.*†	Trapping efficiency, %*			
	Chromotropic - sulphuric acid	Water		
	First scrubber‡	First scrubber	Second scrubber	Overall
0.12 (0.01)	100.0 (8.9)	82.2 (8.9)	15.6 (4.5)	97.8 (9.9)
0.37 (0.02)	100.0 (4.7)	77.8 (5.2)	13.3 (2.2)	91.1 (5.6)
1.53 (0.07)	100.0 (3.1)	75.7 (3.0)	13.0 (1.4)	86.7 (3.3)

* The values in parentheses are the 2σ values of triplicate determinations.
† Calculated from the concentration of formaldehyde trapped in chromotropic - sulphuric acid.
‡ No absorbance was detected in the solution of the second scrubber.

to the concentration of formaldehyde was about 70) was reduced to about half if chromotropic - sulphuric acid was replaced by water as the scrubbing solution. However, both methods were found to give a negative deviation, to about the same extent, in the presence of ethanol. Thus, even disregarding its lower sensitivity and collection efficiency, water cannot be used directly as the scrubbing solution for measuring formaldehyde vapour in a clinical laboratory.

The use of chromotropic - sulphuric acid as the scrubbing solution also has another advantage hitherto unreported, namely, the required sampling time could easily be judged by noting the intensity of the colour developed during sampling (changing from being straw yellow to being tinged with pink). Based on the above results, the use of chromotropic - sulphuric acid was preferred to water in formaldehyde determination and it was used in the subsequent studies.

The calibration graph was found to be linear up to a concentration equivalent to 3.7 p.p.m. *V/V* of formaldehyde in a 5-l air sample with a sensitivity of 0.032 absorbance unit per part per million of formaldehyde per litre of air. The molar absorptivity of the complex in the resulting solution was found to be 1.9×10^4 l mol^{-1} cm^{-1}, which is slightly higher than that reported by Sawicki *et al.*[5]

In histopathology and cytology laboratories, we found xylene, methanol ethanol and chloroform vapours to be present in the air using a gas-chromatographic technique. Methanol and chloroform were found not to interfere in the chromotropic - sulphuric acid method even at concentrations of about 1000 and 100 times higher, respectively, than the concentration of formaldehyde vapour. However, xylene and ethanol were found to cause

TABLE II

EFFECTS OF INTERFERENTS ON THE CHROMOTROPIC ACID METHOD AND USE OF PRE-TRAPS FOR REDUCING THEIR EFFECTS

The experimental conditions used for all determinations were identical, *i.e.*, total flow of nitrogen carrier plus formaldehyde generated, 200 ml min^{-1}; and duration of each experiment, 1 h.

Formaldehyde vapour generated, p.p.m.	Interferent	Absorbance, scrubbing in water*†	Absorbance*‡						
			Without pre-trap	Porapak Q	Chromosorb G	Chromosorb W	Chromosorb P	Chromosorb P (AW, DMCS)	Tenax-GC
0.22	Nil	0.077 (0.006)	0.088 (0.006)	0.056 (0.008)	0.081 (0.008)	0.078 (0.006)	0.066 (0.008)	0.080 (0.008)	0.085 (0.005)
0.78	Nil	0.240 (0.010)	0.288 (0.012)	0.224 (0.020)	0.279 (0.025)	0.260 (0.020)	0.232 (0.018)	0.267 (0.019)	0.283 (0.015)
0.22	Xylene vapour (53 p.p.m.)	0.062 (0.005)	0.057 (0.004)	0.046 (0.006)	0.052 (0.006)	0.054 (0.004)	0.063 (0.008)	0.078 (0.007)	0.085 (0.005)
0.78	Xylene vapour (53 p.p.m.)	0.192 (0.010)	0.178 (0.007)	0.163 (0.010)	0.175 (0.006)	0.184 (0.008)	0.228 (0.012)	0.276 (0.012)	0.286 (0.008)
0.22	Ethanol vapour (220 p.p.m.)	0.065 (0.008)	0.075 (0.004)	0.049 (0.004)	0.071 (0.005)	0.072 (0.008)	0.065 (0.007)	0.073 (0.008)	0.085 (0.005)
0.78	Ethanol vapour (220 p.p.m.)	0.202 (0.006)	0.254 (0.006)	0.208 (0.008)	0.236 (0.007)	0.248 (0.008)	0.226 (0.010)	0.264 (0.008)	0.284 (0.008)

* The values in parentheses are the 2σ values of four replicate determinations.
† Molar absorptivity of the complex formed was found to be 1.8×10^4 l mol^{-1} cm^{-1}.
‡ Scrubbing in chromotropic - sulphuric acid, molar absorptivity was found to be 1.9×10^4 l mol^{-1} cm^{-1}.

a negative interference as mentioned previously. Therefore, they must be removed before the determination of formaldehyde when using this method. A pre-trap of polymer sorbent was used for this purpose.

The prerequisite for the sorbent is that it must allow quantitative breakthrough of formaldehyde. Several porous polymers were chosen for this study because of their known low retention indices for formaldehyde.[16] The results of the breakthrough characteristics of formaldehyde vapour in these sorbents are given in Table II. The amount of formaldehyde breakthrough can be taken as the ratio of the absorbance of the scrubbed solutions with and without using the pre-trap. Thus, it was found that although Porapak Q had been reported to be successful in removing some organic interferents in the determination of formaldehyde,[17] it was in fact unsatisfactory as it retained a significant proportion of the formaldehyde vapour especially at low concentrations. Likewise, the breakthrough characteristics of formaldehyde in Chromosorb P were also found to be unsatisfactory.

The efficiencies of the sorbents in the removal of interferents are indicated by the ratio of the absorbance of the solution scrubbed with pure formaldehyde to that scrubbed with formaldehyde plus interferents, both in the presence of the pre-trap. Thus, from Chromosorb G, Chromosorb W, Chromosorb P (AW, DMCS) and Tenax-GC, which allow quantitative breakthrough of formaldehyde vapour, Chromosorb G and Chromosorb W were found to be unsatisfactory in removing ethanol and xylene vapours as shown in Table II. Although Chromosorb P (AW, DMCS) could be used to remove xylene effectively, it was only partially successful for removing ethanol. Tenax-GC was found to be effective in removing both xylene and ethanol.

A detailed study of the dynamic capacity of a 300-mg Tenax-GC pre-trap was carried out. The results are shown in Table III. No breakthrough was observed up to 3–4 h with concentrations of interferents higher than those commonly encountered in clinical laboratory atmospheres. As the normal sampling time for the determination is expected to be less than 1 h, Tenax-GC should be an effective sorbent for the required purpose.

Tenax-GC can also be used to remove many other neutral and basic organic compounds.[18,19] Thus, this modified method will also be useful for the determination of formaldehyde vapour in industrial plant environments where high levels of organic pollutants are expected.

TABLE III

DYNAMIC CAPACITIES OF TENAX-GC TO TRAP XYLENE AND ETHANOL

Interferent	Time/h	Absorbance with 300 mg of Tenax-GC*
Xylene, 105 p.p.m.	1	0.085 (0.005)
	2	0.088 (0.003)
	3	0.088 (0.006)
	4	0.076 (0.006)
Ethanol, 551 p.p.m.	1	0.085 (0.004)
	2	0.087 (0.006)
	3	0.084 (0.004)
	4	0.084 (0.008)

* Absorbance of the solution that scrubbed 0.22 p.p.m. V/V of formaldehyde in the absence of the interferent and the pre-trap of porous polymer was 0.088 (0.006). The values in parentheses are the 2σ values of triplicate determinations.

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Spectrophotometric Determination of Cobalt in Paints and Environmental Paint Samples

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A method for the spectrophotometric determination of cobalt, based on the formation of a coloured chelate ($\lambda_{max.}$ 480 nm, $\epsilon_{max.}$ 2.83×10^4 l mol^{-1} cm^{-1} and colour contrast 134 nm) with benzyl 2-pyridyl ketone 2-pyridylhydrazone, is described. Under the optimum conditions, colour development is instantaneous and the colour is stable for several weeks. Under appropriate working conditions, the method is applicable to the determination of cobalt in manufactured paints and environmental samples of paint. The fundamental solution chemistry of the reagent and a brief description of its more interesting colour reactions are also reported.

Keywords: *Benzyl 2-pyridyl ketone 2-pyridylhydrazone reagent*; *cobalt determination*; *paint analysis*; *spectrophotometry*

Many methods have been developed for the spectrophotometric determination of cobalt,[1] but these have not been applicable to paints. Cobalt compounds are reported to be toxic,[2–5] causing health disorders through ingestion, inhalation and skin contact; some threshold limit values (TLVs) for environmental cobalt[5] are 0.1 mg m^{-3} (USA), 0.5 mg m^{-3} (Germany) and 0.1 mg m^{-3} (Sweden). As the presence of paint mists in industrial workroom environments often causes these TLVs to be exceeded, cobalt levels must be checked periodically; therefore, methods for its determination in paint are needed.

Benzyl 2-pyridyl ketone 2-pyridylhydrazone (BPKPH) has been reported recently as being a highly sensitive reagent for the fluorimetric determination of gallium,[6] zinc[7] and cadmium.[8] In this paper the fundamental solution chemistry of the BPKPH and a superficial description of its more interesting colour reactions, reported in detail elsewhere,[9] are given. The results show that BPKPH is a promising reagent for the spectrophotometric determination of metal ions. A rapid, simple and selective spectrophotometric method for the determination of cobalt in paints and environmental samples of paint is described here. The method demonstrates the reliability and versatility of the reagent for such purposes and presents an alternative to atomic-absorption spectrometry,[10] having a similar sensitivity.

Experimental

Reagents

Benzyl 2-pyridyl ketone 2-pyridylhydrazone. The synthesis of BPKPH has been reported previously.[6] Solutions (1×10^{-3} M) were prepared weekly by dissolving 0.0288 g of BPKPH in 100 ml of absolute ethanol (Merck, analytical-reagent grade).

Cobalt solution. A stock solution containing 6.904 g l^{-1} of cobalt, complexometrically contrasted, was prepared from cobalt(II) nitrate hexahydrate (UCB, analytical-reagent grade). Working solutions were prepared by appropriate dilution of the stock solution.

Acetate buffer solution, pH 4.0, 0.5 M.

Masking solution. A solution 1 M in sodium thiosulphate, 0.13 M in sodium citrate and 1.5 M in ammonium fluoride was prepared in de-ionised, distilled water from analytical-reagent grade reagents.

Apparatus

The following apparatus was used: a Beckman Acta III spectrophotometer; a Beckman, Model DBGT, spectrophotometer; a Beckman, Model SS, Expandomatic pH meter; a Perkin-Elmer, Model 306, atomic-absorption spectrophotometer with a deuterium background

corrector and a Perkin-Elmer, Model 056, recorder; an MSA, Model G, personal sampler with 37-mm three-body cassettes; and a mixed cellulose ester membrane filter (0.8-μm size and 37 mm diameter) mounted on a cellulose support pad.

Procedure

Transfer a suitable aliquot (up to 3.75 ml) containing 6.25–75 μg of cobalt into a 25-ml calibrated flask. Add, with mixing, 2 ml of masking solution, 5 ml of 1×10^{-3} M BPKPH solution, 4 ml of buffer solution and 10 ml of absolute ethanol and dilute to volume with de-ionised water. Measure the absorbance at 480 nm against a reagent blank.

Sampling and Treatment of the Paint

Dry the sample for 3 h in a current of air at 105 °C. Weigh and digest with 10 ml of nitric acid and then 10 ml of perchloric acid. Evaporate to dryness and dissolve the residue by heating with 10 ml of 10% V/V nitric acid. Remove the binder by filtration and, finally, dilute the filtrate to 10 ml with 0.2 M sodium hydroxide solution.

Environmental samples are collected from paint mists in workroom environments, according to the NIOSH manual,[11] using cellulose ester membrane filters that are mounted on cellulose support pads connected to vacuum personal samplers, operating at a flow-rate of 1 l min^{-1}. The paint is then treated on the filter according to the above procedure.

Results and Discussion

Spectral Characteristics and Acid - Base Properties of the Reagent

When measured at different pHs, the absorption spectra of BPKPH solutions (2.5×10^{-5} M) at different ethanol concentrations showed the normal transitions from the doubly protonated form (D) to the singly protonated form (M) and from the singly protonated form to the neutral molecule (N). Fig. 1 shows the spectra obtained when using a 40% V/V concentration of ethanol. The more strongly basic character of the reagent in the excited state resulted in a bathochromic shift in the absorption bands at the longest wavelengths upon successive protonations of the pyridine nitrogen atoms (Table I). The dissociation constants for the three acid - base forms are summarised in Table II. As expected, an increase in the ethanol content of the medium also increases the corresponding pK values.

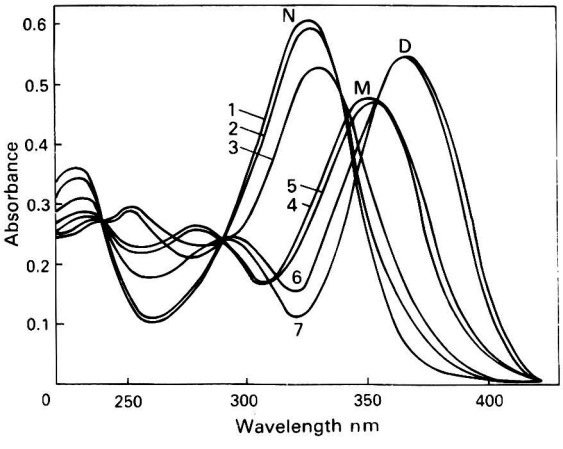

Fig. 1. Absorption spectra of 2.5×10^{-5} M solutions of BPKPH at several pH values and an ethanol concentration of 40% V/V showing the three acid - base forms derived from the compound. pH values: (1) 1.26; (2) 2.41; (3) 2.86; (4) 4.20; (5) 4.70; (6) 7.61; and (7) 8.08.

TABLE I

SPECTROSCOPIC CHARACTERISTICS OF THE ACID - BASE FORMS DERIVED FROM BPKPH IN ETHANOL - WATER MIXTURES

Ethanol content, %	$\lambda_{max.}$/nm			$\epsilon_{max.}$/l mol^{-1} cm^{-1} × 10^{-4}		
	D	M	N	D	M	N
2	370	350	325	3.70	2.90	3.60
40	365	350	322	2.20	1.92	2.44
90	345	335	320	2.15	2.05	2.80

TABLE II

DISSOCIATION CONSTANTS FOR THE DICATION (pK_1) AND MONOCATION (pK_2) DERIVED FROM BPKPH AT DIFFERENT ETHANOL CONCENTRATIONS

The values given in parentheses are the wavelengths (in nanometres) at which the spectrophotometric titrations were plotted.

Ethanol content, %	pK_1	pK_2
2	2.73 (370)	5.55 (325)
40	2.96 (365)	5.85 (322)
90	3.14 (350)	6.20 (320)

Main Colour Reactions of BPKPH

The reagent behaves in a manner broadly similar to other *N*-heterocyclic hydrazones, acting as a tridentate chelating agent. It forms intensely coloured complexes with a number of metal ions and in most of the chelate systems complexation is accompanied by elimination of the imine proton of the ligand. Under basic conditions, most of metal ions form complexes with the anionic form of the ligand that are sparingly soluble in water but solubility is achieved when the medium contains sufficient ethanol. The characteristics of the individual complexes are summarised in Table III. The data were obtained from the appropriate spectra, which were measured in the presence of a five-fold molar excess of the reagent at pH values at which the different complexes form. As shown, the high molar absorptivities and the colour contrast values of the reactions make the reagent suitable for practical purposes.

TABLE III

ANALYTICAL CHARACTERISTICS OF THE MAIN COLOUR REACTIONS OF BPKPH

All values were measured in ethanol - water mixtures containing 20% *V*/*V* of ethanol except those of cobalt for which the ethanol - water mixture contained 60% *V*/*V* of ethanol.

Ion	pH	$\lambda_{max.}$/nm	$\Delta\lambda$*/nm	$\epsilon_{max.}$/l mol^{-1} cm^{-1} × 10^{-4}
Mn	8.7	510	187	3.85
Fe(II)	11.7	407	84	3.27
Fe(III)	11.7	395	72	1.32
Co	4.0	480	134	2.83
Ni	10.0	490	167	4.32
Pd	11.6	535	212	1.45
Cu(II)	11.4	505	182	3.93
Zn	11.1	485	162	5.10
Cd	10.0	497	174	4.61
Hg(II)	10.5	485	162	4.21
Pb	8.0	455	132	0.22

* Colour contrast ($\Delta\lambda$) = $\lambda_{max.}$(complex) − $\lambda_{max.}$(reagent) under identical conditions of measurement.

BPKPH - Cobalt System

Spectral behaviour

The complexation of BPKPH with cobalt was studied over a wide pH range. The yellow - orange complex showed maximum absorbance at 480 nm over the pH range 2–13.5, and no substantial changes in absorbance were found over this range. The work reported here was carried out at pH 4.0 (in the acetate buffer solution).

Reagent concentration and stability of the complex

For complete complexation, a five-fold molar excess of BPKPH is sufficient. Colour development is instantaneous and the colour remains stable for several weeks. Slight variations in absorbance can be found by modifying the concentration of ethanol in the medium. Optimum results are obtained in a 60% *V*/*V* ethanol - water mixture. No changes in absorbance were observed when the order of addition of the reagents was modified.

Characteristics of the complex

Beer's law as obeyed for cobalt levels of up to 3 p.p.m., with an optimum concentration range of 0.25–2.5 p.p.m., as determined by a Ringbom plot. Sandell's sensitivity[12] was found to be 0.0021 μg cm^{-2}. For a series of ten measurements of 1 p.p.m. of cobalt, a relative error of 0.4% and a relative standard deviation of 0.5% were obtained.

Composition of the complex

The metal to ligand ratio in the complex was studied under the established working conditions by both continuous-variation and molar-ratio methods. The results indicated a cobalt to BPKPH ratio of 1:2.

Interferences and application to paint analysis

The effect of foreign ions on the determination of 1 p.p.m. of cobalt by the proposed procedure was studied and the results are summarised in Table IV. The tolerance limit for foreign ions was taken as the amount that caused an error in the absorbance value of no greater than 1%.

TABLE IV

EFFECT OF FOREIGN IONS ON THE DETERMINATION OF 1 p.p.m. OF COBALT

Ion	Amount tolerated/ μg ml^{-1}
$S_2O_3^{2-}$, $C_2O_4^{2-}$, NH_4^+, Cl^-, Br^-, F^-, NO_3^-, ClO_4^-, SO_4^{2-}, citrate	>5000
Pb(II), Mn(II), La(III), Mg(II), alkali metals, APDC*	100†
Cr(III)	25
Cd(II), Hg(II)	10
Zn(II)	2
Ni(II), EDTA	0.1
Fe(III), Cu(II), CN^-	0.05

* Ammonium tetramethylenedithiocarbamate (ammonium pyrrolidinedithiocarbamate).

† Maximum amount tested.

In order to apply the method to the analysis of real environmental samples of paint, prior analyses of a number of them were necessary to determine the ratios of the other metal ions present to cobalt. The reason for this is that usually more than one paint is used in any painting operation. In order to obtain representative values, 40 different environmental samples were analysed by atomic-absorption spectrophotometry, and the results are summarised in Table V. The maximum ratios of the main metal ions present to cobalt were 27:1 for iron, 107:1 for zinc, 1.6:1 for copper, 22:1 for chromium and 78:1 for lead, and according to the preceding interference studies, only iron, zinc and copper were above the tolerance

TABLE V

METAL IONS PRESENT IN ENVIRONMENTAL SAMPLES OF PAINTS AS DETERMINED BY ATOMIC-ABSORPTION SPECTROPHOTOMETRY

Sample No.	Cobalt concentration/ mg m^{-3}	*R**				
		Fe	Zn	Pb	Cr	Cu
1	0.03	6.6	4.3	3.3	—	1.0
2	0.02	8.0	8.5	6.0	—	1.5
3	0.03	13.6	6.6	3.3	—	1.3
4	0.03	4.6	7.0	6.6	—	1.6
5	0.15	27.0	0.9	57.0	0.4	0.3
6	0.10	1.5	0.8	5.0	0.4	0.6
7	0.18	1.2	0.2	1.0	0.1	0.4
8	0.18	1.5	0.2	0.8	0.1	0.4
9	0.12	5.1	63.6	2.6	0.1	—
10	0.09	6.6	107.0	1.8	0.2	—
11	0.12	4.8	0.3	41.3	9.1	—
12	0.11	10.5	1.0	46.3	6.3	—
13	0.04	16.2	1.7	28.5	6.0	0.5
14	0.08	16.0	1.5	29.5	6.8	0.4
15	0.06	14.9	5.7	38.7	3.8	0.5
16	0.04	20.5	7.8	57.8	5.3	0.5
17	0.08	18.0	4.5	35.2	4.4	0.4
18	0.12	8.8	3.5	24.7	3.8	0.2
19	0.12	13.7	0.3	5.5	0.2	0.6
20	0.12	10.5	0.3	4.7	0.1	0.4
21	0.05	4.6	4.0	14.5	2.4	0.4
22	0.07	4.7	2.6	15.4	1.1	0.3
23	0.04	6.5	5.2	26.3	4.0	0.2
24	0.07	8.7	3.7	23.3	0.9	0.3
25	0.88	2.6	0.3	3.2	0.05	0.2
26	0.03	2.6	0.3	64.6	21.7	—
27	0.07	0.9	0.3	77.3	15.7	0.2
28	0.26	5.3	0.9	3.1	0.2	0.1
29	0.09	3.8	2.1	1.8	0.4	0.1
30	0.19	1.8	0.9	22.3	2.4	—
31	0.16	2.4	1.0	25.1	2.6	—
32	0.12	5.7	2.0	16.2	2.6	—
33	0.12	6.2	2.8	18.7	2.7	—
34	0.08	8.2	2.8	20.5	3.4	—
35	0.10	6.1	1.2	5.3	0.9	0.7
36	0.09	6.0	1.2	3.0	0.5	0.8
37	0.09	1.4	1.2	4.1	0.3	—
38	0.07	1.9	2.1	5.6	0.4	—
39	0.07	2.4	1.9	4.6	0.4	—
40	0.07	1.9	1.6	4.7	0.4	—

* R is the ratio of the concentration of the metal ions to cobalt in the environmental samples of paint.

limit. The interferences from these elements were eliminated individually as follows: iron was masked with 1 ml of 3 M ammonium fluoride solution; zinc with 0.25 ml of 1 M sodium citrate solution; and copper with 1 ml of 2 M sodium thiosulphate solution. However, in developing the experimental procedure it was found that the addition of 2 ml of a masking solution containing appropriate amounts of the masking agents was satisfactory. Three synthetic samples each containing the maximum ratios of iron, zinc and copper to cobalt were examined using the proposed procedure. It was found that the optimum results were obtained after a standing time of 30 min, this being recommended for the analysis of paints.

The results of the analyses of ten replicate samples of an individual paint are presented in Table VI. As shown, the results agree with those obtained by atomic-absorption spectrophotometry, showing the reliability of the present method.

TABLE VI

DETERMINATION OF COBALT IN AN INDIVIDUAL PAINT SAMPLE

Sample No.	Mass/g	Cobalt content*/% × 10^2 BPKPH method	Cobalt content*/% × 10^2 Atomic-absorption method
1	0.1867	5.96	6.32
2	0.2044	6.11	6.31
3	0.1423	6.15	6.68
4	0.1354	6.00	6.42
5	0.2250	6.01	6.04
6	0.1131	5.99	6.63
7	0.1815	5.99	6.17
8	0.2149	5.99	6.05
9	0.0789	5.83	5.83
10	0.4820	5.97	5.83

* Value based on the non-volatile component of the paint (pigment and binder).

Table VII shows the determination of cobalt in environmental samples by the spectrophotometric method, the results being expressed in milligrams per cubic metre of cobalt in the environment.

TABLE VII

ANALYSIS OF ENVIRONMENTAL SAMPLES OF PAINT

Sample No.	Volume*/l	Absorbance	Cobalt content/mg m^{-3}
1	70	0.156	0.20
2	76	0.123	0.16
3	70	0.133	0.19
4	70	0.122	0.17
5	84	0.174	0.20
6	84	0.154	0.18
7	65	0.146	0.23
8	65	0.102	0.16
9	53	0.144	0.27
10	53	0.178	0.34
11	45	0.077	0.14
12	68	0.129	0.19

* Volume of air sampled.

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Spectrophotometric Determination of Trace Amounts of Molybdenum with 1,4-Dihydroxyphthalimide Dithiosemicarbazone

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1,4-Dihydroxyphthalimide dithiosemicarbazone reacts with molybdenum(VI) to produce a yellow 1:1 complex in acidic dimethylformamide - water solution. The yellow complex can be extracted into isopentyl alcohol ($\lambda_{max.}$ = 435 nm; ϵ = 9.4 × 10^3 l mol^{-1} cm^{-1}) and used for the spectrophotometric determination of trace amounts of molybdenum in the range 10–95 μg. The interferences of many metallic ions have been examined and a sensitive and selective method for the determination of molybdenum is proposed.

Keywords: *Molybdenum determination*; *spectrophotometry*; *1,4-dihydroxyphthalimide dithiosemicarbazone*

1,4-Dihydroxyphthalimide dithiosemicarbazone (OH-PDT) has been previously described and applied as an indicator reagent in kinetic analysis[1,2] and catalytic titrations,[3,4] based on its autoxidation reaction, catalysed by manganese(II), in alkaline medium.

In this work, OH-PDT has been used as a spectrophotometric reagent and applied to determination of trace amounts of molybdenum. The spectrophotometric characteristics and the development of two sensitive and selective methods (in a homogeneous medium and by extraction with isopentyl alcohol) for the determination of molybdenum(VI) are reported.

The number of chromogenic reagents available for molybdenum is relatively small. Of these, thiocyanate[5] and dithiol[6] are generally used. More recently, some hydroxylated thiosemicarbazones have been utilised as photometric ligands for molybdenum,[7–10] but all of these methods require the presence of tin(II) chloride in mineral acid medium. However, OH-PDT does not react with molybdenum(VI) under similar conditions. In the proposed method, a coloured chelate is formed in moderately acidic medium, the presence of a reducing agent not being necessary. Moreover, the extraction of the chelate gives a high sensitivity and selectivity, similar to those in other methods for the spectrophotometric determination of molybdenum.

Experimental

Reagents

All reagents and solvents were of analytical-reagent grade.

1,4-Dihydroxyphthalimide dithiosemicarbazone. Prepare a 0.03% *m/V* solution in dimethylformamide. The reagent was synthesised from 1,4-dihydroxyphthalimide dioxime and thiosemicarbazide.[1]

Standard molybdenum(VI) solution. Dissolve 1.500 g of molybdenum(VI) oxide in a few millilitres of 0.1 M sodium hydroxide solution, dilute with water, make slightly acidic with hydrochloric acid and dilute to 1 l with water. Dilute this stock solution (1000 g l^{-1}) just before use.

Chloroacetic acid - sodium hydroxide buffer solution, pH 2.6. Add 65 ml of 0.2 M sodium hydroxide solution to 300 ml of 0.2 M chloroacetic acid solution and dilute to 1 l with distilled water.

Apparatus

Spectrophotometer. A Perkin-Elmer 124 spectrophotometer with 1.0-cm silica cells was used.

Digital pH meter. An Orion 701A instrument, with glass - calomel electrodes, was used for pH measurements.

Procedure

Determination of molybdenum(VI) without extraction

To a solution containing 40–225 μg of molybdenum(VI) in a 25-ml calibrated flask, add 5 ml of 0.03% *m/V* OH-PDT solution and 5 ml of pH 2.6 buffer solution and dilute to the mark with distilled water. Measure the absorbance at 425 nm against a reagent blank.

Determination of molybdenum(VI) with extraction

To 1–15 ml of sample solution containing 10–95 μg of molybdenum(VI), in a separating funnel, add 2 ml of 0.03% *m/V* OH-PDT solution and 5 ml of pH 2.6 buffer solution and dilute to 25 ml with distilled water. After mixing, add exactly 8 ml of isopentyl alcohol and shake vigorously for 1 min. Allow the phases to separate and draw off the aqueous layer. Transfer the organic phase into a 10-ml flask containing anhydrous sodium sulphate. Measure the absorbance at 435 nm against a reagent blank similarly prepared.

Prepare a calibration graph by using standard solutions of molybdenum(VI) treated in the same way.

Results and Discussion

Study of the Complex in Aqueous Dimethylformamide Solution

The addition of a 0.03% solution of OH-PDT in dimethylformamide to a solution of molybdenum(VI) ions produces a yellow complex. Absorption spectra of the complex are shown in Fig. 1(A). The complex remains stable for at least 3 h at pH ~ 3 (chloroacetic acid buffer).

Influence of pH

The influence of pH was studied using a series of solutions in the pH range 1–6. At pH <1, OH-PDT is unstable and the molybdenum complex is destroyed. The absorbance *versus* pH curve [Fig. 2(A)] shows a useful working pH range of 2.5–3.5. A buffer solution consisting of chloroacetic acid - sodium hydroxide was adopted for all further work.

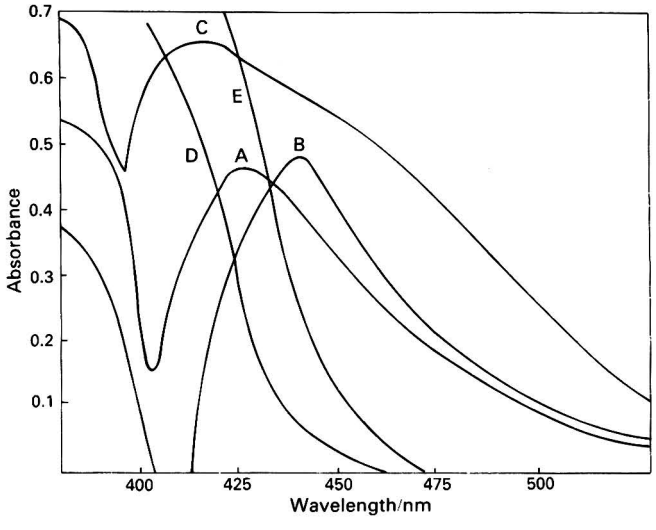

Fig. 1. Absorption spectra of molybdenum complexes with OH-PDT: A, molybdenum(VI) complex in aqueous dimethylformamide medium at pH 3.0; B, molybdenum(VI) complex extracted into isopentyl alcohol at pH 3.0; C, molybdenum(V) complex in aqueous dimethylformamide medium at pH 3.0; D and E, reagent blanks of A and B, respectively. Concentration of molybdenum, 5 p.p.m.; and concentration of OH-PDT, 1.84×10^{-4} M.

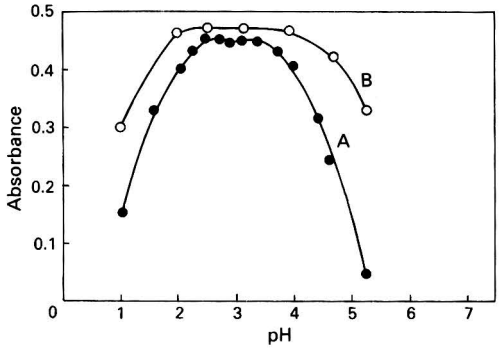

Fig. 2. Influence of pH on the formation of molybdenum(VI) - OH-PDT complex: A, in aqueous dimethylformamide solution at 425 nm (C_{Mo} = 5 p.p.m.); and B, extracted into isopentyl alcohol at 435 nm (C_{Mo} = 5 p.p.m.).

Effect of reagent concentration

The absorbance of the complex was studied as a function of the molar ratio of OH-PDT to molybdenum(VI). A 2-fold molar excess of the reagent over molybdenum was necessary in order to obtain maximum absorbance at 425 nm. A 5-ml volume of 0.03% *m*/*V* solution is recommended as a suitable amount of reagent.

When the molar ratio of reagent to metal is higher than 7 and the concentration of molybdenum exceeds 3 p.p.m., the absorbance decreases and a new complex appears in the solution. The study of the molybdenum(VI) - OH-PDT system under those conditions will be the subject of a future investigation.

Effect of ionic strength and order of addition of reagents

The ionic strength of the solution does not affect the absorbance of the molybdenum(VI) - OH-PDT system. The same constant absorbance values were obtained when 1–5 ml of 0.5 M potassium nitrate solution or 1–5 ml of 0.5 M potassium chloride solution were added.

The order of addition of reagents was found not to be important. The sequence metal ion, reagent and buffer solution was adhered to during the preparation of all measured solutions.

Nature of the complex

The metal to reagent ratio in the molybdenum(VI) complex and the stability constant were determined by the molar ratio[11] and continuous[12] methods (Fig. 3). The stoicheiometry was found to be 1:1 and the stability constant was 2.1×10^5. A study of the retention of this chelate on an anion-exchange resin showed that under these experimental conditions the complex was anionic.

From experimental evidence it was concluded that the reagent forms the yellow complex previously mentioned with molybdenum(VI). The presence of hydroxylamine in the solution before adding the OH-PDT reagent changed the absorbance peak from 425 to 410 nm. When molybdenum(V) was used a green complex was formed [Fig. 1(C)]. The presence of potassium persulphate altered the absorption peak because oxidising agents destroy the reagent.

Solvent Extraction Study

The complex is quantitatively extracted with isopentyl alcohol under the conditions described. The absorption spectrum is very similar to that obtained in aqueous medium and a small bathochromic shift is observed, from 425 to 435 nm [Fig. 1(B)]. The complex is stable for at least 4 h. When isobutyl methyl ketone is used, the stability is higher (24 h) but the

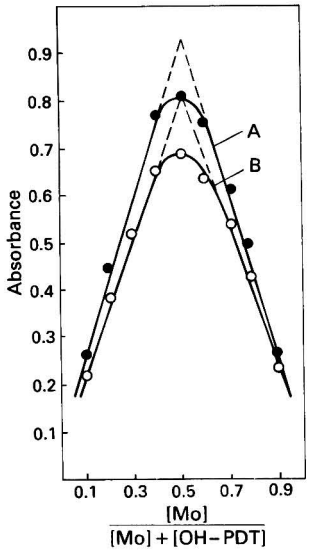

Fig. 3. Stoicheiometry of molybdenum(VI) - OH-PDT complex in aqueous dimethylformamide medium, at pH 3.0 (continuous variation method): A, at 425 nm; and B, at 440 nm. The initial concentration of molybdenum was 1.54×10^{-3} M.

extraction is not quantitative (approximately 80%). In both instances, the presence of dimethylformamide in the medium is necessary in order to increase the stability of the extract.

The extraction of molybdenum(VI) complex is pH dependent [Fig. 2(B)]. The most favourable pH range is 2–4.

The effect of reagent concentration is similar to that reported in homogeneous medium. When a volume of 1.5–3.5 ml of 0.03% OH-PDT solution was utilised, the absorbance remained constant. A 2-ml volume of solution is utilised in the recommended procedure.

The influence of the phase ratio was studied. The extraction of the molybdenum(VI) complex is slightly decreased if the phase ratio (aqueous to organic phase) is increased because of the appreciable solubility of isopentyl alcohol and dimethylformamide in water. When a phase ratio between 1:1 and 1:4 was utilised, the absorbance remained constant. It is concluded that the volume of the aqueous phase should be held approximately constant. A 25-ml volume is utilised in the recommended procedure.

The ionic strength does not affect the extraction of the complex. Salts such as sodium sulphate, potassium perchlorate, potassium chloride and potassium nitrate do not affect the colour intensity, even at a concentration of 2%. The extraction is quantitative from solutions of the complex in chloroacetic acid buffer, a salting-out reagent being unnecessary.

Spectrophotometric Determination of Molybdenum

Based on the experimental work, two methods are reported for the determination of trace amounts of molybdenum involving the formation of the yellow complex with OH-PDT and its extraction into isopentyl alcohol.

Spectrophotometric characteristics

Direct method. In an aqueous dimethylformamide medium, the molybdenum(VI) - OH-PDT system obeys Beer's law from 1.0 to 10.0 p.p.m. of molybdenum. The molar

absorptivity is 9.0×10^3 l mol^{-1} cm^{-1}. A Ringbom plot showed that the range for minimum error in the determination is 1.6–9.0 p.p.m. of molybdenum. The relative error ($P = 0.05$) of the method is $\pm 1.1\%$ and Sandell's sensitivity[13] is 0.011 μg cm^{-2} of molybdenum.

Extraction method. Beer's law is obeyed between 5 and 100 μg of molybdenum under the conditions described. The molar absorptivity is 9.4×10^3 l mol^{-1} cm^{-1} and the range for minimum error in the determination is 10–95 μg of molybdenum. The relative error ($P = 0.05$) of the method is $\pm 1.3\%$ and Sandell's sensitivity is 0.010 μg cm^{-2}.

Interference study

The recommended procedure was used to analyse standard molybdenum solutions in the presence of potential interfering ions. The results for the determination of 50.0 μg of molybdenum are shown in Table I. Determinations by the direct method were carried out at the limiting value of the concentration of foreign ion that caused interference in the extraction method.

TABLE I

RECOVERY OF MOLYBDENUM IN THE PRESENCE OF METAL IONS

Ion added	Mass excess relative to Mo	Amount of molybdenum recovered/μg	
		Extraction method	Direct method
Al(III)	15	49.5	42.0
Ni(II)	15	49.5	85.0
Mn(II)	15	50.0	55.0
Zn(II)	15	50.1	59.0
Cd(II)	15	50.0	57.5
Ti(IV)	15	49.7	42.0
Cr(III)	15	50.1	43.0
Bi(III)	15	50 0	72.0
La(III)	15	50.2	55.0
W(VI)	10	49.5	55.5
Co(II)	2	48.0	>100
Cu(II)	1	22.0	>100
Fe(II)	1	26.0	>100
Fe(III)	1	49.5	>100
Pb(II)	1	50.0	39.0
Hg(II)	1	60.0	99.5
V(V)	1	82.0	100
Alkali and alkaline earth metals	100	50.0	50.0

From the results, it is concluded that the tolerance limits for the extractive determination of molybdenum are greater than those obtained by the direct method. The major interferences were caused by elements that are known to form OH-PDT complexes [Cu(II), Co(II), Fe(II), Fe(III), Hg(II) and V(V)]. Other metal ions, such as Al(III), Ni(II), Mn(II), Zn(II), Cd(II), Ti(IV), Cr(III), Bi(III) and La(III), can be tolerated at up to a 15-fold excess by mass. Tungstate(VI) does not interfere at up to a 10-fold excess.

From the data reported above (spectrophotometric characteristics and interference study), it is concluded that the extraction of the chelate provides the greater sensitivity and selectivity, and the extraction method is therefore proposed.

In order to assess possible analytical applications, it appears that a prior separation of molybdenum before the determination may be necessary in order to improve the specificity in the presence of interfering ions. Extraction with dithizone in carbon tetrachloride is of value in separating a number of heavy metals from molybdenum(VI), if these are not present in large amounts. A 10-fold excess of Cu(II), Co(II), Fe(III) and Hg(II) can be tolerated with prior extraction with 0.1% dithizone solution from aqueous solutions at pH $\sim$ 3. Molybdenum can be also separated from iron, copper, vanadium and lead in the presence of citrate ions.[14]

Ion-exchange separation of molybdenum may also be applicable.[15–18] When used in conjuction with an ion-exchange procedure, the proposed method may be useful in determining the molybdenum content of some steel alloys.

Conclusion

The suitability of the molybdenum(VI) - OH-PDT system for the development of a rapid, simple, accurate and sensitive method for determining small amounts of molybdenum has been demonstrated. The proposed reagent compares well with previously described thiosemicarbazones (Table II). It is evident that OH-PDT is the most sensitive thiosemicarbazone reported for the determination of molybdenum.

TABLE II

CHARACTERISTICS OF MOLYBDENUM - THIOSEMICARBAZONE COMPLEXES

Compound	Condition	$\lambda_{max.}$/nm	Sandell's sensitivity	Reference
Salicylaldehyde thiosemicarbazone	$SnCl_2$, pH <2	550	0.020	7
2-Hydroxybenzophenone thiosemicarbazone	$SnCl_2$, pH 1.3	505	0.033	10
2,2′-Dihydroxybenzophenone thiosemicarbazone	$SnCl_2$, pH 1.3	505	0.030	8
2-Hydroxy-4-methoxy-5-sulphobenzophenone thiosemicarbazone	$SnCl_2$, pH 1.3	505	0.020	9
1,4-Dihydroxyphthalimide dithiosemicarbazone	pH 2–4, extract with isopentyl alcohol	435	0.010	—
Toluene-3,4-dithiol*	H_2SO_4 8–12 N, extract with benzene	680	0.004	6

* Included for the sake of comparison.

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Determination of Iron(II) in Silicates by Gravimetric Titration

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A rapid method, suitable for batch working, is described for determining iron(II) in silicates. Hot 1 + 1 hydrofluoric - sulphuric acid decomposes the subsample in a polypropylene bottle previously filled with carbon dioxide. After dilution with a boric - phosphoric - sulphuric acid mixture, iron(II) is titrated gravimetrically with potassium dichromate solution, in the bottle used for sample decomposition. A sample-preparation procedure that minimises oxidation of iron(II) is described. Results obtained for iron(II) in various international reference rocks by the proposed method agree well with recommended values.

Keywords: *Iron(II) determination*; *silicates*; *gravimetric titration*

French and Adams[1] have published a procedure for determining iron(II) oxide in rocks that involves decomposition of the sample by treatment with a hot 1 + 1 mixture of concentrated hydrofluoric and sulphuric acids. A modification of this procedure is used by the CSIRO Division of Mineralogy, North Ryde, New South Wales.[2] The latter procedure differs from the former procedure mainly in that the sub-sample is decomposed in a narrow-mouthed 125-ml polypropylene bottle previously filled with carbon dioxide, rather than in a 60-ml wide-mouthed polypropylene bottle, and the redox titration is performed with potassium dichromate solution and sodium diphenylamine sulphonate, rather than with cerium(IV) sulphate solution and *N*-phenylanthranilic acid as titrant and indicator, respectively.

The procedure described in this paper is simpler than the two procedures referred to above for the following reasons.

(*i*) The bottle used for decomposing the sub-sample is also the titration vessel.

(*ii*) A convenient gravimetric titration system, consisting of the titrant in a polyethylene dispensing bottle[3] and an electronic top-loading balance, replaces the traditional burette.

The experience of several operators in this laboratory has shown that it is easier to use the above titration system rather than a burette. As the fine tip on the dispensing bottle can deliver individual drops weighing between 0.008 and 0.010 g, the end-point can be located as precisely as with a microburette. The only disadvantage of this gravimetric approach is that an electronic top-loading balance is much more expensive than a burette. However, such a balance has many other uses in the laboratory, so this disadvantage is more apparent than real.

Experimental

Sample-preparation Equipment

This consists of the following items installed in a sample-preparation room with dust extraction facilities for operator safety.

Hydraulic laboratory crusher - breaker with tungsten carbide working faces. Obtainable from Rocklabs, P.O. Box 28–362, Auckland 5, New Zealand.

Jaw crusher with manganese hardened steel plates. As an alternative to the hydraulic laboratory crusher - breaker.

Siebtechnik tungsten carbide - cobalt ring grinder, 300-ml. Weighing about 11.6 kg and obtainable from Siebtechnik, Mühlheim, Federal Republic of Germany.

Wedag laboratory vibrating cup mill, Type MN 954/2. Obtainable from KHD Industrieanlagen AG, Humboldt Wedag, Federal Republic of Germany.

Steel adaptor base. Weighing about 2.7 kg. This allows the Siebtechnik grinder to be used with the Wedag vibrating cup mill.

Apparatus

Narrow-mouthed polypropylene bottles. These are of 125-ml capacity and have screw-caps that give a gas-tight seal. In this laboratory, Nalgene, Catalogue No. 2006–0004, bottles (obtainable from Nalge Company, Division of Sybon Corporation, Rochester, N.Y.) have been found suitable; they can be re-used almost indefinitely. The bottles must be dry and filled with carbon dioxide within 6 h before use.

Glass beakers, 2000-ml. Boiling water in a 2000-ml beaker is used to heat four polypropylene bottles floating upright.

Polyethylene dispensing bottle for use as a weight burette. This has a capacity of 500–600 ml and a fine tip on its dispensing tube capable of delivering drops weighing between 0.008 and 0.01 g.[3]

Magnetic stirrer and PTFE-coated stirrer bars.

Calibrated electronic top-loading balance. This must be capable of weighing up to about 1200 g to the nearest 0.01 g and have a precision of ±0.005–0.01 g.

Reagents

All reagents should be of the appropriate analytical-reagent grade. De-ionised water should be used in the preparation of reagents and throughout the analysis.

Caution—Wear safety glasses and gloves when handling the acids listed below. Handle hydrofluoric acid solutions in a well ventilated fume cupboard. Handle potassium dichromate with care because it is toxic and carcinogenic.[4]

Hydrofluoric acid, sp. gr. 1.13.

Sulphuric acid, sp. gr. 1.84.

Sulphuric acid, 1 + 1. Cautiously add 500 ml of sulphuric acid (sp. gr. 1.84) with stirring to 500 ml of water, cool and dilute to 1000 ml.

Boric acid, solid crystals.

Phosphoric acid, sp. gr. 1.75.

Hot mixture of hydrofluoric and sulphuric acids, 1 + 1. Slowly add 50 ml of sulphuric acid (sp. gr. 1.84) to 50 ml of hydrofluoric acid in a 125-ml polypropylene bottle in a fume cupboard. (Heat and hydrogen fluoride vapour are liberated in this process.) To prevent the acid mixture from cooling before use, stand the sealed bottle in a 500-ml beaker containing water at 60 °C.

Dilute solution of boric, phosphoric and sulphuric acids. Dissolve 75 g of boric acid in 1875 ml of water. Transfer to a 2.5-l polyethylene bottle fitted with a handle for ease of dispensing, add 250 ml of 1 + 1 sulphuric acid and 125 ml of phosphoric acid and mix.

Sodium diphenylamine sulphonate, pellets. No. 32 Analoids from Ridsdale and Co. Ltd., Middlesbrough, England, have been found to be suitable.

Potassium dichromate solution. Weigh 1.2283 g of potassium dichromate into a tared 1100-ml polypropylene beaker. With the aid of a calibrated top-loading balance and a polyethylene dispensing bottle,[3] add 998.78 g of water. Cover and stir magnetically until dissolution is complete. Transfer about half of this solution to the weight burette (see under Apparatus); store the remainder in a 500-ml polyethylene bottle. The concentration of this solution is such that 1.000 g is equivalent to 1.800 mg of iron(II) oxide.

Procedure

With the aid of a hydraulic crusher - beaker or a jaw crusher with hardened steel plates reduce the rock sample to a grain diameter of about 3 mm or less.[5] Place a representative 30-g portion of this material in a 300-ml Siebtechnik tungsten carbide - cobalt ring grinder. Secure the loaded ring grinder in the Wedag mill with the aid of the steel adaptor base; mill for 5 min. Approximately 98% of the resulting powder is less than 45 μm in particle diameter.

Transfer a 0.25-g subsample of powdered rock, weighed to the nearest 0.0002 g, into a 125-ml polypropylene bottle previously filled with carbon dioxide. Add (with a polypropylene measuring cylinder) 10 ml of a 1 + 1 mixture of hydrofluoric and sulphuric acids heated to 60 °C. Immediately tighten the cap of the bottle. Place the bottle upright in a beaker of boiling water and boil for 10 min. (The level of boiling water in the beaker should

be high enough to ensure that the cap of the bottle is higher than the top of the beaker. This will prevent the cap from being heated above about 50 °C; the cap may loosen when heated above this temperature.) Cool the bottle in cold water. Add 90 ml of the dilute solution of boric, phosphoric and sulphuric acids. Immediately seal the bottle, shake and stand it in a cooling bath in readiness for titration.

Any unattacked particles, which may contain iron(II) (especially if they are dark in colour), can probably be seen settling in the bottle at this stage. If a sample does have unattacked particles, as distinct from precipitated fluorides that dissolve after stirring for a few minutes in the presence of boric acid, repeat the procedure, extending the heating period in boiling water to 30 min.

Add one pellet of sodium diphenylamine sulphonate and a PTFE-coated stirrer bar. Stir the contents of the bottle magnetically and titrate gravimetrically with the potassium dichromate solution contained in the polyethylene dispensing bottle[3] until the solution turns from bright green to grey - green. Place the cap on the bottle and shake to include any droplets on the underside of the cap in the titration. Continue to titrate dropwise until the end-point is reached, when the solution turns purple and remains so for at least 15 s.

Calculations

(*i*) Dichromate titre = Mass of dispensing bottle before titration —mass of dispensing bottle after titration

(*ii*) $$\text{Iron(II) oxide (\%)} = \frac{\text{Dichromate titre (g)} \times 0.18}{\text{Mass of subsample}}$$

Results and Discussion

Sample Preparation

French and Adams[1] found that grinding a rock powder with an agate pestle and mortar for 10 min while continuously moistening with acetone produced "a sufficiently fine grain size and no detectable oxidation." As acetone, by rapid evaporation, prevents heating of the sample, this finding is in accord with the statement of Hillebrand *et al.*[6] that the cause of oxidation of iron(II) when rocks are ground in air is probably local heating.

In this laboratory, a Siebtechnik tungsten carbide - cobalt ring grinder is preferred to an agate pestle and mortar for pulverising silicate rocks for summation analysis because it is more rapid and introduces less risk of contamination. Local heating of the sample when being pulverised in this ring grinder is probably negligible if the ring grinder's internal parts do not feel warm immediately after grinding. This situation occurs when the Wedag mill is "weighed down" with a 2.7-kg adaptor base so that one can use the Siebtechnik ring grinder with it; the internal parts of this grinder are still cool to the touch even immediately after 7 min of grinding. As Table I indicates, the adaptor base causes the effective weight of the Siebtechnik ring grinder to be about 21% higher than the Wedag tungsten carbide - cobalt ring grinder. When used on the Wedag mill, the latter ring grinder feels distinctly warm even after grinding for only 1 min.

Table I shows that although the Siebtechnik ring grinder plus adaptor base on the Wedag mill requires about twice the time to achieve a given sample fineness, it yields results for iron(II) oxide in two typical igneous rocks significantly higher at the 95% confidence level than the "all Wedag" system.

Use of Carbon Dioxide-filled 125-ml Bottles

French and Adams[1] used 60-ml wide-mouthed polypropylene bottles as decomposition vessels and found that the copious hydrogen fluoride vapour produced by the violent reaction of the hot acid mixture and the sample was sufficient to expel all the atmospheric oxygen from the bottle and thus to eliminate the negative error due to oxidation of iron(II) during sample decomposition. For titration to be performed in the bottle, its capacity has to be about 125 ml rather than 60 ml.

To ascertain whether the vapour referred to above was sufficient to expel all the atmospheric oxygen from 125-ml bottles, the samples listed in Table II were analysed in quadruplicate with carbon dioxide-filled and air-filled bottles. The caps of the former bottles

were secured immediately after hot acid was added; however, with the latter bottles the French and Adams procedure[1] of waiting for vapour tc appear in the neck of the bottle and then securing the cap was followed. From the results for three of the four samples in Table II, one can conclude with 95% confidence that 125-ml narrow-mouthed polypropylene bottles have to be pre-filled with carbon dioxide in order to avoid oxidation of iron(II) during sample decomposition.

TABLE I

EFFECT OF MASS OF RING GRINDER ON IRON(II) OXIDE RESULT AND GRINDING EFFICIENCY

	Ring grinder used on Wedag mill						
	300-ml Siebtechnik tungsten carbide - cobalt + adaptor base, total mass = 14.38 kg			300-ml Wedag tungsten carbide - cobalt, total mass = 11.87 kg			
Milling time*/min	3	5	7	1	2	3	5
Sample NSWDMR G79/607†—							
FeO (dry basis),% ‡	9.45 ±0.03	9.43 ±0.03	9.42 ±0.03	9.35 ±0.03	9.31 ±0.05	9.26 ±0.03	—
Percentage of sample having particle size <45 μm§ ..	93.9	97.9	99.1	95.1	96.9	97.8	—
Sample NSWDMR G80/847¶—							
FeO (dry basis),% ‡	—	4.50 ±0.04‖	—	—	4.28 ±0.04	—	4.08 ±0.05
Percentage of sample having particle size <45 μm§ ..	—	97.9‖	—	—	96.5	—	94.4**

* For a 30-g portion.
† Very hard, basic, intrusive rock.
‡ Each result is the mean of triplicate determinations ± the 95% confidence limit of the mean, obtained by multiplying the range by 1.3.[7]
§ Particle size was determined by wet sieving.
¶ Porphyritic diorite containing hornblende, quartz and plagioclase.
‖ When a separate 30-g portion of this sample was wetted with 15 ml of acetone in the Siebtechnik ring grinder and ground for 5 min, the result for FeO (dry basis) was 4.57 ± 0.03% and 98.1% of the sample had particle size <45 μm.
** Lower than that obtained after 2 min of grinding because agglomeration occurred during the excessively long grinding time.

In discussing the procedure of French and Adams,[1] Jeffery[9] has stated that although the use of a mixture of hot sulphuric and hydrofluoric acids may be ideal for some rocks, with others it produces a reaction that is too violent, and so should be used with caution. Loss of sample because the reaction is too violent is much less likely in the present procedure because of the use of 125-ml narrow-mouthed bottles instead of 60-ml wide-mouthed bottles.

Blank Determination

If the de-ionised water or the reagents contain appreciable dissolved organic matter, a blank determination is required. As iron(II) has to be present initially for an end-point to occur, a suitable procedure for blank determination is to include in a batch of determinations a rock known to be low in iron(II). The blank is obtained from the difference between the found and expected iron(II) oxide. Blanks thus determined in this laboratory with 30-mg subsamples of the syenite NIM-S have been very low, ranging from −0.01 to +0.03% iron(II) oxide over a 2-year period.

Interferences

In common with other wet-chemical methods for determining iron(II) oxide in silicates, the method described herein suffers from a positive interference by sulphide minerals (except pyrite) and organic matter. The spectrophotometric method of Begheijn[10] is free from interference by organic matter but is less rapid than the method described herein.

Although graphite is not attacked by hot hydrofluoric - sulphuric acids and does not react with potassium dichromate, its presence as a dark suspension in a titration solution renders the end-point indiscernible. This problem is readily overcome by weighing the bottle plus initial solution (approximately 100 ml), centrifuging, transferring most of the supernatant liquid to a fresh, tared bottle, weighing this "gravimetric aliquot" and then titrating it in the usual way. The amount of sample titrated can be readily calculated from the weighings referred to in the previous sentence, provided that the mass of the empty bottle used for decomposing the sample is known.

TABLE II

COMPARISON OF RESULTS OBTAINED USING AIR-FILLED AND CARBON DIOXIDE-FILLED 125-ml NARROW-MOUTHED POLYPROPYLENE BOTTLES

	FeO (dry basis), %		
Sample	Recommended	CO_2-filled bottle*	Air-filled bottle*
Anorthosite AN-G	2.24†	2.29 ± 0.03	2.18 ± 0.06
Granite MA-N	0.31†	0.30 ± 0.03	0.21 ± 0.04
Basalt BE-N	6.77†	6.89 ± 0.07	6.70 ± 0.14
Ammonium iron(II) sulphate ..	18 23 ± 0.09‡	18.3 ± 0.2	17.7 ± 0.2

* Results are the means of quadruplicate determinations ± the 95% confidence limits of the means obtained by multiplying the range of each set of quadruplicate determinations by 0.72.[7]

† Reported by Govindaraju.[8]

‡ 100-mg subsamples of ammonium iron(II) sulphate were analysed. The recommended FeO content of the ammonium iron(II) sulphate (May and Baker, Pronalys analytical reagent) was calculated from its molecular formula, assuming that it was 99.5 ± 0.5% pure, as implied by its label The above results for ammonium iron(II) sulphate are reported on an "as received" basis.

Refractory Iron(II) Minerals

The work of French and Adams[1] showed that results for iron(II) oxide determined by the procedure described herein will have a negative error if the sample contains iron(II) minerals, such as garnet and staurolite, that are resistant to acid attack. Maxwell[11] also includes tourmaline, ilmenite and magnetite in this category. French and Adams[1] showed that staurolite was only slightly decomposed even after 90 min of digestion; however, garnet was almost completely decomposed when the heating period in boiling water was increased from 10 to 30 min.

As mentioned under Procedure, visual examination of the titration solution will probably reveal unattacked minerals. Increasing the heating period to 30 min will sometimes result in such samples being completely decomposed, especially if they are finely ground as described in this paper.

Reference Rocks

Table III gives the mean results for several reference rocks. These results were obtained by including a 0.25-g subsample of a given reference rock in a batch of determinations of iron(II) oxide in rocks submitted for summation analysis. Table III also gives the number of determinations carried out on a particular reference rock and the 95% confidence limits of the mean results. Each determination on a given reference rock was carried out on a separate day.

Throughput of Pre-pulverised Samples

Using the method described, an analyst can readily perform two batches of 12 determinations, *i.e.*, 24 determinations, in a 7-h working day.

Conclusion

The above method is convenient and rapid, and is used in this laboratory for the determination of iron(II) oxide in silicate rocks. Accurate results are obtained provided that the rocks do not contain any of the following: iron(II) minerals incompletely attacked by hot 1 + 1 hydrofluoric - sulphuric acids; organic matter; and sulphide minerals attacked by the above acid mixture.

TABLE III

DETERMINATION OF IRON(II) OXIDE IN SEVERAL REFERENCE ROCKS

Rock	FeO (dry basis), % Recommended	FeO (dry basis), % This procedure*	No. of determinations (this procedure)
Anorthosite AN-G ..	2.24†	2.29 ± 0.03	7
Granite MA-N	0.31†	0.30 ± 0.01	7
Basalt BE-N	6.77†	6.89 ± 0.04	7
Basalt BCR-1	8.96‡	9.05 ± 0.02	2
Norite NIM-N	7.47§	7.48 ± 0.06	4
Granite NIM-G	1.30§	1.28 ± 0.04	4
Syenite NIM-S	0 30§	0.31 ± 0.02	4
Basalt G75/1323¶ ..	—	8.42 ± 0.02	20

* Results are the means ± the 95% confidence limits of the means.
† Reported by Govindaraju.[8]
‡ Reported by Abbey.[12]
§ Reported by Steele and Hansen.[13]
¶ Basalt G75/1323 is an in-house reference rock used in this laboratory as a control sample.

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Substitution in Cellulose Ethers

Part I. Determination of Glucose Units According to Number and Type of Ether Substituents Using Quantitative Thin-layer Chromatography

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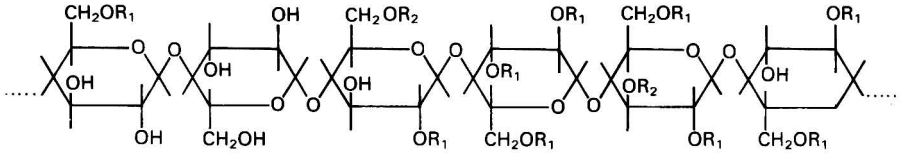
A procedure for the quantitative characterisation of the distribution of substituents in cellulose ethers is presented. After total hydrolysis in dilute sulphuric acid the sample is separated by thin-layer chromatography on silica gel according to number and type of substituents attached to the glucose unit. Quantitative evaluation of the chromatograms is performed by scanning photometry in the visible range. The procedure allows the determination of the percentages of tri-, di-, mono- and unsubstituted monomeric units in alkyl and alkylhydroxyalkyl celluloses. Data on the distribution of the hydroxyalkyl substituents in mixed ethers were also obtained. The reproducibility of the thin-layer chromatographic separation and that of the entire procedure are discussed.

Keywords: *Cellulose ethers*; *distribution of substituents*; *determination of glucose units*; *thin-layer chromatography*; *scanning photometry*

The importance of cellulose ethers has steadily increased in recent years. These polymers, with the structure shown below, are used as binders, emulsifiers, film formers, etc., on a large scale and their application in the chemical industry is also increasing. In view of this development there is a growing demand for a method that is capable of characterising such cellulose derivatives in a more detailed manner by providing sufficient information not only about the average degree of substitution, but also on the distribution of the ether groups over the anhydroglucose units.



where R_1 = alkyl and R_2 = R_1 or hydroxyalkyl.

The methods used at present are still based mainly on ether cleavage by hydriodic or hydrobromic acid and subsequent determination of the ether groups, *e.g.*, by titration or gas chromatography.[1–4] Nuclear magnetic resonance spectroscopy has also been shown to be applicable to the determination of the degree of substitution.[5] In our laboratory, pyrolysis gas chromatography has been employed successfully for the quantitative evaluation of the proportions of various ether substituents.[6] A review of the present state of cellulose ether analysis was published by Bartelmus and Ketterer.[3]

A great limitation to the above methods is the fact that only the total proportion of the ether groups and consequently an average degree of substitution of the monomeric glucose

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units can be determined. They do not provide any data on the inhomogeneity in the distribution of the substituents. A number of workers have indicated ways of overcoming this limitation by making use of gas chromatography[7–9] and thin-layer chromatography (TLC) and spectrophotometry.[10] However, for several reasons these techniques did not find widespread application.

In this paper we report on a procedure that allows the determination of the proportions of tri-, di-, mono- and unsubstituted anhydroglucose units in alkyl and alkylhydroxyalkyl celluloses, based on depolymerisation by acid hydrolysis and the subsequent determination of the components in the hydrolysate by quantitative TLC.

Experimental

Samples

The cellulose ethers investigated were technical samples of methyl cellulose (MC), ethyl cellulose (EC) and mixed difunctional ethers, such as methylhydroxypropyl cellulose (MHPC) and ethylhydroxyethyl cellulose (EHEC).

Hydrolysis

A 100-mg amount of the dried sample is placed in a glass ampoule and 7 ml of sulphuric acid, usually 1% *V*/*V*, are added. The mixture is allowed to stand at room temperature for several hours until the solution becomes clear. Before being sealed, the ampoule is purged with nitrogen for a few minutes. The closed system is then heated at 140 °C for 1–2.5 h, the duration depending on the kind of sample. After re-opening the ampoule, calcium carbonate is added to neutralise the solution and water is removed by evaporation to dryness. Finally, the hydrolysate is extracted with 80% aqueous methanol, the solvent is evaporated and a slightly brownish substance remains. The completeness of hydrolysis is checked via the recovery and by means of TLC.

Thin-layer Chromatography

For qualitative purposes 20 × 20 cm glass plates were coated with a 0.25-mm layer of silica gel G (Stahl) (E. Merck, Darmstadt, Federal Republic of Germany). A 250-μg amount of the hydrolysate from a 5% *m*/*V* solution in 80% aqueous methanol was spotted at the start with a microlitre syringe. Chloroform - acetic acid - water (60 + 70 + 10) served as the solvent system and the migration distance was 15 cm. The separated components can be stained by spraying the plates, heated to 100 °C, with a 0.5% *m*/*V* solution of potassium permanganate in 1 N sodium hydroxide solution or with a 0.1 M solution of vanadium(V) oxide in 1 M sulphuric acid prior to keeping the plates at 150 °C for about 15 min.

Quantitative evaluation of the chromatograms was carried out using an ERI 65m scanning photometer (VEB Carl Zeiss Jena, German Democratic Republic), which requires plate dimensions of 187 × 38 mm. A 10-μl volume of a 5% *m*/*V* solution of the hydrolysate was applied as a streak of length 30 mm. After development to a height of 14 cm with the solvent system chloroform - acetic acid - water (60 + 70 + 10 or 75 + 60 + 5) the separated zones were stained by spraying the dry plates with 25% *V*/*V* sulphuric acid and subsequently heating them at 260 °C for about 2 min.

Results and Discussion

Hydrolysis

Many workers have described the hydrolytic cleavage of the β-1,4-glucosidic bond in cellulose and its derivatives.[8–17] In the current work this step has been performed in dilute sulphuric acid at 140 °C,[15] a procedure distinguished by the easy isolation of the cleavage products. For most of the cellulose ether samples investigated complete hydrolysis was achieved by treatment for 1 h in 1% sulphuric acid at 140 °C. In some instances the time had to be extended to 2–2.5 h and/or 2% sulphuric acid had to be used. The completeness of the cleavage can be checked reliably by TLC (for details, see Fig. 1). The hydrolysates of alkyl celluloses are separated into glucose and the corresponding mono-, di- and trialkylglucoses. In the chromatograms of mixed ethers additional components containing

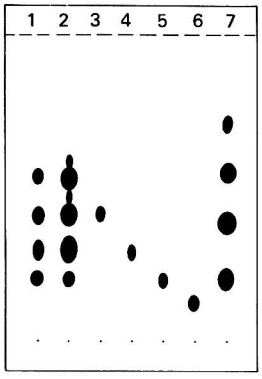

Fig. 1. TLC monitoring of cellulose ether hydrolysis: 1, MC hydrolysate; 2, MHPC hydrolysate; 3, dimethylglucose; 4, methylglucose; 5, glucose; 6, cellobiose; and 7, EC hydrolysate. Solvent system, chloroform - acetic acid - water (60+70+10) and staining reagent, a 0.5% *m/V* solution of potassium permanganate in 1 N sodium hydroxide solution at 100 °C.

hydroxyalkyl substituents are detected. Hydrolysis can be regarded as complete if no spots appear at R_F values lower than that of glucose. The yields of water-soluble cleavage products were mostly close to 100%, in some instances being between 90 and 100%.

Conditions of Quantitative TLC

It is generally known that the sample solution has to be applied as a thin streak in order to ensure minimum broadening of the zones being separated. Therefore, a semi-automatic device was built in our laboratory, by means of which the sample is sprayed as a linear band in a stream of nitrogen. Another simple means for the generation of linear starting bands is to use so-called concentration zones. For this purpose we used the two-chamber coating device according to Radowitz[18] to prepare plates having a 3–4 cm long layer of Kieselguhr at the lower edge followed by the usual silica layer. With the given solvent Kieselguhr is virtually inert to the glucoses investigated, so that the boundary of transition from Kieselguhr to silica gel actually becomes the start of the chromatographic process. The method of application of the substance on to the non-adsorbing layer is therefore of minor importance. Both methods described have proved suitable for the generation of thin and concentrated starting bands as required in quantitative TLC.

Among the variety of solvent systems proposed for similar mixtures of substances, chloroform - acetic acid - water (60 + 70 + 10)[17] was found to be the most efficient. The components of alkyl cellulose hydrolysates are well separated according to the increasing number of alkoxyl groups. With mixed difunctional ethers, such as MHPC and EHEC, the proportions of the three solvents had to be changed to 75 + 60 + 5 in order to obtain satisfactory resolution. On the other hand, the proportions 40 + 80 + 20 are necessary for the separation of lower glucose oligomers. This illustrates the fact that the mixtures of chloroform, acetic acid and water represent a versatile mobile phase for the separation of many different cellulose ether hydrolysates. The use of other solvent systems suggested in the literature[19,20] lowers the resolution markedly.

All separations were carried out in normal chambers at a defined relative humidity in order to improve the reproducibility in general and that of the R_F values in particular. The effect of pre-conditioning of the plates over the solvent system was also studied. The best results

were obtained by pre-conditioning for 1 h over chloroform - acetic acid - water, in the presence of 42.6% *m/m* sulphuric acid for the generation of a defined and constant relative humidity in the chamber. Table I shows the reproducibility of R_F values measured for the components of hydrolysed MHPC in nine different chromatograms. Standard deviations are between 0.01 and 0.045 R_F unit, which is sufficient for the quantitative evaluation by measurement of peak areas. Considering the order of elution derived from the chromatograms of reference substances, the zones can be characterised by the mean R_F values.

TABLE I

PRECISION OF R_F VALUES FOR NINE SEPARATIONS OF AN MHPC HYDROLYSATE

For names of components and TLC conditions, see legend to Fig. 4.

	Component									
	1	2	3	4	5	6	7	8	9	10
Mean R_F value	0.09	0.17	0.23	0.32	0.36	0.40	0.50	0.56	0.63	0.70
Standard deviation ($n = 9$) ..	0.01	0.015	0.02	0.02	0.03	0.035	0.04	0.04	0.045	0.04

As quantitative evaluation of the chromatograms by scanning photometry was intended, a suitable spray reagent had to be chosen. Such a reagent should be sufficiently sensitive and at the same time give an unambiguous correlation between the amount of substance per zone and the measured reflectance signal. Moreover, good contrasts between spots and background and stability of the coloration over a certain period of time are most desirable. Although various reagents for sugars and their derivatives have been described, our experiments have shown that almost all of them did not fulfil the requirements that would justify their use in the photometric evaluation of our thin-layer chromatograms. This applied to Fehling's solution, aniline - diphenylamine - phosphoric acid, *m*-phenylenediamine, 3,5-dinitrobenzoic acid and orcinol - sulphuric acid. Basic potassium permanganate solution[21] showed high sensitivity, but the colours of the spots and the background were not stable enough. This disadvantage was not observed with vanadium(V) oxide,[20] but it was very difficult to obtain a low-noise background and the necessary reproducibility of coloration. Finally, sulphuric acid proved to be the best reagent. After being sprayed with 25% *V/V* sulphuric acid the plates are heated at 260 °C for about 2 min,[22] until black zones appear on the white background. The intensity of the spots remains practically constant for at least 24 h and this period can be prolonged considerably by storing the plates in a dark place.

Photometric Evaluation of Thin-layer Chromatograms

After staining, the chromatograms show exclusively black and white contrasts. This circumstance makes scanning photometry of the plates more convenient, as the densitometer can be run without additional colour filters. The correlation between the reflectance peak area and the amount of substance per zone has been investigated for glucose, 3-*O*-methylglucose and 2,3-*O*-dimethylglucose. The results show that the corresponding calibration graphs for these three components are very close to each other, *i.e.*, the response factors within the concentration range of interest (5–40 μg per zone) differ only insignificantly. With the given precision of this method it is therefore not necessary to introduce correction factors. Mass proportions of the components considered in this work are derived from the calibration graph, which was found to be of approximately parabolic shape with an almost linear section in the most relevant concentration range.

In order to exclude errors that could result from chromatographic wall effects, only a 10 mm wide strip in the central part of the separated zones was scanned. This step reduced the influence on the final result of non-linear band shapes and deviating R_F values in the marginal areas of the zones.

Alkyl Celluloses

Figs. 2 and 3 show typical chromatograms of MC and EC hydrolysates. The identities of the peaks were established using pyrolysis gas chromatography, nuclear magnetic resonance spectroscopy and reference substances. Results obtained with a sample of MC are presented

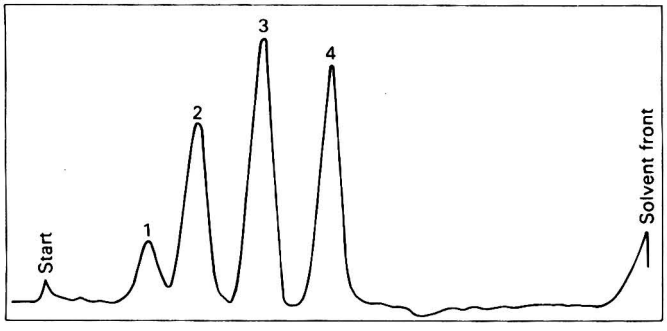

Fig. 2. Scanned chromatogram of an MC hydrolysate: 1, glucose; 2, methylglucose; 3, dimethylglucose; and 4, trimethylglucose. Solvent as in Fig. 1 and staining reagent, 25% *V*/*V* sulphuric acid at 260 °C.

in Table II. The average degree of substitution (*DS*) of the monomeric units is calculated according to the equation

$$DS = (c_{mono} + 2c_{dl} + 3c_{trl})/100$$

where c_{mono}, c_{dl} and c_{trl} are the percentages of the mono-, di- and trisubstituted anhydroglucose units, respectively.

For evaluation of the procedure, various samples were analysed by both hydrolysis - TLC and according to Zeisel ether cleavage.[1] Comparison of the corresponding *DS* values shows that the degrees of alkoxyl substitution obtained by our procedure are generally higher by up to 5%. Other workers who attempted to determine the *DS* of alkylcelluloses by alternative methods also found values slightly higher than those of the Zeisel ether cleavage.[5,10] A possible reason for this could be the presence of measurable amounts of hydrobromic acid in the hydriodic acid used for the cleavage.

Thus, in addition to the determination of the average degree of substitution, quantitative TLC is able to provide information about the inhomogeneous distribution of the substituents over the monomeric units of the cellulose chain. This distribution is known to bear a direct relationship to certain properties of the polymer.

As can be seen from Table II, standard deviations for the determination of single components are between 1.1 and 2.0 units, and for the *DS* it is 0.05.

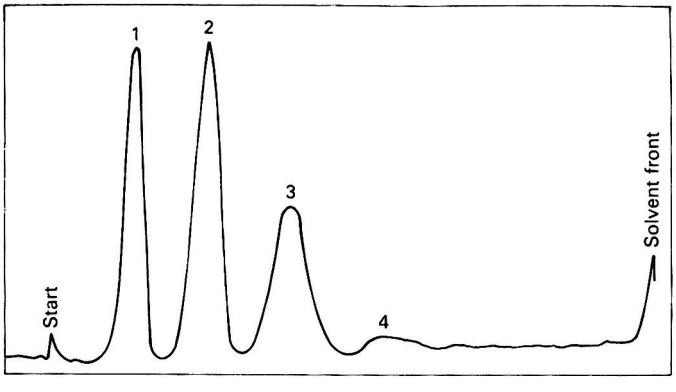

Fig. 3. Scanned chromatogram of an EC hydrolysate: 1, glucose; 2, ethylglucose; 3, diethylglucose; and 4, triethylglucose. Solvent and staining reagent as in Fig. 2.

TABLE II

RESULTS OF TEN DETERMINATIONS WITH A SAMPLE OF MC

	Unsubstituted glucose, % *m/m*	Methylglucose, % *m/m*	Dimethylglucose, % *m/m*	Trimethylglucose, % *m/m*	*DS*
	10	25	35	30	1.85
	10	23	35	32	1.89
	12	28	33	27	1.75
	12	24	34	30	1.82
	11	25	33	31	1.84
	10	25	33	32	1.87
	9	25	35	31	1.88
	10	28	36	26	1.78
	10	28	32	30	1.82
	9	23	38	30	1.89
Mean value	10	25	34	30	1.84
Standard deviation ..	1.1	2.0	1.8	2.0	0.05

Mixed Difunctional Ethers

The separations of hydrolysed mixed ethers are given in Figs. 4 and 5. In addition to the peaks of the alkylglucoses, additional peaks appear in the chromatograms, which must be assigned to those monomeric units carrying hydroxyalkyl substituents. As reference substances of the alkylhydroxyalkylglucose type are not available from commercial sources, the identification of these minor peaks is extremely difficult, as they represent only small amounts of substance. Moreover, there is a considerable number of possible individual species that could be present owing to the ability of the hydroxyalkyl groups to form longer chains by adding more alkylene oxide molecules.

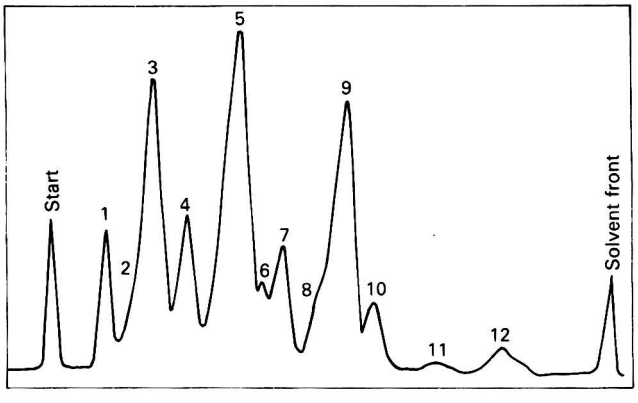

Fig. 4. Scanned chromatogram of an MHPC hydrolysate: 1, glucose; 2, glucose with one unit of propylene oxide (PO); 3, methylglucose; 4, methylglucose with one PO unit; 5, dimethylglucose; 6, methylglucose with two PO units; 7, dimethylglucose with one PO unit; 8, dimethylglucose with three PO units; 9, trimethylglucose; 10, dimethylglucose with three PO units; 11, dimethylglucose with four PO units; and 12, tetramethylglucose. Solvent system, chloroform - acetic acid - water (75+60+5) and staining reagent as in Fig. 2.

We attempted to solve this problem by means of an increment model that allows the calculation of the contribution to the total R_F of each single methyl group and each propylene oxide unit, and consequently the prediction of R_F values of the relevant mixed difunctional glucose ethers under the chromatographic conditions described above. The model is based on R_F measurements with pure methyl- and hydroxypropylglucoses. As a result, the assignment of peaks 4, 6, 7, 8, 10 and 11 in the chromatogram of MHPC hydrolysate (Fig. 4) has

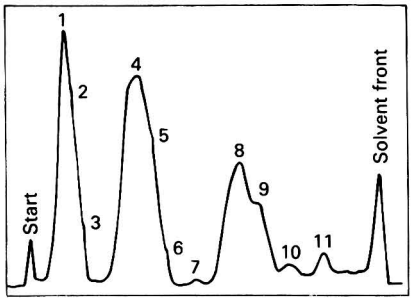

Fig. 5. Scanned chromatogram of an EHEC hydrolysate: 1, glucose; 2, glucose with one unit of ethylene oxide (EO); 3, glucose with two EO units; 4, ethylglucose; 5, ethylglucose with one EO unit; 6, ethylglucose with two EO units; 7, ethylglucose with three EO units; 8, diethylglucose; 9, diethylglucose with one EO unit; 10, diethylglucose with two EO units; and 11, triethylglucose. Solvents as in Fig. 4 and staining reagent as in Fig. 2.

been accomplished by comparison of predicted and measured R_F values. The correctness of the model has been confirmed for methylhydroxypropylglucose (peak 4) after the preparation of this mixed glucose ether. Work is continuing in this field.

From the chromatograms qualitatively assigned this way, quantitative data on the distribution of methoxyl groups and the average degree of methoxylation, DS_{Me}, are obtained. The results of nine determinations by hydrolysis - TLC with a sample of MHPC as presented in Table III emphasise the reproducibility of the whole procedure. An estimation of the molar hydroxypropyl substitution, MS_{HP}, can also be made, although with lower accuracy, as the peaks of some components overlap.

TABLE III

RESULTS OF NINE DETERMINATIONS WITH A SAMPLE OF MHPC

	Concentration of glucose ethers, % *m/m*										
		Methylglucose			Dimethylglucose				Tri-methyl-glucose	Tetra-methyl-glucose	
	Glucose	Alone	+ PO*	+ 3PO*	Alone	+ PO*	+ 3PO*	+ 4PO*			DS_{Me}
	4	16	8	4	26	9	7	1	24	1	1.90
	6	20	8	4	26	7	5	0.5	22	1	1.79
	5	18	9	6	24	7	7	0.5	23	1	1.83
	4	18	8	4	26	8	7	0.5	23	1	1.86
	5	18	9	4	24	9	6	0.5	23	1	1.83
	5	16	9	4	24	9	7	1	24	1	1.87
	5	16	9	5	24	8	7	1	23	2	1.87
	5	19	9	4	25	7	5	1	24	1	1.84
	6	20	9	3	25	6	5	1	22	3	1.84
Mean value	5	18	9	4	25	8	6	0.8	23	1.3	1.85
Standard deviation	0.7	1.6	0.6	0.9	0.9	1.1	1.0	0.3	0.8	0.7	0.03

* PO = propylene oxide unit.

The components involved in the chromatograms of EHEC hydrolysates have been established by an analogous approach (for details see the legend to Fig. 5). It is obvious that in this instance a quantitative evaluation of the hydroxyethyl substituents is impossible because of the poor resolution.

The degrees of methoxyl and ethoxyl substitution obtained by the Zeisel method are again lower than the corresponding *DS* values obtained by the hydrolysis - TLC procedure, the differences being up to 10%. Such large differences must be expected, as it has been established by other workers that the Zeisel method leads to low *DS* values with mixed ethers that have hydroxyethyl or hydroxypropyl substituents because of the formation of ethylene or propylene in the cleavage.[3,4]

The results obtained demonstrate that the procedure described provides a detailed quantitative description of the substutition in mixed cellulose ethers also. The thin-layer chromatograms are highly characteristic for a given sample, so that they can be regarded as the "fingerprint" of the corresponding cellulose ether.

Conclusions

The procedure proposed for the quantitative TLC of hydrolysates has been applied successfully to the characterisation of a large number of alkyl and alkylhydroxyalkyl celluloses. Its value consists in the more detailed information provided about the inhomogeneity of the substitution in the polymer, which means real progress with respect to the traditional methods based on ether cleavage. The procedure has been shown to be reproducible and accurate and it possesses all the attributes necessary for it to be widely employed as an effective method in research on and routine analysis of cellulose ethers.

The results of further investigations on cellulose ethers using high-performance liquid chromatography will be published in Part II.

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Stability Indicating Assay for Dipyrone

Part I. Separation and Quantitative Determination of Dipyrone and Its Degradation Products by Thin-layer Chromatography

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A thin-layer chromatographic procedure is described for the separation and quantification of dipyrone *in situ* in the presence of three of the major degradation products. The repeatability and detection limits are given and the applicability of the assay in checking the stability of tablets and injections is shown.

Keywords: *Dipyrone determination*; *degradation products determination*; *thin-layer chromatography*; *tablet stability*; *injection stability*

Sodium *N*-(1,5-dimethyl-3-oxo-2-phenylpyrazolin-4-yl)-*N*-methylaminomethanesulphonate monohydrate [dipyrone, metamizol, Novalgine (Hoechst)] is an analgesic and antipyretic drug. The compound readily undergoes hydrolysis and oxidation according to the general scheme summarised by Pellerin and Letavernier.[1] The degradation pathways are given in Fig. 1. In the solid state, dipyrone[2] can be oxidised into 4-formylaminoantipyrine (**IV**). In acidic solution[2] dipyrone is transformed into 4-formylmethylaminoantipyrine (**V**). In aqueous neutral solution dipyrone is in equilibrium with sodium hydroxymethane sulphonate and 4-methylaminoantipyrine (**I**). This latter compound may be oxidised and hydrolysed by two possible routes. One pathway[2–4] leads to methylrubazonic acid (**VIII**) through 4-aminoantipyrine (**II**) and iminobisantipyrine (**VII**). An alternative pathway[3] leads to bismethylantipyrinylmethane (**VI**) obtained from reaction of **I** and **V**. Another possible degradation compound, which occurs on prolonged storage has been identified[2] as 4-hydroxyantipyrine (**III**). **I**, **II** and **III** have also been identified as metabolites.[5] **I** and **II** have been detected in injections of dipyrone as being the two main decomposition products.

Some of the proposed analytical methods for identification and quantification of the decomposition products of dipyrone are not reliable, as discussed in reference 1: either the extraction procedure used may displace the hydrolysis equilibrium, noted in Table I of reference 1, or the methods lack specificity (*e.g.*, in ultraviolet determination where the by-products may have similar spectral characteristics to the parent compound). These disadvantages do not apply to chromatographic methods; however, gas - liquid chromatography cannot be used because of thermal decomposition in the injector.[1] A high-performance liquid chromatographic procedure developed by Tamura *et al.*[6] is not convenient when applied to the trace level determination of degradation products in pharmaceutical formulations. Ebel and Rost[7] developed a thin-layer chromatographic procedure for the determination of dipyrone and one degradation compound, and requires the use of two different internal standards. Preliminary work in our laboratory indicated that this proposed mixed solvent system could not achieve the separation of dipyrone and **I**, **II** and **III**. The work described in this paper was undertaken to develop an improved thin-layer chromatographic method to separate and determine *in situ*, with only one internal standard, dipyrone, **I**, **II** and **III**.

Experimental

Apparatus

The chromatograms were evaluated using a chromatogram spectrophotometer (Zeiss PM QII) equipped with a chart recorder (B.B.C. Goerz) and an electronic integrator (Minigrator, Intersmat).

Materials

Pre-coated TLC silica gel F_{254} plates (Merck, Darmstadt, West Germany), thickness 0.25 mm, were used.

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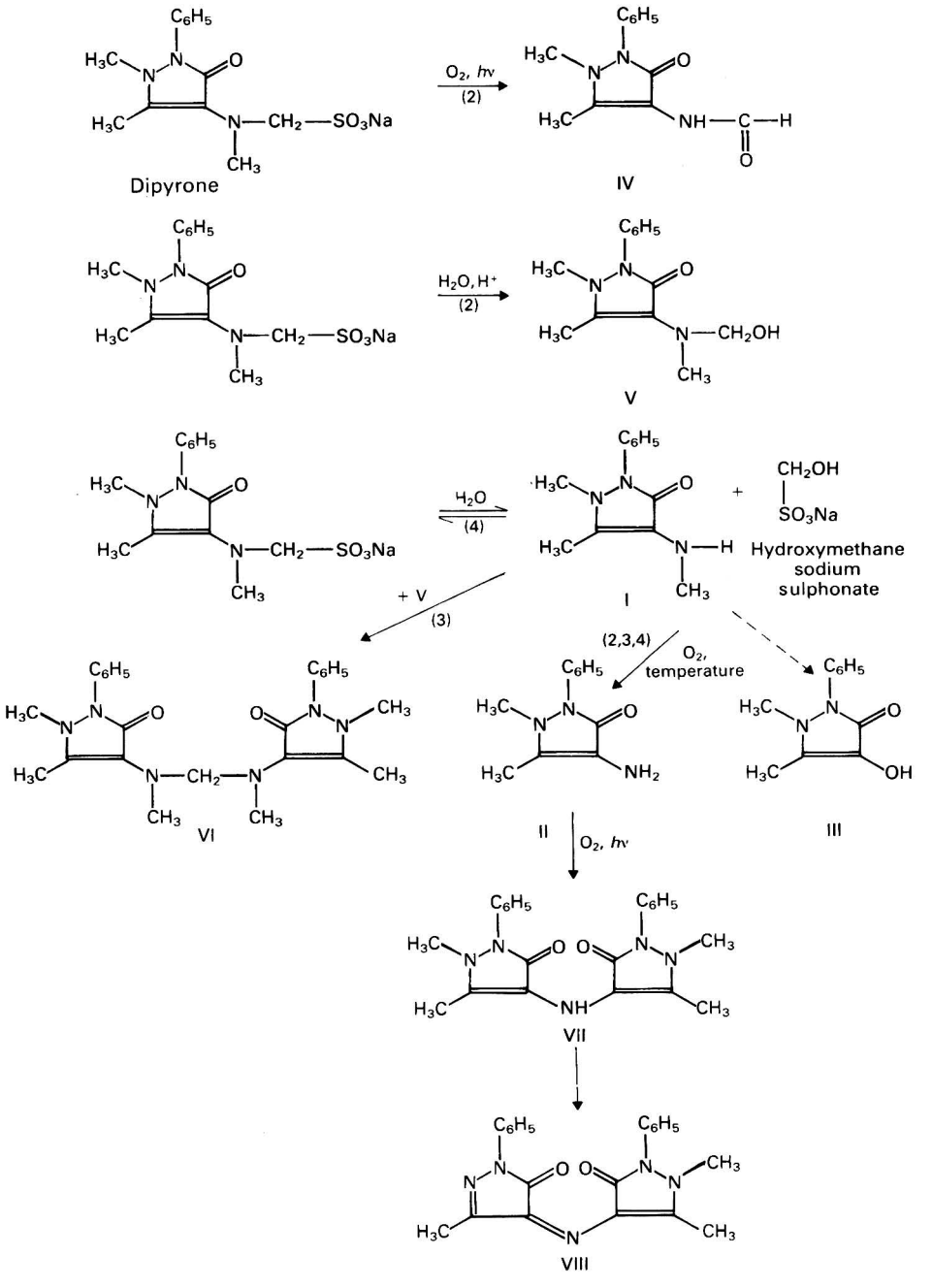

Fig. 1. Degradation pathways of dipyrone. **I**, 4-Methylaminoantipyrine; **II**, 4-aminoantipyrine; **III**, 4-hydroxyantipyrine; **IV**, 4-formylaminoantipyrine; **V**, 4-formylmethylaminoantipyrine; **VI**, bismethylantipyrinylmethane; **VII**, iminobisantipyrine; and **VIII**, methylrubazonic acid. (2), According to Pechtold; (3), according to Wagner; and (4), according to Ono. Broken lines indicate hypothetical pathways of degradation.

Methanol, ethanol, benzene, chloroform and ammonia solution were of analytical-reagent grade. Dipyrone, **I**, **II** and **III** were gifts from Hoechst Laboratories and were used as received. Theophylline was purchased from Sigma Chemical Co. Tablets (500 mg of dipyrone per tablet) and injections (2.5 g of dipyrone per 5 ml of solution) were commercial formulations obtained from Hoechst Laboratories.

Standard solutions

Methanolic stock standard solutions were prepared: 3 mg ml^{-1} of dipyrone; 2 mg ml^{-1} of **I**; 2 mg ml^{-1} of **II**; and 2 mg ml^{-1} of **III**. These solutions were diluted with methanol to give a concentration range from 0.3 to 1.5 mg ml^{-1} for dipyrone and from 0.2 to 1.0 mg ml^{-1} for **I**, **II** and **III**. These solutions were then diluted with an equal volume of internal standard solution (0.3 mg ml^{-1} of theophylline in methanol). The final concentration range was from 0.15 to 0.75 mg ml^{-1} for dipyrone and 0.1 to 0.5 mg ml^{-1} for **I**, **II** and **III**.

The rapid degradation of the dipyrone was avoided by using methanol as the solvent; in the mixed standard solutions the presence of degradation products stabilises the hydrolysis equilibrium between dipyrone and **I** (shown in Fig. 1).

Test solutions

The average mass of dipyrone per tablet was determined from 10 ground tablets. An accurately weighed amount, equivalent to 100 mg of dipyrone, was transferred into a 100-ml calibrated flask. The powder was mixed with about 50 ml of methanol, and agitated using an ultrasonic bath for 2–3 min. The mixture was then diluted to 100 ml with methanol. The resulting suspension was filtered through a Whatman GF/C paper, or equivalent, and the filtered solution was then diluted to the required concentration.

The dipyrone injection was suitably diluted with methanol. In both instances, internal standard solution was added to correspond to the calibration graph.

Chromatography

Aliquots of 2 μl of the reference solutions and the test solutions were applied to the thin-layer chromatographic plate using a syringe (10-μl Hamilton syringe, calibrated at 0.1-μl intervals). The developing tank was saturated with a mixed solvent system: ethanol - chloroform - benzene - 10% ammonia solution in the proportions 50 + 30 + 18 + 2. The plate was developed to a height of around 15 cm and then dried in a stream of air. The spots were revealed under ultraviolet light at a wavelength of 254 nm and quantified by direct reflectance spectrometric measurements using a chromatogram spectrophotometer at 273 nm (the wavelength of maximum absorbance for all the compounds).

Results and Discussion

The R_F values of the compounds are reported in Table I.

TABLE I

R_F VALUES FOR DIPYRONE, **I**, **II** AND **III**

Compound	R_F	R_F*	R_F†
Dipyrone	0.18	0.54	1
I	0.59	1.78	3.27
II	0.52	1.57	2.88
III	0.44	1.33	2.44

* R_F values relative to theophylline (internal standard).
† R_F values relative to dipyrone.

The chromatogram of one of the standard solutions is shown in Fig. 2.

Table I and Fig. 2 show the good separation of dipyrone from its degradation products. The resolution (R) was calculated using the equation

$$R = \frac{2\,(d_2 - d_1)}{W_1 + W_2}$$

where $d_2 - d_1$ is the distance between the components measured at the peak maximum and W_2 and W_1 are the peak widths. The lowest resolution, between **I** and **II**, is better than 0.8.

The peak skew was evaluated using the asymmetry coefficient A_s:

$$A_s = \frac{b}{a}$$

where b is the distance after the peak maximum and a the distance before the peak maximum, both a and b being measured at 10% of the total peak height. The asymmetry coefficient was 1.6 for dipyrone, 1.2 for theophylline, 1.2 for **I**, 1.1 for **II** and 1.7 for **III**.

The resolution and the asymmetry coefficients we found should be suitable for the quantitative determination of each compound.

A calibration graph was plotted of the peak-area ratio of the solute to the internal standard against the concentration of dipyrone, **I**, **II** and **III**. The calibration graph was found to be rectilinear in all instances. The coefficients of the linear regression analysis calculated from six replicate assays were better than 0.996 for each compound. The calibration graphs passed through the origin, except for **III** (this result could be associated with the weak absorbance of this compound on the plate).

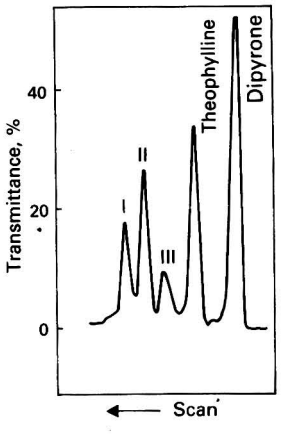

Fig. 2. Chromatogram of a standard solution: 1.05 mg ml^{-1} of dipyrone; 0.35 mg ml^{-1} of **I**; 0.43 mg ml^{-1} of **II**; 0.50 mg ml^{-1} of **III**; and 0.16 mg ml^{-1} of theophylline. Reflectometric measurements at 273 nm; scanning speed, 50 mm min^{-1}; and chart paper speed, 30 mm min^{-1}.

Detection Limits

These were evaluated by the amount of sample that yields a detector response equal to twice the detector noise, and were 0.04 μg for dipyrone, 0.03 μg for **I** and **II** and 0.1 μg for **III**.

Repeatability

This was checked with five identical samples spotted on the same plate. Results are given in Table II.

TABLE II

REPEATABILITY DATA FOR DIPYRONE AND DEGRADATION PRODUCTS

Compound	Standard deviation, $\sigma_{n-1}/\mu g$	Average peak-area ratio of solute to internal standard, R	Relative standard deviation, $\sigma_{n-1/R}$, %
Dipyrone ..	0.0172	1.608	1.07
I	0.0263	0.817	3.21
II	0.0167	1.069	1.55
III	0.0196	0.467	4.19

Recovery Studies

The recovery studies on dipyrone, **I**, **II** and **III** were carried out on synthetic tablets and injections of dipyrone with and without added amounts of **I**, **II** and **III**. Average results of duplicate assays on tablets and injections are listed in Table III. Satisfactory recoveries of dipyrone, **I**, **II** and **III** were found in both of the pharmaceutical formulations, and the accuracy of the method therefore supports the proposed procedure.

TABLE III

RECOVERY DATA FROM SYNTHETIC FORMULATION MIXTURES

Preparation		Amount added/ mg	Amount found/ mg	Relative error, %
Tablets—				
Placebo + dipyrone		500.00	504.25	+0.8
Placebo +	dipyrone	500.00	501.00	+0.2
	I	2.50	2.45	−2.0
	II	2.50	2.49	−1.0
	III	2.50	2.58	+0.5
Placebo +	dipyrone	500.00	492.25	−1.5
	I	7.50	6.90	−6.5
	II	7.50	7.49	0.0
	III	7.50	7.89	+1.5
Injections—				
Placebo + dipyrone		2500.00	2560.00	+2.41
Placebo +	dipyrone	2500.00	2440.00	−2.4
	I	25.00	27.45	+6.0
	II	25.00	25.10	+0.4
	III	25.00	25.10	+0.4
Placebo +	dipyrone	2500.00	2377.00	−4.8
	I	75.00	77.48	+3.3
	II	75.00	72.66	−3.1
	III	75.00	75.26	+0.3

A placebo prepared for tablets and injections showed that there was no interference from the excipients.

For dipyrone and phenylbutazone, both derivatives of pyrazole, a possible oxidation *in situ* on the silica plate may be expected as suggested by Macek.[8] For phenylbutazone, qualitative and quantitative thin-layer chromatography could not be carried out without a special procedure.[9] When the methanolic mixed standard solutions of dipyrone, **I**, **II** and **III** were applied on the silica plate, no oxidation was noted.

To determine trace amounts (about 0.1 μg) of degradation products in the test solutions high loadings (20–60 μg) of dipyrone have to be applied. With a 40-μg loading of dipyrone, no degradation was noted when development was rapid, but trace amounts of **I**, which is the first oxidation compound, may be detected (always less than 0.5% with respect to dipyrone) when the time of analysis is extended. To prevent this uncertainty a blank determination, corresponding to an equal loading of reference dipyrone, has to be applied simultaneously with the test loading.

Because of the concentration range of dipyrone involved in the standard solutions, no blank is required.

The described procedure was applied to detect and quantify dipyrone and its eventual degradation products in commercial tablets and injections. The dipyrone content (average of duplicate assays) was found to be 494.0 mg (98.8% of the labelled content) in tablets and 2375.25 mg (95.01% of the labelled content) in injections. The amount of **I** was 2.5 mg in tablets (0.5% with respect to the theoretical dipyrone content) and 50.0 mg in injections (2.0% with respect to the theoretical dipyrone content); **II** and **III** were not detected in either formulation.

The thin-layer chromatographic procedure, applied to check the stability of commercial formulations stored at ambient temperature, showed that dipyrone does not undergo any significant degradation. As reported in the literature,[7,10] **I** is detected in both pharmaceutical formulations; the concentration of **I** is higher in injections than in tablets probably because of hydrolysis. Compound **II** was not detected within the limit of detection, in contrast to the data of Pellerin and Letavernier.[1] We presume that the detection of **II** would be related to the decomposition of **I**, which may occur when the dipyrone solution was injected into the gas chromatograph. Thin-layer chromatography can be performed without this drawback.

Conclusions

The proposed thin-layer chromatographic procedure can be easily used on a routine basis to check the stability of dipyrone in drugs; the use of theophylline as an internal standard allows an accurate determination of dipyrone and three major degradation products in pharmaceutical formulations.

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Stability Indicating Assay for Dipyrone

Part II.* Separation and Quantitative Determination of Dipyrone and Its Degradation Products by High-performance Liquid Chromatography

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A rapid, sensitive and reproducible procedure for separation and quantification of dipyrone and three of the degradation products is proposed, using high-performance liquid chromatography on a reversed-phase column and ultraviolet detection. The method is about ten times more sensitive (detection limit 3–5 ng) than thin-layer chromatography and requires only a 12-min elution time. This method has been applied to the analysis of tablets and injections.

Keywords: *Dipyrone determination*; *degradation products determination*; *high-performance liquid chromatography*; *reversed-phase chromatography*

In the preceding paper,[1] we discussed the determination of sodium *N*-(1,5-dimethyl-3-oxo-2-phenylpyrazolin-4-yl)-*N*-methylaminomethanesulphonate monohydrate [dipyrone, metamizol, Novalgine (Hoechst)] and we have shown that chromatographic methods are suitable for the determination of dipyrone and its degradation products. Tamura *et al.*[2] have used high-performance liquid chromatography to perform a pharmacokinetic study of dipyrone, but no information was given on the accuracy, linearity, repeatability and the detection limit of the procedure, and the resolution of the peaks did not seem to be sufficient to detect dipyrone in the presence of trace amounts of the degradation compounds. In this paper a rapid high-performance liquid chromatographic procedure on a reversed phase is proposed for separating and quantifying dipyrone and three major degradation products: 4-methylaminoantipyrine (**I**), 4-aminoantipyrine (**II**) and 4-hydroxyantipyrine (**III**) (for structures of **I**, **II** and **III** see Part I[1]). Repeatability and accuracy are tested and the applications of the method are given. The results are compared with those obtained from the thin-layer chromatographic method given in Part I.

Experimental

Apparatus

A high-performance liquid chromatograph (Spectra-Physics, SP 8000) equipped with a variable-wavelength ultraviolet detector (Schoeffel, Model SF 770) and a 10-μl automatic loop injection system was used. A reversed-phase column (10-μm RP-18, 25 cm long and 4.6 mm i.d., Merck) and a home-made pre-column (10-μm RP-18 stationary phase, 5 cm long) were used.

Reagents and Materials

Triethylamine and methanol were of analytical-reagent grade; distilled water was filtered through a 0.45-μm filter (Millipore). Dipyrone, **I**, **II** and **III** were gifts from Hoechst Laboratories and were used as received. Phenacetin was of analytical-reagent grade. Tablets (500 mg of dipyrone per tablet) and injections (2.5 g of dipyrone per 5 ml of solution) were commercial formulations (Hoechst Laboratories).

Standard solutions

A mixed stock standard solution in methanol was prepared, which was 120 μg ml^{-1} in each of dipyrone, **I**, **II** and **III**. This solution was suitably diluted with methanol to give a concentration range from 3 to 12 μg ml^{-1} of each compound, then diluted with an equal

* For Part I of this series, see p. 61.

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volume of internal standard solution (phenacetin, 2 μg ml^{-1} in methanol). The final concentration range of the standard solutions was from 1.5 to 6.0 μg ml^{-1} for each compound.

The mobile phase [methanol - water - triethylamine (50 + 50 + 0.025)] cannot be used as the solvent because of the instability of the standard and test solutions in this mixture.

Test solutions

Test solutions were obtained from tablets and injections by the procedure described in Part I. The solutions were suitably diluted with methanol and internal standard solution (phenacetin in methanol) was added to compare with the calibration graph.

Chromatography

A 10-μl aliquot of each standard solution and 10 μl of test solution were injected under the following isocratic conditions: mobile phase, methanol - water - triethylamine (50 + 50 + 0.025); flow-rate, 0.3 ml min^{-1}; pressure, 57 $\pm$ 1 bar; chart recorder speed, 0.5 cm min^{-1}; and detector sensitivity, 0.04 a.u.f.s. The chromatograms were recorded at 228, 244 and 254 nm.

Results and Discussion

The wavelengths of maximum absorbance in the mobile phase are 228 nm for dipyrone and 244 nm for **I**, **II** and **III**. We recorded the chromatograms at these maxima as well as at 254 nm, because many chromatographic systems are equipped with a fixed-wavelength detector set at 254 nm.

A specimen chromatogram of a standard solution is shown in Fig. 1.

Table I lists the retention times of each compound.

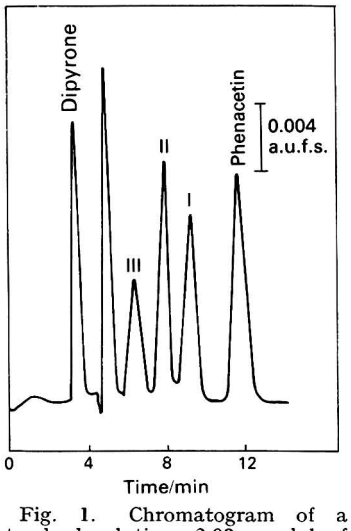

Fig. 1. Chromatogram of a standard solution: 2.93 μg ml^{-1} of dipyrone; 2.85 μg ml^{-1} of **I**; 3.03 μg ml^{-1} of **II**; 3.93 μg ml^{-1} of **III**; and 1.09 μg ml^{-1} of phenacetin. Mobile phase, methanol - water - triethylamine (50 + 50 + 0.025); flow-rate, 0.3 ml min^{-1}; pressure, 57 bar; detector sensitivity, 0.04 a.u.f.s.; chart recorder speed, 0.5 cm min^{-1}; and wavelength (λ), 244 nm.

TABLE I

RETENTION TIMES FOR DIPYRONE, **I**, **II** AND **III**

Compound	Retention time/min	Retention time relative to phenacetin*/min	Retention time relative to dipyrone/min
Dipyrone	3.26	0.27	1.00
I	8.83	0.74	2.70
II	7.66	0.64	2.34
III	5.58	0.47	1.71
Phenacetin	11.83	1.00	3.62

* Internal standard.

Fig. 1 and Table I show the good separation of each compound. This satisfactory resolution (always better than 0.96) should allow the procedure to be used quantitatively.

Calibration graphs were plotted of the peak-area ratios of the solutes to the internal standard against the concentrations of dipyrone, **I**, **II** and **III**. The calibration graphs were found to be rectilinear at 228, 244 and 254 nm and passed through the origin in all instances, when the peak areas and the peak heights were used. The coefficients of the linear regression analysis were better than 0.998 for each compound. The detection limits (evaluated by the amount of sample that yields a detector response equal to twice the detector noise) was 0.004 μg at 228 nm for dipyrone, 0.003 μg at 244 nm for **I** and **II** and 0.010 μg at 244 nm for **III**. At a wavelength of 254 nm (the best fit for a simultaneous determination of the four compounds), the detection limits for dipyrone, **I**, **II** and **III** do not differ appreciably from those observed at their individual wavelengths of maximum absorbance.

The repeatability, checked by five replicates, is given in Table II.

TABLE II

REPEATABILITY DATA FOR DIPYRONE AND DEGRADATION PRODUCTS

Compound	Standard deviation, $\sigma_{n-1}/\mu g$	Average peak-area ratio of solute to internal standard, R	Relative standard deviation, σ_{n-1}/R, %
Dipyrone	0.00263	0.515	0.51
I	0.00198	0.67	0.30
II	0.01142	0.737	1.55
III	0.02778	0.902	3.08

Recovery studies on dipyrone, **I**, **II** and **III** were carried out on laboratory prepared tablets and injections of dipyrone with and without added amounts of **I**, **II** and **III**. Average results of duplicate assays on tablets and injections are listed in Table III.

Inert interference was checked with the placebo of the tablets and injections. No interference was noted.

Satisfactory recoveries of dipyrone, **I**, **II** and **III** were found and demonstrate the accuracy of the proposed high-performance liquid chromatographic procedure.

The proposed procedure is faster (elution time 12 min), easier and ten times more sensitive than thin-layer chromatography. The repeatability obtained is better than that for thin-layer chromatography for all the compounds, especially for dipyrone and **I**, which is the first step of the hydrolytic process of decomposition and is the major degradation product.

This method was applied to the determination of dipyrone, **I**, **II** and **III** in the pharmaceutical formulations analysed in Part I; the tablets and injections had the same reference numbers as those used previously.

The dipyrone content was found to be 489.5 mg (97.9% of the labelled strength) in tablets and 2383.8 mg (95.35% of the labelled strength) in injections. The amount of **I** was found to be 2.4 mg in tablets (0.48% with respect to theoretical dipyrone content) and 50.75 mg in injections (2.03% with respect to theoretical dipyrone content). Compounds **II** and **III** were not detected.

These data confirm the results found using the thin-layer chromatographic procedure.

TABLE III

RECOVERY DATA FROM SYNTHETIC FORMULATION MIXTURES

Preparation	Amount added/ mg	Amount found/ mg	Relative error, %
Tablets—			
Placebo + dipyrone	500.00	502.18	+0.4
Placebo + dipyrone	500.00	496.00	−0.8
Placebo + I	2.50	2.51	+0.4
Placebo + II	2.50	2.50	0.0
Placebo + III	2.50	2.50	0.0
Placebo + dipyrone	500.00	506.00	+1.2
Placebo + I	7.50	7.25	−0.3
Placebo + II	7.50	7.69	+2.6
Placebo + III	7.50	7.35	−1.9
Injections—			
Placebo + dipyrone	2500.00	2482.50	−0.7
Placebo + dipyrone	2500.00	2422.00	−3.1
Placebo + I	25.00	25.42	+1.6
Placebo + II	25.00	25.23	+0.9
Placebo + III	25.00	25.94	+3.7
Placebo + dipyrone	2500.00	2451.40	−1.9
Placebo + I	75.00	75.44	+0.5
Placebo + II	75.00	78.38	+4.5
Placebo + III	75.00	77.09	+2.8

Conclusions

As demonstrated, this method can be used as a stability indicating assay for pharmaceutical formulations; because of the selectivity of the reversed phase, the detection limits and the rapidity of the procedure, it can be easily applied to the determination of dipyrone, **I**, **II** and **III** in drugs.

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

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Determination of the Active Ingredient Content of Technical and Formulated Dinobuton, Dinoseb, Dinoterb and DNOC by High-performance Liquid Chromatography, Spectrophotometry and Gas - Liquid Chromatography

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A reversed-phase high-performance liquid chromatographic (HPLC) method is described for the determination of the active ingredient content of technical and formulated dinobuton, dinoseb, dinoterb and DNOC. The results obtained by HPLC are compared with those obtained using gas - liquid chromatography for dinoterb and spectrophotometry for the other compounds.

Keywords: *Dinobuton, dinoseb, dinoterb, DNOC analysis; formulation analysis; high-performance liquid chromatography*

The dinitrophenol pesticides 2-*sec*-butyl-4,6-dinitrophenol isopropyl carbonate (dinobuton), 2-*sec*-butyl-4 6-dinitrophenol (dinoseb), 2-*tert*-butyl-4,6-dinitrophenol (dinoterb) and 4,6-dinitro-*o*-cresol (DNOC) belong to a group of compounds that have been extensively used as herbicides, fungicides, acaricides and insecticides.

Early methods proposed for the determination of the active ingredient content of dinitrophenol pesticides were based on gravimetric determination after a preliminary extraction (DNOC),[1,2] or determination of the amount of titanium(III) salt required to reduce available nitro groups (*e.g.*, dinobuton).[3] Both methods are non-specific and not suitable for modern formulations. Bouwmann and Westenberg[4] reported a method for the analysis of dinoseb that involved the separation of impurities on a cellulose column, followed by spectrophotometric determination; a modified version of this was later published by the Collaborative International Pesticides Analytical Council.[5] This method was not, however, wholly satisfactory and subsequent methods for the analysis of DNOC, dinoseb and dinobuton[6] have been based on a method described by Lynch,[7] which was originally applied to dinobuton. This method involves the separation of impurities on a column of neutral alumina followed by spectrophotometric determination.

For dinoseb and DNOC these impurities are eluted from the column with chloroform, followed by acetonitrile - propan-2-ol. Finally, the dinitro pesticide is eluted as its butylamine salt. The analysis of dinobuton involves a preliminary selective adsorption of free dinoseb on a column of neutral alumina (grade V), the dinobuton solution eluted from this column subsequently being de-esterified to dinoseb on a column of neutral alumina (grade 1). Impurities are then eluted as for dinoseb analysis and the active ingredient content is ultimately derived from spectrophotometric analysis of the dinoseb butylamine salt. These methods are relatively straightforward when applied to technical samples and oil-based formulations, but the analysis of ammonium and alkanolamine salts requires an additional solvent extraction stage. Further, the successful use of the method appears to be wholly restricted to one manufacturer's alumina.[6] The analysis of dinoterb involves methylation of the sample with diazomethane prior to gas - liquid chromatographic (GLC) analysis with flame-ionisation detection.[8]

Reversed-phase high-performance liquid chromatography (HPLC) is a useful alternative to the above methods, and the method proposed here was found to give results comparable to those obtained by spectrophotometry and GLC.

Roseboom and Herbold[9] and Roseboom *et al.*[10] have reported reversed-phase HPLC procedures that involve the use of ion-pairing agents for the determination of nitrophenol residues. However, these papers have appeared only recently hence no consideration could be given to them in setting up the method reported.

Experimental

Apparatus

A Waters Associates, Model 6000A, constant-volume solvent-delivery system was used. A variable-wavelength ultraviolet monitor (Cecil Instruments, Model CE212), fitted with a 5-μl flow cell (1-cm light path), was used as a detector. The monitoring wavelengths were 240 nm for dinobuton, dinoseb and DNOC and 267 nm for dinoterb. The column used was a laboratory-prepared 15-cm column (stainless steel, 4.6 mm i.d.) packed with Spherisorb ODS, 5 μm (Phase Separations), coupled to a Rheodyne 7120 syringe-loading sample injector.

Reagents

All reagents were of analytical-reagent grade unless specified.

Acetone.

Dinobuton, dinoseb, dinoterb, DNOC. Analytical reference-grade materials (obtainable from the National Physical Laboratory) were used.

Methanol.

Water, glass distilled.

Tetramethylammonium bromide. Hopkin and Williams material was used.

Mobile phases. Prepare a solution containing tetramethylammonium bromide (9.2 g l^{-1} in methanol - distilled water). Adjust the methanol to water ratio for analysis of each pesticide as follows: 70 + 30 for dinoseb, dinobuton, dinoseb in dinobuton; 50 + 50 for dinoterb; and 30 + 70 for DNOC.

Internal standard solutions

Prepare a solution containing approximately 2 g l^{-1} of acetophenone, propiophenone or butyrophenone in the mobile phase appropriate to the pesticide to be analysed.

Injection of a solution of internal standard alone should produce no additional peaks with retention times close enough to interfere with the measurement of the peak arising from the corresponding dinitrophenol pesticide. Conversely, injection of a solution of dinitrophenol pesticide should give no peak that could interfere with the measurement of the appropriate internal standard peak.

Dinitrophenol pesticide standard solutions

(*i*) *Analysis of active ingredient content.* Prepare solutions of each analytical reference-grade pesticide (1 mg ml^{-1}) in methanol. To 20 ml of a standard solution add the appropriate internal standard as follows: DNOC, 5 ml of acetophenone solution; dinobuton, 10 ml of butyrophenone solution; dinoseb, 5 ml of butyrophenone solution; and dinoterb, 5 ml of propiophenone solution; then dilute to 50 ml with methanol. If necessary, concentrations should be adjusted so that pesticide and internal standard peak heights are approximately equal.

(*ii*) *Analysis of free dinoseb in dinobuton.* Prepare a solution containing analytical reference-grade dinoseb (1 mg ml^{-1}) in methanol and dilute to a concentration of 2 μg ml^{-1} with methanol.

NOTE—The linearity of response for both pesticide and internal standard should be checked over the intended working range before commencing quantitative analysis.

Procedures

Technical and formulated pesticides, except oil-based formulations

Prepare duplicate solutions of sample containing the equivalent of 1 mg ml^{-1} of active ingredient in methanol. To 20 ml of each of these solutions add the appropriate volume of internal standard solution (see preparation of standard solutions) and dilute to 50 ml with methanol. Inject suitable volumes (about 5 μl) of standard and sample solutions on to the HPLC column, using the mobile phase given above, and a flow-rate of 1–2 ml min^{-1}. Bracket duplicate injections of one sample solution with injections of standard solution and determine the average response ratios (peak height of pesticide to peak height of internal standard) for each solution. Compare the response ratios and hence calculate the active ingredient content for the duplicate sample solutions.

Oil-based pesticide formulations

Proceed as above but substitute acetone for methanol to dissolve these formulations; the same internal standard solutions may be used.

Analysis of dinoseb in dinobuton

Prepare duplicate solutions of dinobuton (10 mg ml^{-1}) in methanol and dilute to 0.01 mg ml^{-1} with methanol. Inject a suitable volume for analysis on to the HPLC column, compare the peak height of any dinoseb seen with that of the known standard solution and calculate the free dinoseb content of the sample.

Results and Discussion

Reversed-phase HPLC is a suitable technique for the analysis of dinitrophenolic pesticides provided that an ion-pairing agent (in this instance tetramethylammonium bromide) is incorporated into the mobile phase. Quantitative analysis of active ingredient contents is aided by the use of internal standards and the series of phenones acetophenone, propiophenone and butyrophenone was found to be suitable for this purpose. Further, the addition of small amounts of the ion-pairing agent to sample solutions, converting the nitrophenols into their ion-paired form, was found to improve the repeatability. The internal standard solutions exhibit maximum absorption at 240 nm and at this wavelength the ultraviolet absorption of dinobuton, dinoseb and DNOC does not vary greatly with small changes in wavelength. A similar coincidence does not exist for dinobuton, so 267 nm (the dinobuton absorption maximum) was adopted as the analytical wavelength. Representative chromatograms obtained using the above conditions are shown in Fig. 1. During the course of the work some changes in the relative retention times of dinitrophenols and internal standards were noticed, but these did not affect the day to day analysis or the accuracy of the method.

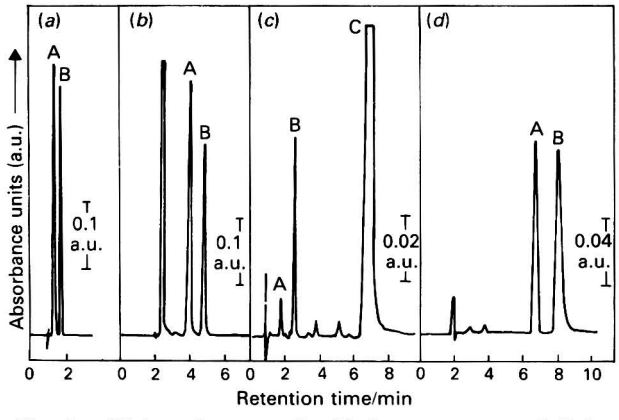

Fig. 1. High-performance liquid chromatograms of dinitrophenol pesticides: (*a*) DNOC ammonium salt [DNOC (A) and acetophenone (B)]; (*b*) dinoseb in oil [dinoseb (A) and butyrophenone (B)]; (*c*) dinoseb in dinobuton [dinoseb (A), butyrophenone (B) and dinobuton (C)]; and (*d*) dinoterb ammonium salt [propiophenone (A) and dinoterb (B)].

The HPLC method was applied to the analysis of a number of batches of technical and formulated dinitrophenol pesticides and the results obtained were compared with those from spectrophotometric (or GLC) analysis performed at the same time. The results given in Table I are from duplicate sub-samples of pesticide, each result being an average of duplicate injections on to the HPLC column. Statistical analysis performed on these dinoseb and DNOC results showed no significant difference between the HPLC and spectrophotometric methods (at the 95% confidence level). The dinoseb HPLC results gave a repeatability standard deviation of 0.46% *m/m*, the figure for DNOC being 0.14% *m/m*. There were

TABLE I

COMPARISON OF TECHNIQUES FOR THE DETERMINATION OF DINITRO PESTICIDES

Sample type	Dinoseb content, % *m/m* HPLC	Dinoseb content, % *m/m* Spectrophotometry
Dinoseb technical 1	98.2, 99.5	98.5, 98.3
Dinoseb technical 2	96.9, 98.3	97.9, 97.7
Dinoseb ammonium salt	18.5, 18.6	18.1, 18.2
Dinoseb alkanolamine salt 1	18.3, 18.1	17.4, 17.9
Dinoseb alkanolamine salt 2	17.1, 17.1	17.2, 17.7
Dinoseb in oil 1	10.5, 10.5	10.6, 10.4
Dinoseb in oil 2	10.3, 10.2	9.9, 10.8
Dinoseb in oil 3	10.0, 10.1	10.2, 10.3
Dinoseb in oil 4	10.3, 10.2	10.3, 10.3
Dinobuton technical 1	0.01, 0.01	0.01, 0.01
Dinobuton technical 2	0.02, 0.02	0.05, 0.05
Dinobuton technical 3	0.03, 0.02	0.02, 0.02

	DNOC content, % *m/m* HPLC	DNOC content, % *m/m* Spectrophotometry
DNOC technical 1	96.6, 96.7	97.1, 97.7
DNOC technical 2	98.4, 98.5	98.1, 98.7
DNOC ammonium salt 1	46.4, 46.1	47.2, 47.4
DNOC ammonium salt 2	39.5, 39.8	38.9, 38.7
DNOC in oil	1.8, 1.8	1.9, 2.0

	Dinobuton content, % *m/m* HPLC	Dinobuton content, % *m/m* Spectrophotometry
Dinobuton technical 1	100.3, 100.1	100.1
Dinobuton technical 2	99.1, 99.7	99.9
Dinobuton technical 3	98.3, 98.2	98.7, 98.5

	Dinoterb content, % *m/m* HPLC	Dinoterb content, % *m/m* GLC
Dinoterb technical	92.1, 92.2	89.4, 90.6
Dinoterb ammonium salt	23.2, 23.6	23.4, 23.2

insufficient data for a statistical analysis of the results from the dinobuton and dinoterb samples. One analysis in which a discrepancy was noted was that of the dinoseb content of dinobuton technical 2. The HPLC result on the sample was confirmed by an HPLC analysis of the dinoseb fraction obtained for spectrophotometric analysis, and it is thought that some interference occurs in the spectrophotometric analysis of this particular sample that gives rise to the higher result.

The HPLC method therefore offers a convenient, specific means of analysing dinitrophenol pesticides and can be applied to a wide range of technical and formulated materials. It has provided a check on the accuracy of the spectrophotometric method that was being tested collaboratively by the Dinitro Pesticides Panel of the Pesticides Analysis Advisory Committee and offers a useful alternative procedure.

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Determination of Acid and Hydroxybenzonitrile Herbicide Residues in Soil by Gas - Liquid Chromatography after Ion-pair Alkylation

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The herbicides are extracted from soil with saturated calcium hydroxide solution. After clean-up the residues are ethylated using iodoethane and tetrabutylammonium hydrogen sulphate as counter ion. Liquid - liquid partition and the use of a macroreticular resin column were compared as clean-up steps and the reaction conditions for optimum yield of ethyl ester or ether were evaluated. Recoveries in excess of 80% were achieved for 2,4-D, dicamba, 3,6-dichloropicolinic acid, dichlorprop, picloram, 2,4,5-T, fenoprop, 2,3,6-TBA, bromoxynil and ioxynil.

Keywords: *Herbicide determination*; *soil*; *gas - liquid chromatography*; *alkylation*

Acid herbicides such as phenoxyalkanoic and benzoic acids and the hydroxybenzonitrile herbicides are widely used in agriculture and are often formulated as mixtures. They may also be mixed in the spray tank or used in sequence, so it is likely that residues of more than one of these compounds may be present in the soil.

Many methods have been reported for the extraction of these compounds from soil. Khan[1] used acidified acetone followed by methylation with diazomethane for the simultaneous determination of 2,4-D, dicamba and mecoprop residues, as did Bache and Lisk[2] for ioxynil. Abbott *et al.*[3] developed a method for MCPA, MCPB, 2,4-D, dichlorprop and 2,4,5-T in which dilute sulphuric acid and diethyl ether were used for extraction. Work in this laboratory has shown that diethyl ether - chloroform - acetic acid is a suitable extractant for 2,4,5-T, 2,4-D, dichlorprop and dicamba[4] and saturated calcium hydroxide solution is efficient for picloram[5] and 3,6-dichloropicolinic acid.[6] In the work reported here calcium hydroxide solution was chosen as the extractant because it is the cheapest and least toxic of the chemicals used.

All of the methods discussed use liquid - liquid partition for clean-up. However, Smith and Hayden[7] and Johnson *et al.*[8] have shown that the macroreticular resin XAD-2 is an efficient adsorber of 2,4-D from air and aqueous solution, so the XAD-2 method was compared with liquid - liquid partition.

After extraction and clean-up the acid and hydroxybenzonitrile herbicides are either too polar or insufficiently volatile to be determined directly by gas - liquid chromatography, and a suitable derivative must therefore be prepared. Amongst the methods reported are the preparation of methyl esters using diazomethane,[1,2,9,10] boron trichloride - methanol reagent[11] or iodomethane with alkali metal carbonate catalysis under anhydrous conditions.[12] Other esters have been prepared by reaction of herbicide acids with the appropriate alcohol.[6,13,14] Some workers have prepared esters with enhanced electron-capturing properties to improve detection limits, such as straight-chain halogenated esters[15] or strongly halogenated aromatic esters such as pentafluorobenzyl.[16–20] Various silyl esters have also been prepared.[21,22]

Each of these methods has some disadvantage. Diazomethane is toxic, carcinogenic and explosive. Boron trichloride - methanol will not alkylate hydroxybenzonitriles. The silyl derivatives tend to condense in the electron-capture detector and decrease its sensitivity. The yield of ester from the acid-catalysed reaction of acid and alcohol, although reproducible, is usually of the order of only 80%. Pentafluorobenzylation and alkali metal carbonate-catalysed alkylation require anhydrous conditions. The use of halogenated reagents also has the disadvantage of transferring electron-capturing properties to impurities or co-extractants that may interfere with measurement of the herbicide.

Extractive or ion-pair alkylation is an alternative method that has been reported for the derivatisation of many pharmaceutical products.[23–26] This paper describes the application

of the procedure to herbicides. The ethyl ester was selected as previous work suggested that the methyl ester was unlikely to be resolved from co-extractants.[6,13]

Experimental

Reagents

Tetrabutylammonium hydrogen sulphate (TBHS) was obtained from Sigma London Chemical Co. (Poole, Dorset). All other chemicals were purchased from BDH Chemicals (Poole, Dorset). All solvents were distilled in glass before use.

Herbicides

Herbicides were either obtained pure from the manufacturer or recrystallised from the commercial product. The acid herbicides 2,4-D (2,4-dichlorophenoxyacetic acid), dicamba (3,6-dichloro-2-methoxybenzoic acid), 3,6-dichloropicolinic acid, dichlorprop [(±)-2-(2,4-dichlorophenoxy)propionic acid], picloram (4-amino-3,5,6-trichloropicolinic acid), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), fenoprop [(±)-2,4,5-trichlorophenoxypropionic acid] and 2,3,6-TBA (2,3,6-trichlorobenzoic acid) were used. The hydroxybenzonitriles were bromoxynil (3,5-dibromohydroxybenzonitrile) and ioxynil (4-hydroxy-3,5-diidobenzonitrile).

Soils

Soils from two locations were used for the fortification studies. Table I gives some details of their compositions. Each soil was air dried and passed through a 3-mm sieve prior to fortification. Aqueous solutions of the herbicides were prepared from methanolic solutions containing 1 mg ml^{-1} of herbicide so that, when sufficient solution was added to the soil to achieve 75% water-holding capacity, the concentration of herbicide in soil was 1.0 or 0.1 $\mu g\ g^{-1}$. Soils were fortified in triplicate and allowed to stand in a cold room at 4 °C for 48 h before extraction.

TABLE I

SOME PROPERTIES OF THE SOILS USED

Property	Soil 1	Soil 2
Organic carbon, %	1.6	4.1
pH	7.0	5.1
Clay, %	16	16
Silt, %	11	16
Sand, %	73	68
Water-holding capacity, %	16.6	27.0

Extraction

Approximately 1 g of calcium hydroxide was added to a 10-g sample of the fortified soil and the mixture extracted with 50 ml of de-ionised water by shaking on a wrist-action shaker for 1 h. The resulting soil slurry was filtered through a Whatman No. 42 filter-paper. Each fortified sample was analysed twice.

Clean-up

Liquid - liquid partition

A 10-ml volume of the extract was transferred into a separating funnel, acidified to a pH of less than 2 and extracted with two 25-ml portions of dichloromethane. The dichloromethane layers were combined and concentrated to about 1 ml under reduced pressure whilst warming in a water-bath at 45 °C. The remaining solvent was removed with a gentle stream of dried air.

XAD-2

XAD-2 resin was washed with distilled water to remove salts and fine beads of less than 20 mesh were decanted. The resin was refluxed for 8 h with methanol, filtered through a

Buchner funnel and washed with methanol to remove extraneous organic matter. A 300 × 15 mm i.d. glass chromatographic column fitted with a glass frit (porosity 1) was filled to a depth of 20 mm with resin. A 50-ml volume of de-ionised water was run through the column at a rate of 5 ml min^{-1} and the flow was stopped when the liquid level was just above the level of the resin. A 10-ml volume of the aqueous extract was run through the column at 2 ml min^{-1} and allowed to drain. The herbicides were eluted with 25 ml of methanol, then a further 10 ml of methanol, at 2 ml min^{-1}. The methanol eluate was concentrated to about 1 ml under reduced pressure whilst warming in a water-bath at 45 °C and the remaining solvent was removed with a gentle stream of dried air.

Preparation of Standards

Preparation of ethyl esters

Approximately 1 g of pure acid herbicide was refluxed in 50 ml of ethanol for 5 h using 1 ml of concentrated sulphuric acid as catalyst. The reaction mixture was allowed to cool and diluted with 200 ml of distilled water. Approximately 3 g of sodium carbonate were added to convert any underivatised herbicide into the water-soluble sodium salt. On dilution, except for picloram, the ester separated from the solution was an oily layer at the bottom of the reaction flask. The excess of solution was decanted and the ester was washed twice with distilled water, then dried over anhydrous sodium sulphate. Picloram produced a solid ester, which was filtered through a Whatman No. 1 filter-paper, rinsed with water and dried at 105 °C.

Preparation of ethyl ethers

Approximately 1 g of hydroxybenzonitrile herbicide was dissolved in 25 ml of 1.0 M sodium hydroxide solution and about 1 g of TBHS and 20 ml of iodoethane were added. The reaction mixture was shaken for 5 h on a wrist-action shaker, then transferred into a separating funnel. The lower organic layer was run into a conical flask and concentrated by heating in a water-bath at 60 °C until no further reduction in volume took place. The resulting viscous liquid, which became solid on cooling, was recrystallised from ethanol to yield the ether.

Standard solutions

A 10-mg amount of the prepared ester or ether was dissolved in 10 ml of methanol. Aliquots of these solutions were diluted with hexane to give solutions containing 0.2, 0.1 or 0.05 ng μl^{-1}.

Chromatography

A Pye GCD chromatograph fitted with a nickel-63 electron-capture detector and a 1.5 m × 4 mm i.d. glass column was used. The conditions employed were as follows: column packing, 5% SE-30 on Chromosorb W HP (80–100 mesh); carrier gas, oxygen-free nitrogen at a flow-rate of 60 ml min^{-1}; temperatures, column 180 °C, injector 210 °C and detector 300 °C; attenuation, 1024; detector current, 5×10^{-10} A. Injections of 5 μl were made using a Pye S8 Autojector and a Perkin-Elmer Sigma 10 Chromatography Data Station was used to measure peak area and retention time.

Ethylation of Residues

Preliminary experiments

The preliminary experiments designed to optimise reaction conditions were carried out with 2,4-D. In each instance 5 μg of 2,4-D were shaken for 1 h with 1 ml of a 10% solution of iodoethane in dichloromethane and 1 ml of the appropriate TBHS solution. A 100-μl volume of the lower organic layer was transferred into a test-tube and the solvent allowed to evaporate. The residue was dissolved in 5 ml of hexane and the yield of ester was determined chromatographically by comparison with the standard solutions.

To assess the effect of counter-ion concentration on the yield of ester, solutions of TBHS at 0.05, 0.1, 0.2 and 0.4 M concentrations were prepared in both 0.1 and 0.5 M sodium hydroxide solution. The effect of pH on yield was determined by preparing a 0.1 M TBHS solution and adding small amounts of sodium hydroxide to give a range of pH between 1.5 and 12.6.

The reaction time for maximum yield was determined using a 0.1 M solution of TBHS in 0.5 M sodium hydroxide solution. Samples were shaken for 15, 30 and 45 min and 1, 1.5 and 2 h. A graph of yield against shaking time was constructed and the time to maximum yield was obtained by interpolation. This procedure was repeated for each of the herbicides studied.

Results and discussion of preliminary experiments

Gyllenhaal *et al.*[24] used a 0.1 M solution of TBHS in carbonate buffer of pH 10 for the methylation of sulphonamide drugs. Hartvig and Fagerlund[26] used a 0.1 M solution of TBHS in a phosphate buffer of pH 11 for methylation of 8-hydroxyquinolines. In the preliminary experiments TBHS concentrations of about 0.1 M were chosen. The yield of 2,4-D ethyl ester was found to increase with increasing concentration of TBHS in 0.5 M sodium hydroxide solution but decreased with increasing concentration of TBHS in 0.1 M sodium hydroxide solution. These apparently contradictory results were thought to be due to the low pH in the less concentrated sodium hydroxide solutions. Fig. 1 shows the variation in yield of 2,4-D ethyl ester after a reaction time of 1 h at different pH values. A pH of 12.6 corresponded to a concentration of 0.1 M TBHS in 0.1 M sodium hydroxide solution, although the yield of 95.9% was less than that achieved in the previous experiment in which 0.5 M TBHS in 0.5 M sodium hydroxide solution gave a yield of 100%. A 0.1 M solution of TBHS in 0.5 M sodium hydroxide solution was chosen for the recommended procedure as the background response also increased with increasing TBHS concentration. The background signal was probably due to organic impurities in the reagents because it could be reduced by extracting the TBHS in sodium hydroxide solution with dichloromethane.

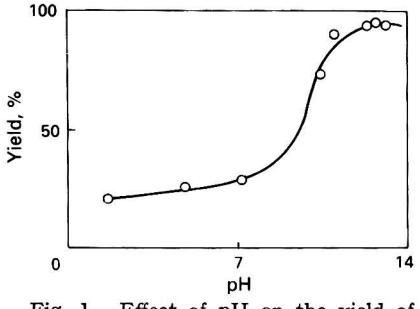

Fig. 1. Effect of pH on the yield of 2,4-D ethyl ester.

Table II gives the yields, times to maximum yield, retention times and least detectable amounts of the herbicide esters or ethers prepared using the conditions subsequently adopted for the recommended procedure. The yields given are the means of five determinations for each herbicide and in no instance was the standard error of the mean greater than 2%. The least detectable amount is based on a peak giving a response of twice the background signal.

Recommended procedure

Dissolve the cleaned-up extract in 1 ml of a 10% solution of iodoethane in dichloromethane and add 1 ml of a 0.1 M solution of TBHS in 0.5 M sodium hydroxide solution. Shake the mixture for 1 h on a wrist-action shaker. Allow the organic layer to settle in the bottom of the reaction flask. Take a 100-μl volume of the lower organic layer, allow the solvent to evaporate and dissolve the residue in hexane for chromatography.

TABLE II

YIELDS, TIMES TO MAXIMUM YIELD, RETENTION TIMES AND LEAST DETECTABLE AMOUNTS OF ALKYLATION PRODUCT

Herbicide	Yield, %	Time to maximum yield/min	Retention time/min	Least detectable amount/ng
2,4-D	95.9	37	3.75	0.05
Dicamba	99.0	56	3.04	0.005
3,6-Dichloropicolinic acid	94.5	60	2.16	0.01
Dichlorprop	100	60	5.52	0.05
Picloram	99.6	15	14.57	0.002
2,4,5-T	90.2	45	5.76	0.005
Fenoprop	95.9	17	6.40	0.005
2,3,6-TBA	92.8	20	3.03	0.005
Bromoxynil	98.8	45	3.88	0.05
Ioxynil	91.2	15	8.52	0.05

Results and Discussion

Table III gives the recoveries of the herbicides from soil using calcium hydroxide extraction and the recommended ethylation procedure. Clearly the recovery is over 80% for all of the herbicides and adequate at both levels of fortification using the liquid - liquid partition clean-up. However, if XAD-2 is used for the clean-up, the results are variable with the 1.0 $\mu g\ g^{-1}$ treatment and with the 0.1 $\mu g\ g^{-1}$ treatment the background response masked any herbicide peak that may have been present. The method using liquid - liquid partition is obviously suitable as a multi-residue method but, as the retention times in Table II show, 2,3,6-TBA is not resolved from dicamba and the bromoxynil and 2,4-D derivatives have very similar retention times. When it is suspected that both 2,3,6-TBA and dicamba or both 2,4-D and bromoxynil are present in a sample a confirmatory test would be needed. A suitable method is transesterification,[27] in which 2,3,6-TBA and bromoxynil remain unchanged whereas dicamba and 2,4-D are transesterified to either propyl or butyl esters, depending on the alcohol used.

TABLE III

RECOVERY OF HERBICIDES FROM SOIL USING ALTERNATIVE CLEAN-UPS

Herbicide	Treatment/ $\mu g\ g^{-1}$	Mean recovery, %* Partition Soil 1	Partition Soil 2	XAD-2† Soil 1	XAD-2† Soil 2
2,4-D	1.0	92.9(2.0)	90.0(1.96)	76.8(6.7)	56.3(9.6)
	0.1	94.7(2.9)	100.0(<0.1)	— —	— —
Dicamba	1.0	95.3(0.1)	100.0(3.4)	110.4(3.6)	100.4(13.7)
	0.1	95.3(3.2)	97.6(0)	— —	— —
3,6-Dichloropicolinic acid	1.0	96.7(5.4)	91.3(5.2)	66.7(32.2)	21.6(23.1)
	0.1	93.2(5.2)	83.0(4.7)	— —	— —
Dichlorprop	1.0	80.0(2.4)	87.2(2.0)	96.4(3.6)	77.1(7.2)
	0.1	88.6(2.6)	94.0(2.1)	— —	— —
Picloram	1.0	99.2(4.4)	99.2(2.2)	62.7(6.8)	68.1(2.4)
	0.1	97.8(2.2)	89.8(<0.1)	— —	— —
2,4,5-T	1.0	103.8(2.7)	100.0(3.0)	88.9(6.97)	73.5(5.9)
	0.1	95.2(2.5)	98.2(2.9)	— —	— —
Fenoprop	1.0	90.0(1.7)	91.3(1.7)	93.6(5.7)	92.8(15.4)
	0.1	84.7(0.1)	93.4(1.7)	— —	— —
2,3,6-TBA	1.0	92.7(2.0)	96.7(1.8)	89.8(43.4)	48.1(36.4)
	0.1	94.1(2.3)	91.2(1.2)	— —	— —
Bromoxynil	1.0	90.6(3.2)	88.6(3.1)	79.3(6.2)	48.4(13.6)
	0.1	92.3(3.1)	93.4(3.2)	— —	— —
Ioxynil	1.0	98.0(2.9)	81.1(2.3)	51.4(9.7)	38.9(15.0)
	0.1	99.8(7.2)	85.5(6.2)	— —	— —

* Recovery figures are means of six determinations. Figures in parentheses are standard deviations.
† XAD gave insufficient clean-up to measure recoveries at the 0.1 $\mu g\ g^{-1}$ level.

The monochlorinated phenoxyalkanoic acids MCPA, mecoprop and MCPB can also be esterified with iodoethane but are not included in this method as the low sensitivity of the electron-capture detector towards their alkyl esters makes the method unsuitable for the determination of residues of these compounds.

During the development of this method it was found that the background response increased if heat was applied to the evaporation of the solvent after alkylation. This may have been due to esterification of impurities in the reagents but with the hydroxybenzonitrile herbicides it could also have been due to alkaline hydrolysis of the benzonitrile to the corresponding carboxylic acid and alkylation of that acid to its ethyl ester.

The practical limit of determination for these herbicides is between 0.01 and 0.05 $\mu g\ g^{-1}$ depending on the background response from the soil extract.

Conclusion

The method described gives good recoveries from soil of all of the herbicides studied and is suitable as a multi-residue method provided that the limitations of the chromatographic system are recognised. Both of the clean-up methods used are capable of giving good results at the 1 $\mu g\ g^{-1}$ level but XAD-2 is inadequate for smaller amounts of herbicides in soil. This poor clean-up and the higher variability in recovery shows XAD-2 to be unsuitable for residue determinations.

The application of ion-pair alkylation gives accurate, reproducible results and the method is simple and rapid, so it is well suited for routine analysis.

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Determination of Trace Amounts of Nitrite by Derivatisation and Gas Chromatography

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A gas-chromatographic method for the determination of nitrite is described. Nitrite is converted into substituted benzene derivatives by reaction with substituted anilines and hypophosphorous acid, and the resulting benzene derivatives are determined by gas chromatography. The derivatisation yields of nitrite were studied by using anilines with various substituents that have a high response to an electron-capture detector. Nitroanilines give high yields and *m*-nitroaniline is the most suitable derivatisation reagent for determining trace amounts of nitrite. By using *m*-nitroaniline and an electron-capture detector, the detection limit and determination range of nitrite are 0.5 ng ml^{-1} and up to 1.00 μg ml^{-1}, respectively, which are much lower than those of the widely used colorimetric method for the determination of nitrite (detection limit 16 ng ml^{-1}). River water samples containing nitrite were analysed by both this gas-chromatographic method and the colorimetric method.

Keywords: *Nitrite determination*; *trace analysis*; *gas chromatography*; *derivatisation*

The toxicity of nitrite towards humans is due primarily to the possibility that it can cause methaemoglobinaemia in infants and form carcinogenic nitrosamines under certain conditions. The determination of nitrite at trace levels is, therefore, of great interest. Nitrite is usually determined by a colorimetric method,[1] based on the formation of an azo dye produced by diazotisation of sulphanilic acid and subsequent coupling with *N*-(1-naphthyl)ethylenediamine.

Recently, nitrite has also been analysed by chromatographic methods involving derivatisation and several reviews have been published.[2–6] In the method described in our previous papers,[7,8] nitrite was converted into an aryl halide by reaction with a substituted aniline in the presence of a copper(II) halide and the aryl halide was determined by gas chromatography with an electron-capture detector (ECD). Several groups have reported another gas-chromatographic method, based on the oxidation of nitrite to nitrate and nitration of benzene (or a benzene derivative) in the presence of sulphuric acid as catalyst.[9–11] However, this method is non-specific for nitrite and requires vigorous derivatisation conditions. Other methods have been described that involve the formation of triazole derivatives by reaction of nitrite with *o*-phenylenediamine[12,13] or hydralazine.[14,15]

It is well known that nitrite diazotises aromatic amines in acidic media and that the resulting diazonium ions are reduced by hypophosphorous acid to form benzene derivatives.[16,17] The reactions are formulated as follows:

$$NO_2^- + Ar\text{–}NH_2 \xrightarrow{HX} Ar\text{–}N_2^+X^-$$

$$Ar\text{–}N_2^+X^- + H_3PO_2 + H_2O \longrightarrow Ar\text{–}H + H_3PO_3 + HX + N_2$$

This paper describes a method for the determination of nitrite by derivatisation gas chromatography in which trace amounts of nitrite are converted into nitrobenzene by the above reactions, and the resulting nitrobenzene is subsequently determined by gas chromatography with an ECD.

Experimental

Apparatus

The gas chromatographs used were a Shimadzu (Kyoto, Japan) Model GC–4BM equipped with a nickel-63 ECD and a Model GC–3BF equipped with dual flame-ionisation detectors (FID).

In the analysis of nitrite at trace levels, the GC–4BM instrument was used. A stainless-steel column (3 m × 3 mm i.d.) was packed with 5% PEG-HT on 60–80-mesh Uniport HP; this column packing material was purchased from Gasukuro Kogyo (Tokyo, Japan). Nitrogen was used as the carrier gas at a constant flow-rate of 40 ml min^{-1}. The detector, injection port and column temperatures were maintained at 250, 250 and 180 °C, respectively.

In the analysis of nitrite at relatively high concentrations, the GC–3BF instrument was used. A glass column (2.1 m × 3 mm i.d.) was packed with the same column packing material as mentioned above. The injection port and column temperatures were varied depending on the converted benzene derivative. The nitrogen flow-rate was 30 ml min^{-1} and the hydrogen and air pressures were 0.5 and 1.0 kg cm^{-2}, respectively. The peak areas were integrated by a digital integrator (Shimadzu Chromatopac-E1A).

Reagents

All reagents were of analytical-reagent grade and were used without further purification unless otherwise stated. Hexane and de-ionised water were distilled before use.

Sodium nitrite. This was dried in an oven at 110 °C for 1 h before being accurately weighed.

Nitrite standard solution. A 1.0% solution of nitrite was prepared by dissolving dried sodium nitrite in water. The titre was determined with standard potassium permanganate solution.[1] Solutions containing nitrite at lower concentrations were prepared by dilution of this standard solution.

Procedure

The recommended procedure for the determination of nitrite at trace levels (below 1.0 μg ml^{-1}) is as follows. A 0.5-ml volume of 4.0×10^{-4} M *m*-nitroaniline solution dissolved in 1.0 N hydrochloric acid was added to 1.0 ml of aqueous sample in a reaction vessel (*ca.* 10 ml) fitted with a glass stopper. After a few minutes, 0.3 ml of 4.0 M hypophosphorous acid solution was introduced into the vessel. The vessel was then allowed to stand for 2 h in a water-bath controlled at 80 °C. At the end of the reaction period, 1.0 ml of hexane containing *m*-nitrotoluene (2.0×10^{-6} or 2.0×10^{-5} M) as an internal standard was added. After the resulting nitrobenzene had been extracted by shaking for 10 min at room temperature, the organic layer was separated from the aqueous layer. An aliquot (2.5 or 0.6 μl) of the organic layer was injected into the gas chromatograph equipped with an ECD and the converted nitrobenzene was measured by an internal standard method. When the nitrite concentration in the sample was lower than 0.10 μg ml^{-1}, a 2.0×10^{-6} M solution of *m*-nitrotoluene in hexane was used and the injection volume was 2.5 μl. On the other hand, 2.0×10^{-5} M *m*-nitrotoluene solution was used and 0.6 μl of hexane extract was injected in the determination of concentrations of nitrite higher than 0.10 μg ml^{-1}.

In the study using the FID, the concentrations of substituted aniline in 1.0 N hydrochloric acid solution and hypophosphorous acid in the aqueous solution were 1.5×10^{-2} and 1.5 M, respectively, the reaction time was 15 min and the extraction was performed by using 1,2-dichloroethane instead of hexane. Except for these derivatisation reaction conditions, the procedure was the same as for the procedure using the ECD, although the internal standard was not used.

Results and Discussion

Selection of Substituted Aniline

In order to find a suitable derivatisation reagent, the derivatisation yields of nitrite were obtained by using various substituted anilines. The derivatisation yield was calculated from the following equation:

$$\text{Derivatisation yield (\%)} = \frac{PA_c - PA_b}{PA_s} \times 100$$

where PA_c = peak area of the substituted benzene derivative converted from aqueous nitrite solution, PA_b = peak area for the blank (pure water was used instead of the nitrite

solution) and PA_s = peak area for the standard solution (the concentration of substituted benzene derivative is the same as that of nitrite in the aqueous solution).

Derivatisation yields were measured by using the gas chromatograph equipped with an FID, and PA_b was constantly zero whichever substituted aniline was used. The derivatisation yields thus obtained are shown in Table I. It is apparent that nitroanilines and *o*-aminobenzonitrile are suitable derivatisation reagents. Nitrobenzene converted from nitroanilines gives a high response to the ECD, whereas the response of benzonitrile converted from *o*-aminobenzonitrile is less satisfactory.

TABLE I

DERIVATISATION YIELDS FOR NITRITE USING VARIOUS ANILINE DERIVATIVES

The concentration of nitrite solution was 3.0×10^{-3} M.

Aniline derivative	Yield, %	Aniline derivative	Yield, %
Aniline	1	*o*-Nitroaniline	102
o-Chloroaniline	86	*m*-Nitroaniline	90
m-Chloroaniline	62	*p*-Nitroaniline	97
p-Chloroaniline	68	*o*-Aminobenzonitrile	100
p-Bromoaniline	49	2,4-Dinitroaniline	89
o-Iodoaniline	4	2,6-Dibromoaniline	89
m-Iodoaniline	2	2,4,6-Tribromoaniline	1
p-Iodoaniline	12	Pentafluoroaniline	0

Of the three nitroanilines, *o*-nitroaniline gives the highest derivatisation yield. Therefore, *o*-nitroaniline was used as the derivatisation reagent. However, when using *o*-nitroaniline, there is a serious problem that in the extraction step a large excess of *o*-nitroaniline is extracted into the hexane layer together with the converted nitrobenzene. *o*-Nitroaniline, when detected with the FID, does not interfere in the determination of nitrite, although the large peak of *o*-nitroaniline appears a relatively long time after the peak of nitrobenzene. When the ECD is used, on the other hand, a large amount of *o*-nitroaniline causes serious problems as it enters the ECD: contamination of the detector, reduction of its sensitivity and an increase in noise. These problems cannot be overcome by washing the hexane layer with concentrated hydrochloric acid. The same applies to *p*-nitroaniline. In contrast, *m*-nitroaniline is hardly extracted from the aqueous reaction mixture into the hexane layer. Therefore, *m*-nitroaniline was selected as the derivatisation reagent in the procedure using the ECD, although the derivatisation yield in this instance was not quantitative (90%).

Optimum Derivatisation Conditions

In order to carry out the derivatisation under the optimum reaction conditions, the effects of reaction temperature, reaction time and concentrations of *m*-nitroaniline and hypophosphorous acid solutions on the derivatisation yield of nitrite were studied for 0.10 μg ml^{-1} nitrite solution. In this series of studies using the ECD, the other conditions used were identical with those described under Experimental. The effect of reaction temperature on the derivatisation yield was examined (Fig. 1), and it was found that the yield increases with an increase in reaction temperature. Considering the boiling-point of water, the reaction temperature was fixed at 80 °C. Fig. 1 also shows the effect of reaction time. The yield becomes constant with reaction times longer than 1 h. Therefore, the reaction time was fixed at 2 h.

Fig. 2 shows the effects of the concentrations of *m*-nitroaniline and hypophosphorous acid solutions on the yields. The yields reach a constant value when the *m*-nitroaniline and hypophosphorous acid concentrations are higher than 2.0×10^{-4} and 2.0 M, respectively. From these results, the optimum derivatisation conditions described under Experimental were chosen.

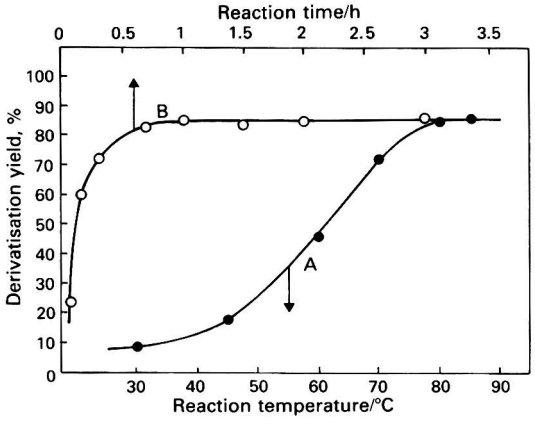

Fig. 1. Effects of reaction temperature (A) and reaction time (B) on derivatisation yield.

Calibration Graph and Derivatisation Yield

Calibration graphs of nitrobenzene peak area *versus* nitrite concentration were constructed for nitrite concentration ranges of 0.10–1.00 and 0.01–0.10 μg ml^{-1} and are shown in Fig. 3(A). The calibration graph is a straight line in the concentration range 0.20–1.00 μg ml^{-1}, but not in the range 0.01–0.20 μg ml^{-1}. Similarly, the relative peak area of nitrobenzene was plotted against the nitrobenzene concentration in a standard solution containing the internal standard [Fig. 3(B)]. The graph was not linear at nitrobenzene concentrations below 4.35 μM. From these results, it is concluded that the curvature of the nitrite calibration graphs is not due to the derivatisation reaction but to the response of the ECD to nitrobenzene. The small unknown peak was given by the blank without nitrite.

A commonly accepted definition of the detection limit is the concentration of analyte giving a signal twice the average noise. Using this definition and the average of the blank peak area, a detection limit of 0.5 ng ml^{-1} was found. This detection limit is low compared with that of the widely used colorimetric method (16 ng ml^{-1}).[1] It may be possible to detect nitrite at concentrations lower than 0.5 ng ml^{-1} by the use of aniline derivatives with substituents that gave a higher response than nitroaniline to an ECD, for example, dinitroaniline or bromonitroaniline.

The derivatisation yield for 2.0 $\times$ 10^{-6} M (about 0.1 μg ml^{-1}) nitrite was found to be

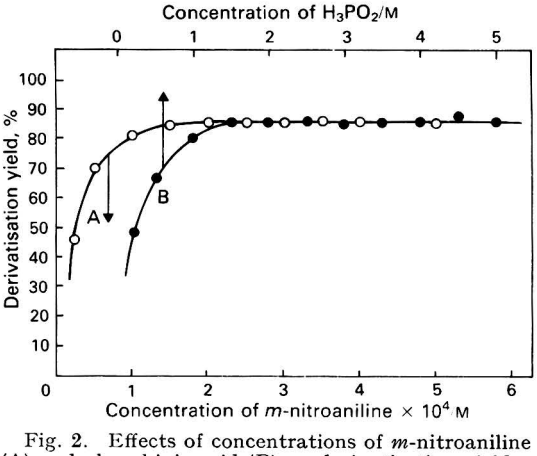

Fig. 2. Effects of concentrations of *m*-nitroaniline (A) and phosphinic acid (B) on derivatisation yield.

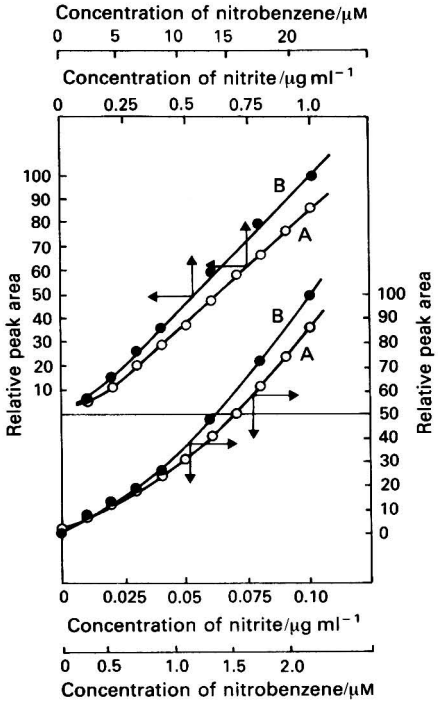

Fig. 3. Calibration graphs for nitrite (A) and for nitrobenzene standard solution (B).

86.0 ± 1.6% from five replicate measurements. This value is close to the yield shown in Table I (90%), which suggests that the yield hardly varies when the nitrite concentration is reduced from 3.0×10^{-3} to 2.0×10^{-6} M.

Interference Study

The proposed gas-chromatographic method was tested in the presence of several ions normally found in environmental samples. In Table II, the peak area of nitrobenzene produced from the standard solution containing only 0.10 μg ml^{-1} of nitrite was arbitrarily assigned a value of 100. The concentrations of the ions (100 μg ml^{-1}) added to the standard nitrite solution were much higher than those in environmental samples. The results indicated that these anions, except for nitrate, do not interfere in this method. Moreover, chloride added as sodium chloride at a concentration of 2.0% to the standard solution has no effect, which suggests the possibility of applying this method to the analysis of seawater. However, nitrate interferes positively at a concentration of 100 μg ml^{-1}, although this interference is not found at 10 μg ml^{-1}. A small peak appeared when 100 μg ml^{-1} nitrate solution without nitrite was analysed by this gas-chromatographic method, and was slightly greater than the blank peak. Therefore, it seems that the nitrate interference is due to nitrite present as an impurity in the sodium nitrate used. The concentration of nitrite in sodium nitrate used is lower than 0.01% *m/m*, calculated from the positive error.

Applications

River water samples were collected from the rivers running through the city of Osaka and were analysed both by the gas-chromatographic method and by the colorimetric method.[1] The results obtained are given in Table III and a typical gas chromatogram obtained in the analysis of river water samples is shown in Fig. 4. Table III indicates that the gas-chromatographic methods tends to give slightly lower values than those measured by the colorimetric method (the mean bias is 0.03), the reason for which is unknown.

TABLE II

RESULTS OF INTERFERENCE STUDY

The concentration of nitrite was 0.10 μg ml^{-1}.

Ion	Concentration/ μg ml^{-1}	Relative peak area*	95% Confidence interval
Standard	—	100.0 ± 2.0	97.5–102.5
Cl^-	20000	101.2 ± 2.3	98.3–104.1
Cl^-	100	99.4 ± 2.1	96.8–102.2
Br^-	100	100.4 ± 2.2	97.7–103.1
F^-	100	98.8 ± 1.9	96.4–101.2
SO_4^{2-}	100	97.8 ± 1.3	96.2–99.4
HCO_3^-	100	99.1 ± 1.5	97.2–101.0
$H_2PO_4^-$	100	98.6 ± 2.5	95.5–101.7
NO_3^-	100	111.9 ± 1.3	110.3–113.5
NO_3^-	10	99.6 ± 1.8	97.4–101.8
NH_4^+	100	100.4 ± 1.1	99.0–101.8

* Mean ± standard deviation for five replicate analyses (between batch). The peak area of nitrobenzene converted from the standard solution was arbitrarily assigned a value of 100.

TABLE III

RESULTS OF STUDY OF INTERCOMPARISON OF METHODS

Sample	Nitrite determined/μg ml^{-1}: Colorimetry	Gas chromatography: Mean ± s.d.*	Gas chromatography: Relative standard deviation, %
A	0.54	0.53 ± 0.013	2.5
B	0.57	0.54 ± 0.006	1.1
C	0.46	0.42 ± 0.009	2.1
D	0.69	0.62 ± 0.007	1.1
E	0.58	0.54 ± 0.004	0.7
F	0.61	0.58 ± 0.007	1.2

* Mean ± standard deviation for five replicate analyses (between batch).

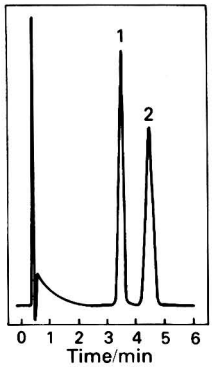

Fig. 4. Gas chromatogram obtained from the analysis of river water sample: 1, nitrobenzene; and 2, *m*-nitrotoluene.

Conclusion

The colorimetric method usually requires pre-treatment to remove turbid and/or coloured materials, if present. On the other hand, the gas-chromatographic method is not affected by such impurities. The sample volume needed for analysis is only 1.0 ml. The proposed method is precise and gives a much lower detection limit than those of the colorimetric and the derivatisation gas-chromatographic methods that have been reported previously.[7–15] Consequently, the proposed gas-chromatographic method is suitable for the practical analysis of environmental and biological samples. Nitrite at relatively high concentrations can be determined similarly by using *o*-nitroaniline and an FID. A straight-line calibration graph passing through the origin is obtained with nitrite concentrations in the range 0.60–3.00 μmol ml^{-1}.

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Determination of Germanium in Coal Ashes by Hydride Generation and Flame Atomic-absorption Spectrophotometry

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This paper describes the determination of germanium by atomic-absorption spectrophotometry with direct introduction into a dinitrogen oxide - acetylene flame of the germanium(IV) hydride generated by reducing with sodium tetrahydroborate(III) solution. A comparative study of the sensitivity of the germanium determination has been carried out and a study of interferences from lead(II), arsenic(III) and -(V), iron(II) and -(III), tellurium(IV), selenium(IV), antimony(III), tin(II), tartrates, oxalates and fluorides is described. The sensitivity of the proposed method is 0.012 μg ml^{-1} and the detection limit is approximately 100 times better than obtained with silica tube atomisation at a wavelength of 265.14 nm. The method has been applied to the determination of germanium in lignite ashes with an average germanium recovery of 97.65% and a relative standard deviation of 2.87 %.

Keywords: *Hydride generation*; *dinitrogen oxide - acetylene flame*; *atomic-absorption spectrophotometry*; *germanium determination*; *coal ashes*

The increased reliance on coal to satisfy the nation's growing energy demands superimposed on acute environmental awareness by the public has aroused concern about the pollution consequences. Interest in emissions of trace elements received impetus from published reports of widespread atmospheric dissemination of these substances as a consequence of fossil fuel use.[1] Of the fossil fuels, coal is considered the major source of atmospheric pollution[2] and, compared with oil and uranium use, probably constitutes a greater public risk.[3] Fuel combustion in stationary sources accounts for 21% of total air pollution emissions and particulates represent 13% of the air pollutants.[4]

Reports on the beneficial action of germanium(IV) oxide on the formation of erythrocytes and the increase in haemoglobin in blood have been described and with regard to sanitary standards 2 mg m^{-3} of germanium or germanium(IV) oxide in air are allowed in working premises. Divalent germanium compounds are considerably more toxic.[5]

Germanium is determined in coals, usually after the carbonaceous part of the sample has been burnt by a dry or wet method. However, methods are described for direct determination using d.c. arc emission.[6] Uncontrolled incineration of the coal can lead to loss of germanium in the form of germanium(II) oxide, and in the presence of a high sulphur content as germanium(II) sulphide.[7] If the temperature is increased rapidly, there are always losses: at 450–500 °C up to 5%, at 750 °C up to 11%, at 900 °C up to 20–37% and at 1000 °C up to 100%. The following incineration conditions have been proposed for lignite coal: the sample is heated at 110 °C for 1 h, then for 2 h at 225 °C, the temperature is then slowly increased to 450 °C and the heating is continued at this temperature for 5 h.[8]

For the determination of germanium in coal ashes gravimetric,[9] titrimetric,[10] spectrophotometric,[11] fluorescence,[12] polarographic,[13] and arc emission spectroscopic[5,6] methods have been employed. The most widely employed method is the phenylfluorone spectrophotometric method.[14]

Therefore, analytical methods that show sufficient sensitivity, together with the selectivity for avoiding interferences of the matrix, are necessary. The majority of the existing methods and in particular the spectrophotometric ones suffer from a lack of selectivity and long separation methods are necessary.

Separation by hydride generation is frequently used for determining arsenic, bismuth, lead, selenium, tellurium, tin and antimony, and less frequently for germanium in real matrices. There are few reported applications of the determination of germanium by atomic-absorption spectrophotometry; Yanagisawa *et al.*[15] determined germanium in synthetic fibres

at concentrations of 0.01–0.02%, after extracting into 4-methylpentan-2-one from 7.5 M hydrochloric acid. When hydride generation is used, the gas phase is transferred, generally, by means of a current of inert gas, up to an electrothermal atomiser, silica tube or hydrogen flame and the atomic absorption is measured.

The use of sodium tetrahydroborate(III) reported by Braman *et al.*[16] is a landmark in the development of the hydride generation and was reinforced by Schmidt and Royer's contribution.[17] In 1973 the use of sodium tetrahydroborate(III) was extended to the determination of germanium by Pollock and West[18] and by Fernandez.[19] All of the above methods use the argon - hydrogen flame. Several workers have reported the use of hydride collection devices, a liquid-nitrogen trap,[20–22] the balloon method[23] and rigid or semi-rigid containers.[18,24] The collection time for germanium(IV) hydride was reduced to 2–3 min by Fernandez's method.[19] However, these are specialised techniques and seem to be slow and complex for routine use, and it is not always necessary to collect the liberated hydride; most commercially available systems now allow direct introduction of the hydride into the atomiser.[25]

The relationship between hydrochloric acid concentration [using sodium tetrahydroborate(III) as the reducing agent to generate germanium(IV) hydride] and sensitivity were studied by Fernandez.[19] He reported that signal size remained almost constant with increasing acid concentration for arsenic, bismuth, antimony and tellurium. The optimum acid strength for germanium determination is 1–3 M. However, Macklen[26] has observed that reduction with sodium tetrahydroborate(III) can also give rise to the formation of elemental germanium, especially when starting from solutions in hydrochloric acid, in an analogous way to the process that takes place with nickel, aluminium, antimony, mercury, iron and thallium.

The major contribution to the information on interferences has been made by Smith[27] who undertook a general study of the effects of 48 elements at concentrations of 1 mg ml^{-1} on the determination of 1 $\mu g\ ml^{-1}$ of arsenic, 0.5 $\mu g\ ml^{-1}$ of bismuth, 2.0 $\mu g\ ml^{-1}$ of germanium, 10.0 $\mu g\ ml^{-1}$ of tellurium, 2.0 $\mu g\ ml^{-1}$ of selenium and 1.0 $\mu g\ ml^{-1}$ of antimony. This work was carried out by using sodium tetrahydroborate(III) pellets and an argon - hydrogen flame, and gave a germanium atomic-absorption supression of greater than 50% in the presence of arsenic, gold, cadmium, cobalt, iron, nickel, palladium, platinum, rhodium, ruthenium, tin, antimony and selenium and between 10–50% in the presence of bismuth, copper, iridium and tellurium. Smith uses a hydrochloric acid medium.

This paper describes the determination of germanium by atomic-absorption spectrophotometry, with direct introduction into a dinitrogen oxide - acetylene flame of the germanium(IV) hydride generated by reduction with sodium tetrahydroborate(III) solution and a study focused on obtaining the optimum analytical conditions for the determination of germanium in lignite ashes. It incorporated a study of the sensitivity of the atomic-absorption spectrophotometric determination of germanium in aqueous, ethanol, acetone and hexane solutions after extracting germanium(IV) chloride[28] and germanium(IV) hydride from a gas phase medium. The interferences of lead(II), arsenic(III) and -(V), iron(II) and -(III), tellurium(IV), selenium(IV), antimony(III), tin(II), tartrates, oxalates and fluorides on the hydride generation method and atomic-absorption spectrophotometric determination of germanium were also studied.

Experimental

Reagents

All solutions were prepared from analytical-reagent grade chemicals and doubly distilled water and stored in polyethylene bottles.

Standard germanium(IV) solution, 1000 $\mu g\ ml^{-1}$. Prepare by dissolving 0.36 g of germanium(IV) oxide in 10 ml of 0.1 N sodium hydroxide solution, then acidify with 15 ml of 0.1 N hydrochloric acid and dilute to 250 ml with water. The stock solution is stable for at least 1 month. The working solutions are obtained by diluting this solution immediately before use.

Sodium tetrahydroborate(III) solution, 1–5%. Prepare by dissolving 0.5–2.5 g in 50 ml of water, containing one pellet of sodium hydroxide. The liquid is vacuum filtered through a

0.45-μm membrane filter. According to the method proposed by Knechtel and Fraser,[29] the solution prepared in this way can then be used for up to 3 weeks.

Acetic acid - sodium acetate buffer solution, 0.1 M.

Interfering ion solutions. The cation and anion solutions used in the study of interferences are prepared from analytical-reagent grade chemicals.

Apparatus

The atomic absorption is measured with a Pye Unicam SP–9 atomic-absorption spectrophotometer, equipped with a 50-mm slot burner and a Westinghouse germanium hollow-cathode lamp, working at an intensity of 10 mA. The area of the atomic-absorption peak is integrated with an SP–9 computer, with an integrating time of 14 s. The generation of germanium(IV) hydride is carried out in a 50-ml spherical glass vessel (2.5-mm thick wall) with two mouths, both of which have been closed by means of a septum membrane and one of which is pierced by an extended nebuliser capillary (Fig. 1). The sodium tetrahydroborate(III) solution is injected through the other septum membrane. After the sodium tetrahydroborate(III) solution has been injected the atomic-absorption peak is obtained for a full 10 s. The shaking of the solution and the use of a carrier gas is not necessary, because the aspiration process and the hydrogen pressure produced in the reduction are sufficient to produce the atomic-absorption peak very quickly. The absence of a carrier gas is an important factor with a view to the sensitivity obtained. There is no dilution effect in the gas phase generated and the atomic population in dinitrogen oxide - acetylene, and subsequently, in the beam of the hollow-cathode lamp, is considerably increased, and is hence greater than if nitrogen or argon is used to carry the germanium(IV) hydride into the atomiser. The hydride generation system is very simple and inexpensive.

The instrument operating conditions for measuring the atomic absorption are dinitrogen oxide flow-rate 5.0 l min^{-1}; acetylene flow-rate 4.2 l min^{-1}; burner height 6 mm; spectral slit width 0.08 mm; and wavelength 265.14 nm.

Procedure for Germanium Determination in Lignite Ash

The lignite sample is incinerated in a platinum crucible, under the following conditions: lignite is heated at 110 °C for 1 h, then for 3 h at 300 °C, the temperature is then increased slowly to 475 °C and the heating is continued at this temperature for 4 h.

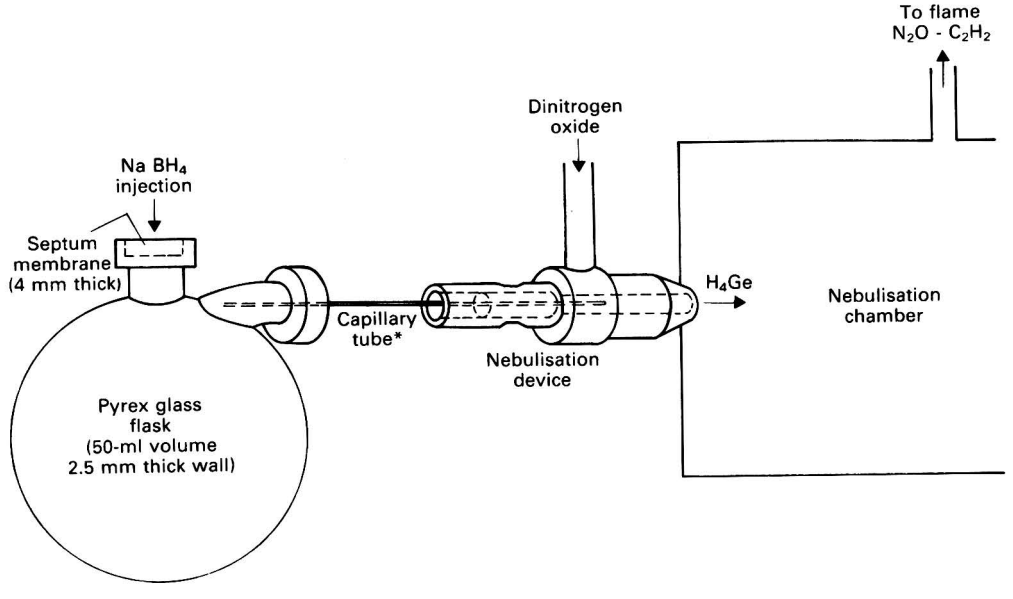

Fig. 1. Germanium hydride generation vessel. The original capillary tube* of the nebulisation device was extended by 40 mm.

A 0.5-g sample of the ashes obtained (23–27% of the sample according to the source) is treated with 20 ml of hydrofluoric acid - hydrochloric acid mixture (1 + 1 *V/V*) in a PTFE vessel and heated to 50 °C. The heating is prolonged until the residue is almost dry. This is then dissolved in the 0.1 M acetic acid - sodium acetate buffer solution and made up to 100 ml with the buffer solution.

A 5-ml aliquot of this solution, containing not more than 20 μg of germanium, is placed in the reduction vessel and 4 ml of a 3.5% *m/V* solution of sodium tetrahydroborate(III) are injected. A 1% *m/V* sodium tetrahydroborate(III) solution is the optimum concentration for determining a pure germanium solution; however, in order to avoid any interference from iron the use of a 3.5% solution is necessary. The atomic absorption of the gas phase generated is measured at 265.14 nm in the dinitrogen oxide - acetylene flame.

The calibration graphs are obtained from a pure standard germanium solution (5 ml of 0.2–4.0 μg ml^{-1} of germanium in 0.1 M acetic acid - sodium acetate solution), treated in the same way as the samples.

Results and Discussion

The optimum medium for generating the germanium(IV) hydride is the 0.1 M acetic acid - sodium acetate solution (pH 4.75). If the medium is more acidic a larger and quicker hydrogen production is generated, which dilutes the germanium(IV) hydride in the gas phase giving smaller atomic-absorption signals. However, at higher pH values an incomplete reduction of germanium is produced, some remaining in the form of elemental germanium, in the hydrochloric acid medium. The results obtained at different pH values are given in Fig. 2.

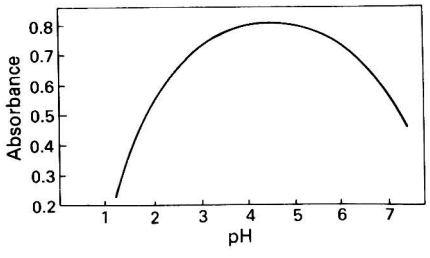

Fig. 2. Atomic absorbance of germanium-(IV) hydride with varying pH. Amount of germanium, 2 μg; total sample volume, 5 ml; volume of sodium tetrahydroborate(III) solution injected, 4 ml of 1% *m/V*.

The optimum volume and concentration of the sodium tetrahydroborate(III) solution for determining up to 20 μg of germanium contained in a sample volume of 5 ml are 4 ml at 1% *m/V* for a pure germanium solution. The calibration lines are valid for up to 20 μg of germanium. Some atomic-absorption peaks are shown in Fig. 3.

The reproducibility of the method is excellent. The atomic-absorption results for 25 germanium solutions of the same concentrations, and reduced to germanium(IV) hydride, give a relative standard deviation of 1.88% for 1.0 μg ml^{-1} of germanium (for a total volume of 5 ml of sample) considering the height of the peaks, whilst integrating the closed areas under the peaks (14-s integration time) starting from just after sodium tetrahydroborate(III) solution is injected gives a relative standard deviation of 0.17%.

The sensitivity of the method (0.0044 absorbance unit) is 0.012 μg ml^{-1} and the detection limit (twice the value of the background noise) is 0.0038 μg ml^{-1}. These values are comparables to those obtained by electrothermal atomic-absorption spectrophotometry and better than those obtained by other hydride generation systems, see Table I.

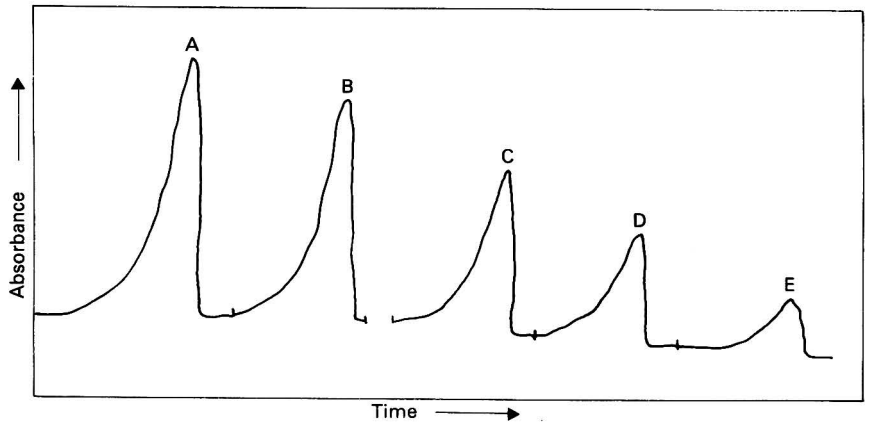

Fig. 3. Atomic-absorption peaks for germanium. Total sample volume, 5 ml; pH, 4.7; and 4 ml of 1% *m*/*V* sodium tetrahydroborate(III) solution. Amount of germanium: A, 10 μg; B, 8 μg; C, 6 μg; D, 4 μg; and E, 2 μg.

TABLE I

FLAME ATOMIC-ABSORPTION SPECTROPHOTOMETRIC DETERMINATION OF GERMANIUM BY HYDRIDE GENERATION

Reducing agent	Collection vessel	Atomiser system	Detection limit/ μg ml^{-1}	Sensitivity/ μg ml^{-1}	Reference
$NaBH_4$ pellet	Balloon	Air - H_2 flame	0.2	0.27	18
$NaBH_4$ solution, 1% *m*/*V*	None	Silica tube	0.5	1.0	30
$NaBH_4$ solution, 1% *m*/*V*	None	N_2O - C_2H_2 flame	0.0038	0.012	This paper

To reinforce the results of this study on the sensitivity of the proposed method, the sensitivity of the determination of germanium has been obtained by nebulising germanium standard solutions in water, water - ethanol (1 + 1 *V*/*V*) and water - acetone (1 + 1 *V*/*V*) media, in hexane after extracting germanium as germanium(IV) chloride from a 7.5 M hydrochloric acid[28] medium and direct introduction of germanium(IV) hydride into a dinitrogen oxide - acetylene flame. The results are given in Table II.

TABLE II

SENSITIVITY OF FLAME ATOMIC-ABSORPTION SPECTROPHOTOMETRIC DETERMINATION OF GERMANIUM

Conditions and germanium concentrations (p.p.m.) that give a sensitivity of 0.0044 absorbance unit.

Phase atomised	N_2O flow-rate/ l min^{-1}	C_2H_2 flow-rate/ l min^{-1}	Burner height/ mm	Monochromator slit width/ mm	Wavelength/nm 259.25	265.14	270.96	275.46
Water	6.7	4.0	4	0.1	3.75	0.75	22.50	7.50
Water - acetone ..	6.0	3.8	4	0.1	0.15	0.08	0.37	0.16
Water - ethanol ..	6.5	4.2	6	0.1	0.20	0.10	0.56	0.28
$GeCl_4$ - hexane	6.9	4.0	6	0.1	0.08	0.045	0.18	0.093
H_4Ge	5.0	4.2	6	0.08	0.02	0.012	0.042	0.023

Interferences

The interferences in methods that determine elements after the formation of the volatile hydrides are generally due to two causes: the reduction of a foreign ion in preference to the

element being determined and consequent consumption of the sodium tetrahydroborate(III), or reduction to the elemental state, which can absorb the volatile hydride and impede its movement towards the atomisation system or catalyse the decomposition of the volatile hydride. It is also necessary to take into account the presence of anions that may form complexes with germanium.

Up to 10 mg of lead(II), arsenic(III), and -(V), tellurium(IV), selenium(IV), iron(II) and -(III), antimony(III), tin(II), oxalates, tartrates and fluorides were added to 5 ml of solutions containing 8 μg of germanium and 4 ml of 1% sodium tetrahydroborate(III) solution and then injected, having carried out the reduction process under the conditions described previously. The results are given in Table III.

TABLE III

INTERFERENCES IN THE ATOMIC-ABSORPTION SPECTROPHOTOMETRIC DETERMINATION OF GERMANIUM BY HYDRIDE GENERATION

Ion studied	Species present	Amount of ion studied/mg per 5 ml: 1	4	10
		Suppression of Ge absorbance peak, %*		
Pb^{2+}	$Pb(NO_3)_2$	0	0	1.1
As^{3+}	As_2O_3 (NaOH solution)	0.8	2.3	4.4
As^{5+}	Na_3AsO_4	0	0	0.6
Fe^{2+}	$FeSO_4(NH_4)_2SO_4.6H_2O$	7.5	40.5	100
Fe^{3+}	Fe (HCl solution)	7.0	49.5	100
TeO_3^{2-}	H_2TeO_3	4.5	60.0	100
SeO_3^{2-}	Na_2SeO_3	5.0	61.0	100
Sb^{3+}	$SbCl_6^{3-}$	8.0	75.3	100
Sn^{2+}	$Sn(OH)_4^{2-}$	7.6	68.7	100
$C_4H_4O_6^{2-}$	$NaKC_4H_4O_6$	9.5	47.3	100
$C_2O_4^{2-}$	$Na_2C_2O_4$	8.7	53.7	100
F^-	NaF	0	0	0.4

* Average value of ten determinations.

Lead(II), arsenic(III) and -(V) and fluoride at the 2 mg ml^{-1} level do not interfere. No severe interferences occurred with 0.2 mg ml^{-1} of iron(II) and -(III), tellurium(IV), selenium-(IV), antimony(III), tin(II), oxalates or tartrates. However, interferences did occur with 0.8 mg ml^{-1} of each of these species and with 2 mg ml^{-1} the germanium atomic-absorption signal was totally suppressed.

The interference of iron, which is very important in the determination of germanium in lignite ashes (the iron contents are about 1.6 mg in the 5 ml of final sample solution), may be prevented by masking with the fluorides added during the acid attack and using a more concentrated solution of sodium tetrahydroborate(III) solution (3.5% *m*/*V*). Oxalate and tartrate interferences, as was to be expected, result from the masking of germanium at pH 4.75, and the reduction to germanium(IV) hydride is prevented. The concentrations of tellurium, selenium, antimony and tin in coal ashes are lower than those which produce a suppression of the atomic-absorption signal.

Germanium Determination in Lignite Ashes

The values obtained for the germanium content of some lignites supplied by the Spanish Institute of Carbochemistry (C.S.I.C.) were compared with the values obtained from the same samples by germanium(IV) chloride into hexane from a 7.5 M hydrochloric acid medium and atomic-absorption determination by nebulising the organic phase,[28] using the same incineration process for obtaining the lignite ashes. The results are given in Table IV.

The proposed method has also been verified by standard additions to the samples before the incineration process. The average recovery of the germanium added was 97.65%, Table V.

The germanium contents in the lignites analysed are concordant with the geomorphological characteristics of the deposits that were the source of the lignite samples.

The procedure can easily be extended with minor modifications to a variety of other matrices such as plants, natural water, wastewater or environmental samples.

TABLE IV

DETERMINATION OF GERMANIUM IN LIGNITE ASHES

Sample	Ge found in ashes, p.p.m.*		Relative standard deviation, %	
	$GeCl_4$ in hexane	GeH_4	$GeCl_4$ in hexane	GeH_4
Calaf 1	94.5	91.6	1.9	2.3
Calaf 2	81.0	82.0	2.0	2.2
Calaf 3	84.6	86.8	1.8	2.5
Utrillas 1	122.8	119.6	3.2	3.8
Utrillas 2	115.7	117.8	3.6	3.7
Utrillas 3	93.0	92.2	3.3	3.9
P.G.R. 1†	74.5	76.2	2.1	2.3
P.G.R. 2†	62.4	63.3	2.2	2.7
P.G.R. 3†	74.2	72.2	2.0	2.9
S. Tomas 1 ..	75.3	76.4	2.1	2.4

* Average value of ten determinations.
† P.G.R. = Puentes Garcia Rodriguez.

TABLE V

STANDARD-ADDITIONS METHOD, GERMANIUM ADDED BEFORE LIGNITE INCINERATION

Sample	Ge found in ashes, p.p.m.	Ge added, p.p.m.	Total Ge found, p.p.m.*	Ge recovery, %
Calaf 1	91.6	100	189.7	98.1
Utrillas 1	119.6	100	216.8	97.2
P.G.R. 1	76.2	75	148.7	97.5
S. Tomas	76.4	75	149.2	97.8

* Average value of ten determinations.

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Determination of Mercury Vapour in Air Using Electrothermal Atomic-absorption Spectrometry with an Electrostatic Accumulation Furnace

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The "electrostatic accumulation furnace for atomic-absorption spectrometry" technique has been tested for the determination of mercury vapour in the atmosphere. Even if the electrostatic capture of mercury vapour is much more difficult than for particles, an efficiency higher than 90% can be achieved. A calibration procedure is proposed. Under appropriate experimental conditions, a detection limit (signal to noise ratio = 3) of 50 ng m^{-3} was obtained.

Keywords: *Electrothermal atomic-absorption spectrometry*; *electrostatic precipitators*; *particulate matter analysis*; *mercury vapour determination*

In a previous paper[1] a new apparatus called an electrostatic accumulation furnace for electrothermal atomic spectrometry (EAFEAS) was described for the precise, simple and fast determination of lead in air particulate matter. The operating principle is the coupling of an "*in situ*" pre-concentration step, electrostatic capture of particulate matter in a graphite cylinder (the furnace), with a measuring step, electrothermal atomisation in an atomic-absorption spectrometer. The calibration of the apparatus was accomplished with aqueous solutions of the metal to be analysed by assuming that the capture efficiency was near 100% and that all particles were captured in a small central section of the furnace. This assumption was based on experimental evidence and on the well known fact[2,3] that such systems can be operated easily at about 100% efficiency. Up, to now however, there is no direct evidence that the above assumption is valid. Measurements are under way using specific techniques for counting the particles entering and leaving the furnace and for localising the point where they are captured.

The proposed apparatus can also be used for the determination of mercury vapour in the atmosphere as mercury vapour can be captured with electrostatic precipitators,[4] and a gaseous standard with a known concentration of the metal can be obtained easily. In this way calibration can be made under the same experimental conditions and meaningful results can be obtained. At the same time this permits comparison of the capture efficiency of the apparatus under two very different conditions, *i.e.*, with particles and with atoms. In particular, the efficiency for mercury atom capture should represent the lower limit for particle capture[2,3] and its experimental measure can be used as indirect confirmation of the validity of the procedure described in reference 1 for lead particles.

In this paper the influence of potential and flow-rate on the capture efficiency is analysed and a calibration technique described. A measure of the efficiency of the system is also given.

Experimental

Apparatus

All the absorption measurements were performed with a Perkin-Elmer 460 spectrometer equipped with an Intensitron lamp operated at 253.8 nm. The slit width gave a band pass of 0.2 nm. Deuterium background correction was always used. The output was stored on a Gould, Advance 4000, storage oscilloscope with option 4001, from which a hard copy of the transient could be obtained with the aid of a strip chart recorder when necessary. Peak heights were used throughout.

Tygon tubes were used for connecting the various devices.

Graphite cylinders (used as furnaces) were made from U-7 graphite rods (Ultra Carbon Corp.).

Reagents

Mercury(II) stock solution, 1000 p.p.m., *pH* 1. Prepared from mercury metal and concentrated nitric acid. From this solution 200 and 50 p.p.m. solutions were prepared weekly and more dilute solutions daily.

Sodium tetrahydroborate(III) solution, 2%. Prepared freshly and used only after any mercury present had been stripped off by bubbling nitrogen through the solution.

Nitrogen. U.P.P. grade nitrogen, pre-purified over a BASF oxygen absorber, was used during electrothermal atomic-absorption spectrometry (EAAS) measurements.

Results and Discussion

Influence of Potential and Flow-rate

The experimental set-up and methodology are practically the same as those described for lead.[1] The only part of the apparatus that has been slightly modified is the metallic cylinder from which the air is sucked through the furnace (see part g in Fig. 1 of reference 1); for this an additional gas outlet enables a constant flow-rate of air through the cylinder to be maintained despite the small flow drawn through the furnace during the sampling step. The measurements on particulate matter were repeated in order to eliminate possible signal shifts that changes in the apparatus (such as the needle and the furnace) could have introduced. Air with a constant content of mercury vapour, was obtained (see Fig. 1) by mixing the nitrogen flow, enriched with mercury vapour (*vide infra*), with air purified over metallic zinc. The ratio between the flow-rates of air and nitrogen was always higher than 25 in order to avoid significant changes in the air composition. A Matheson 7400 Rotameter was used as a flow regulator. The content of mercury vapour could be varied by changing the flow-rates or the temperature of the thermostated mercury saturator. Similar results were also obtained by sampling the laboratory air. However, the first system was preferred because of the reduced content of particulate matter and the possibility of changing the mercury concentration with good precision.

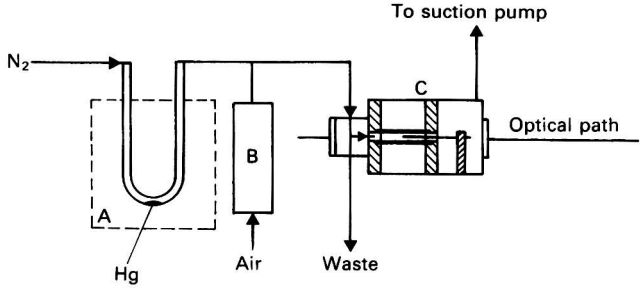

Fig. 1. Schematic layout of the system used to generate air with a constant content of mercury vapour and to study the influence of potential and flow-rate on the capture efficiency. A, Thermostatically controlled mercury enriching Pyrex tube; B, air trap filled with zinc pellets; C, EAFEAS device (for a complete description refer to reference 1).

Fig. 2 shows the absorbance, normalised to the maximum, as a function of the applied potential, at constant flow-rate, for lead in particulate matter (curve A) and for mercury vapour (curve B). In the same figure the current flowing in the circuit is also reported (curve C). The large error bars shown in curve C arise from the intrinsic instability and variation with time and air composition of the corona discharge.[5] The most revealing feature of Fig. 2, apart from the similarity of the curves A and B, is the shift of B towards higher potentials. The coincidence between the onset of a measurable current and an appreciable variation in the capture efficiency (compare curve C with curves A and B) is a clear indication that the charging of the particles or of the mercury vapour is the main factor governing the capture efficiency. In fact the field strength does not change substantially either when passing from the minimum to the maximum capture efficiency for both curves A and B, or when passing from curve A (particles) to curve B (atoms).

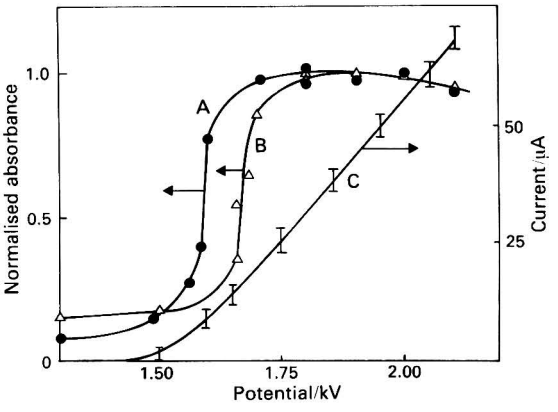

Fig. 2. Influence of the applied potential on the absorption signal of lead and mercury (at constant flow-rate) and on the current (C) flowing in the electric circuit. A, Particles, flow-rate of 20 cm s^{-1}; and B, mercury vapour, flow-rate of 6 cm s^{-1}. The arrows on the graphs indicate the ordinate to which the graphs must be referred.

Fig. 3 shows the influence of the flow-rate, at constant applied potential, on the lead and mercury absorption signals. Here the differences are much more pronounced, as the signal relevant to lead particles is constant for all the velocities studied whereas for mercury vapour a flat maximum is reached only at rather low flow-rates. The values of the flow-rates reported in this paper are average values obtained by dividing the air volume passed in a second by the electrostatic accumulation furnace hole section. The differences between curves A and B in Figs. 2 and 3 can be explained easily, according to theory, if we assume that mercury vapour actually behaves as very small particles,[2,3] as the capture efficiency is an increasing function of particle dimension. As a consequence, with mercury vapour our apparatus can be fully tested as a decrease in efficiency with increasing flow-rate is measurable in the range of flow-rates used, whereas for particles even at velocities beyond 200 cm s^{-1} there is no sign of a decrease. The explanation is, in our opinion, simple. The number of particles below 0.2 μm in air is falling rapidly to zero[6] and therefore there is a big gap between the smallest particles and atoms, which is reflected by a big difference in radial velocity.[1] Moreover, the particles containing lead have a distribution[7] with a peak at values higher than 0.2 μm.

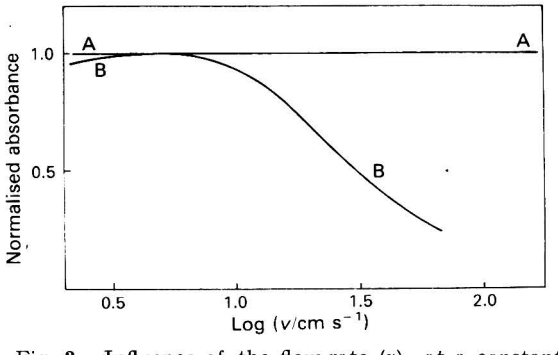

Fig. 3. Influence of the flow-rate (v), at a constant applied potential, on the absorption signal. A, Particles, applied potential 1.8 kV; and B, mercury vapour, applied potential 1.95 kV.

The non-zero signal observed at low potentials (see Fig. 2), and at high velocity (see Fig. 3) for mercury vapour can be ascribed to diffusion capture, which is also responsible for the small decrease of the signal at low flow-rates (Fig. 3). The diffusion coefficient[8] of mercury in nitrogen at 25 °C is 0.16 $cm^2 s^{-1}$ and it is reasonable to assume that its value is not significantly different in the atmosphere. As the inside diameter of the furnace is 0.325 cm the amount of mercury captured by diffusion can be high and will increase with decreasing flow-rates. Under our circumstances, this capture mechanism will be more predominant at the entrance of the furnace. Considering that the peak height in EAAS is an increasing function of the heating rate and that the heating rate decreases from the centre to the extremities[1] of the furnace, the decrease of the peak height observed at low flow-rates can be ascribed to the larger amount of the analyte deposited at the entrance.

The flow-rate and potential values are chosen in the region where the signal is highest and its variation practically independent of their value (see Figs. 2 and 3, respectively). Different flow-rates and potentials have also been studied, although the results are not shown here as they agree with theory.

Calibration Procedure

The calibration of the EAFEAS technique for mercury determination is of paramount importance as the assumption of complete capture of mercury vapour in a small section of the furnace is even more drastic than with lead particles. The calibration is restricted to mercury vapour (volatile mercury compounds and mercury associated with particles have not been considered). For this purpose the apparatus described in reference 1 was modified as shown schematically in Fig. 4. It is composed basically of three parts: the furnace, the furnace container for the electrostatic accumulation step and the spectrometer positioner, which serves to accommodate and heat the furnace during the electrothermal atomisation step and/or to accommodate the absorption cell that measures the mercury concentration in the nitrogen stream during the accumulation step (see later).

The furnace is composed of two brass blocks (A) screwed to a glass-reinforced PTFE ring (B). The graphite tube (C) is kept tightly on the furnace axis by two graphite screws (D). Cooling holes (E) are machined in the brass blocks of the furnace.

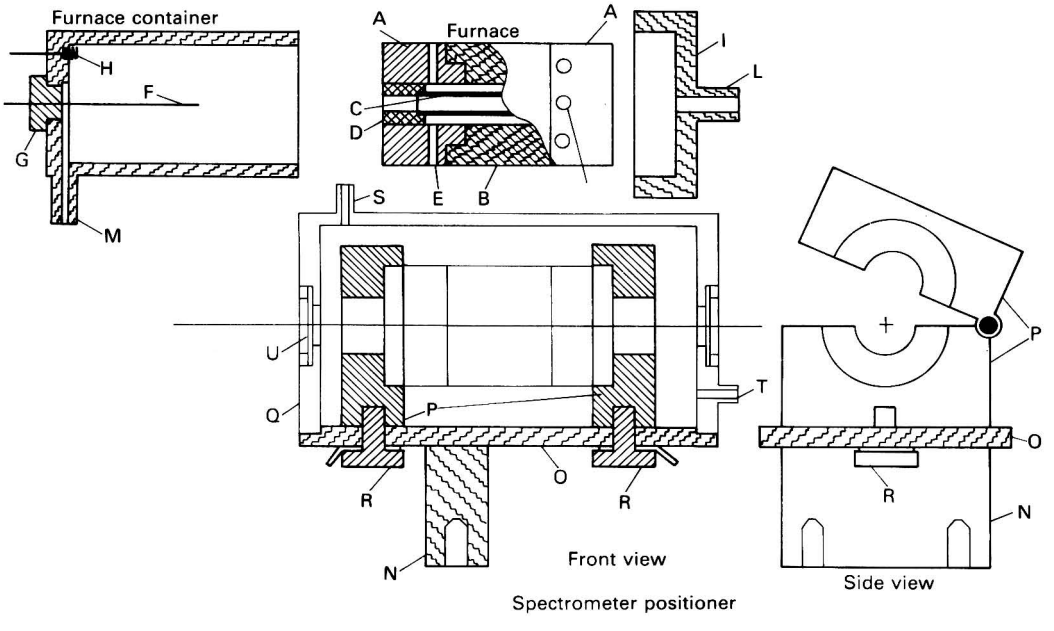

Fig. 4. Schematic view of the electrostatic accumulation furnace, the furnace container for the accumulation step and of the spectrometer positioner. For a detailed description see text.

The PTFE container (which accommodates the furnace during the accumulation step) has a tungsten tapered wire (F) mounted just along the axis of the furnace. The wire can be moved to the optimum depth[1] inside the furnace simply by rotating the screw (G) to which it is fixed. A soldered spring contact (H) ensures the electrical connection with the furnace (C) via the brass blocks (A). A screw cup (I) closes the container once the furnace has been introduced. Tube attachments (L and M) connect the container to the sample gas line and to the suction pump, respectively.

The spectrometer positioner is basically composed of an adapter (N) for the spectrometer flame mount, a Plexiglass base (O), two brass plates (P) and a Plexiglass cover (Q). The two brass plates (P), fixed to the base by two screws (R) which serve also as electrical leads, are machined in such a way that the furnace (or the absorption cell) fits snugly in. They are connected to a variable constant voltage power supply. The cover ensures an inert atmosphere during the EAAS step. It is equipped with a gas inlet (S), a gas outlet (T) and two quartz windows (U). The cover is, of course, no longer necessary when the absorption cell is used.

The reproducibility of ten replicates, measured with the described apparatus for 1 μl of a 0.1 p.p.m. standard lead(II) solution, was $\pm 5\%$ at the 67% confidence level.

The first calibration attempt was made by capturing in the furnace a small amount (0.3–2 ng) of mercury, generated according to the "cold vapour" method in a small compact PTFE reaction vessel, and stripped with a mixture of oxygen and nitrogen gases (25 + 75% *V*/*V*). The results were very disappointing because of high losses ascribed primarily to absorption of the metallic mercury on the vessel and tubing walls.

A new calibration procedure was consequently set up, involving the following steps:

(*a*) Production of a continuous stream of nitrogen with a constant and known content of mercury vapour.

(*b*) Calibration of an absorption cell with the nitrogen stream generated in step (*a*).

(*c*) Calibration of the furnace by means of the absorption cell calibrated in step (*b*).

The experimental set-up used for steps (*a*) and (*b*) is shown schematically in Fig. 5. A steady flow of a mercury(II) solution (0.1 p.p.m.) acidified to pH 1 with concentrated nitric acid was mixed with a reducing solution of sodium tetrahydroborate(III) and introduced into a Pyrex U-tube maintained at a temperature (150 $\pm$ 10 °C) high enough to vaporise the solution completely. A constant flow of nitrogen entrained the mercury and water vapours. The water vapour was removed, except for the equilibrium vapour pressure, by means of a condenser. The nitrogen stream enriched with mercury then passed through the absorption cell. In this way, knowing the concentration of the mercury(II) solution, its flow-rate and the flow-rate of nitrogen, the absorption cell could be calibrated easily provided that the mercury(II) is quantitatively reduced and entrained in the nitrogen stream. In order to

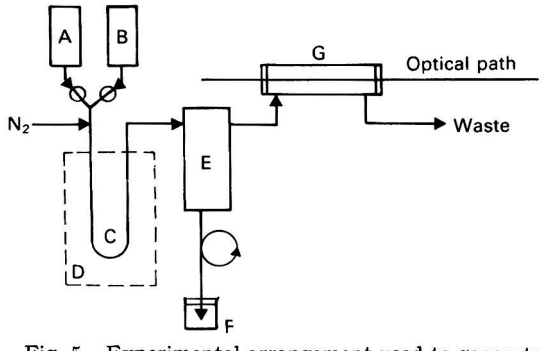

Fig. 5. Experimental arrangement used to generate a nitrogen flow with constant and known mercury concentration and to calibrate the absorption cell. A and B, mercury(II) and sodium tetrahydroborate(III) solution reservoirs; C, Pyrex tube; D, oven; E, condenser; F, water seal; and G, absorption cell.

ascertain if this assumption is valid, a second independent set of measurements was performed by passing the mercury(II) nitrate solution, acidified to pH 2 with concentrated nitric acid, through the Pyrex U-tube heated in the range 350–450 °C. The partial pressure (10^{-7} atm) of the mercury and oxygen gases produced by the decomposition of the mercury(II) nitrate,[9] and possibly of the mercury(II) oxide, is such that the decomposition should be quantitative.[10] With very gentle heating and in carefully controlled conditions, stable nitrates can also be obtained at such temperatures.[11] However, we have not noticed significant changes in passing from 350 to almost 450 °C [such a big change in temperature should have a dramatic effect if decomposition of the mercury(II) nitrate is incomplete]. The results obtained in the two sets of measurements were the same. This may be taken as a sufficiently clear indication that all of the mercury introduced with the solution and reduced by sodium tetrahydroborate(III) passes in the gas stream as mercury vapour. On the other hand, other direct methods such as weighing are impractical because of the small amount involved.

Once the absorption cell has been calibrated it can be used to measure the concentration of mercury in the nitrogen stream and then to calibrate the EAFEAS device. For this purpose a stream containing mercury vapour can be produced as described above or, more conveniently, by passing a stream of nitrogen over a small mercury drop. In order to simulate a real situation as closely as possible, the flow of nitrogen, containing mercury vapour, is mixed with air in a ratio not more than 1 : 25. This is the mixture used to calibrate our EAFEAS device. The flow system is shown in Fig. 6 and a typical calibration graph is given in Fig. 7. From a series of measurements with a mercury concentration of 0.53 μg m^{-3}, a limit of detection of 0.05 μg m^{-3} could be calculated for a signal to noise ratio of 3 by sampling 200 cm^3 of air at 6 cm s^{-1}.

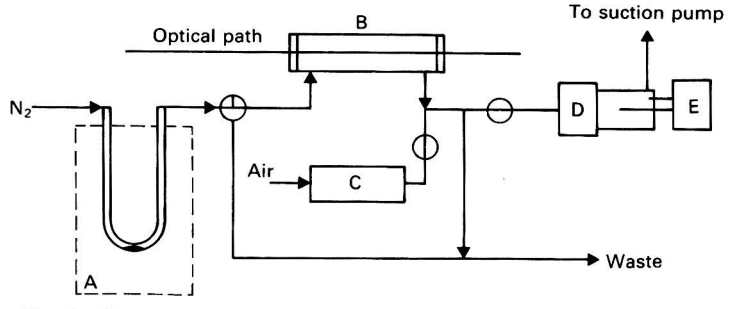

Fig. 6. Experimental arrangement used to calibrate the EAFEAS device. A, Thermostatically controlled mercury enriching Pyrex tube; B, absorption cell; C, trap filled with zinc pellets; D, furnace and furnace container described in Fig. 4; and E, high voltage power supply.

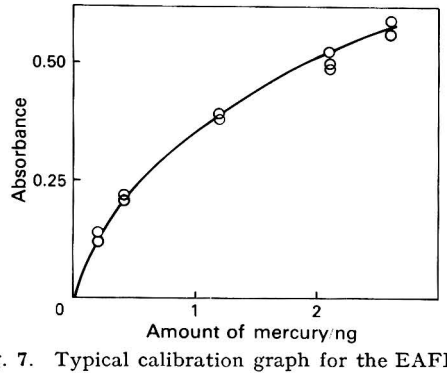

Fig. 7. Typical calibration graph for the EAFEAS.

A comparison could be made between a signal obtained with the same furnace by capturing a certain amount of mercury as vapour or by introducing it as a solution with a device similar to that already described.[1] However, even if constant signals can be obtained, there is evidence[12] that even in very drastic experimental conditions some mercury is lost before the atomisation step. For this reason such measurements were not attempted. A measure of the efficiency of our device can be made easily by rearranging the various parts of Fig. 6. In this instance air instead of nitrogen is passed over the mercury drop in the mercury-enriching tube (A) and the furnace (D) is placed before the absorption cell (B). When the best flow-rate and potential, from Figs. 2 and 3, were chosen the absorption *versus* time graph (Fig. 8) was obtained. Although there is a very high mercury vapour concentration ($\sim$1 mg m^{-3}) present here, nonetheless, the capture efficiency of the electrostatic accumulation furnace is more than 90%. The sluggishness of the signal in reaching steady values may be due to memory effects, diffusion capture and/or re-entrainment. The high concentration used derives from the necessity for a good signal to noise ratio. An indication that the mercury captured is not confined in a small central section of the furnace can be inferred from Fig. 9, in which the signal obtained with a mercury(II) solution (curve A), as suggested in reference 12, and the signal obtained from mercury vapour (curve B) are shown. The higher peak width and the more pronounced tail of curve B can also be explained by the mechanism already presented to justify the decrease of the peak height with low flow-rates (Fig. 3).

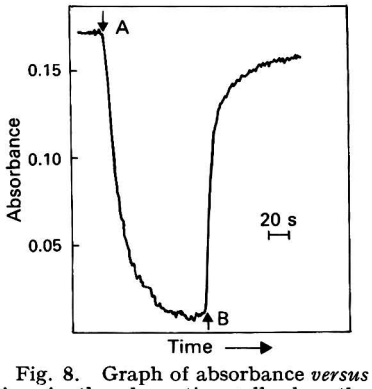

Fig. 8. Graph of absorbance *versus* time in the absorption cell when the potential in the EAFEAS is switched on (A) and then off (B). The experimental arrangement is the one shown in Fig. 6 with the EAFEAS inserted upstream of the absorption cell. Mercury concentration, 0.9 mg m^{-3}; applied voltage, 1.95 kV; and gas flow-rate, 5.5 cm s^{-1}.

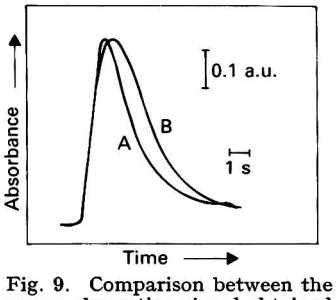

Fig. 9. Comparison between the mercury absorption signal obtained from mercury(II) solution (A) and from mercury vapour (B) electrostatically captured with an applied voltage of 1.95 kV and a flow-rate of 4.5 cm s^{-1}.

Lastly, the level of mercury in the laboratory atmosphere and the influence of air circulation is presented in Fig. 10.

From the above results it can be concluded that the EAFEAS method permits a rather fast and precise measure of very low levels of mercury vapour in the atmosphere. The comparison between the efficiency of electrostatic capture of particles and mercury vapour shows that even if with mercury vapour the capture is more difficult, efficiencies higher than 90% can be reached. Of course, the obvious implication is that with particles an efficiency near 100% is not unreasonable.

The help of the mechanical work-shop technicians V. Sacchetti and G. Cosmai is acknowledged. The work was carried out with partial financial assistance of C.N.R. (Rome).

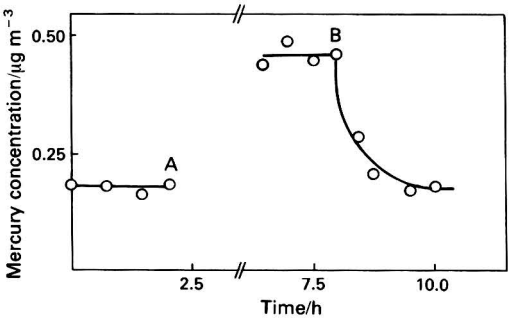

Fig. 10. Level of mercury vapour concentration in the laboratory with and without air circulation: A, windows and door closed; B, windows and door opened.

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Interactions of Major, Minor and Trace Elements on the Carbon Rod Atomic-absorption Spectrophotometric Determination of Micro-amounts of Ytterbium, Holmium, Dysprosium and Thulium

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The electrothermal atomisation of trace amounts of some heavy rare earth elements has been investigated using a carbon rod atomiser. The interferences due to the components (major, minor and trace) of a common silicate matrix have been studied in order to obtain the best analytical conditions for future determinations. The detection limits (using uncoated graphite rods) are 5 pg of dysprosium, 3 pg of holmium and thulium and 0.4 pg of ytterbium.

Keywords: Dysprosium, holmium, thulium and ytterbium determinations; silicate rocks; atomic-absorption spectrophotometry with electrothermal atomisation; interferences

The most useful indicators of geochemical processes are groups of trace elements with similar physico-chemical properties. The rare-earth elements, which all, essentially, have the same chemical properties and display small regular differences in mass and also in ionic radius as a result of the lanthanide contraction, are considered to form the best available group of such elements. An accurate knowledge of the abundance of these elements is of considerable value in evaluating the evolution of igneous rocks, and variations in their abundance have been widely studied[1–4] in investigations of the magmatic behaviour of silicate melts.

Previously,[5] it has been stressed that few analytical results for europium, in particular, and other lanthanides, in general, have been obtained from electrothermal atomic-absorption spectrophotometry, a cheap and readily available technique that is capable of measuring the ultra-trace levels (below about 1 p.p.m.) of rare earth elements that are encountered in silicate rocks. For this reason we studied those lanthanides that (having good flame sensitivity[6]) appeared to be the most likely to give good analytical results. In particular, we investigated the atomic-absorption spectrophotometric behaviour of ytterbium, dysprosium, holmium and thulium.

Experimental

Generally, when a trace element is to be determined by atomic-absorption spectrophotometry, large amounts of the elements present in common silicate rocks enhance or inhibit the behaviour of the atoms of trace elements.

Preliminary analyses carried out on the whole solution of the dissolved sample of some international standard rocks gave erroneous results. The interferences were evaluated by the addition of solutions of pure element salts to holmium, ytterbium, thulium or dysprosium solutions of constant concentration. The concentration of the lanthanide tested was checked at a level high enough to give a good response (about 0.3 absorbance unit) in order to evaluate positive or negative fluctuations. Preliminary tests, performed directly on 0.6 M hydrochloric acid solutions of pure rare earth element oxides, gave the electrothermal atomic-absorption spectrophotometric sensitivities, which enabled the lanthanide concentration necessary to obtain the cited absorbance value to be calculated. These values are shown in Table I.

TABLE I

ANALYTICAL PARAMETERS FOR THE ELECTROTHERMAL ATOMIC-ABSORPTION SPECTROPHOTOMETRIC DETERMINATION OF DYSPROSIUM, HOLMIUM, THULIUM AND YTTERBIUM

Element	Wavelength/nm	Sensitivity/pg
Dysprosium	421.2	5
Holmium	410.4	3
Thulium	371.8	3
Ytterbium	398.8	0.4

The concentration of the solutions added was varied over a range appropriate for each element in common silicate rocks. Interferences caused by those elements usually present in large amounts in a common silicate matrix (henceforth referred to as major elements), were investigated starting from an interfering element to rare earth element ratio, of 1:1 up to 100000:1. Shorter ranges were adopted for those elements usually present in minor or trace amounts (henceforth referred to as minor or trace elements, respectively).

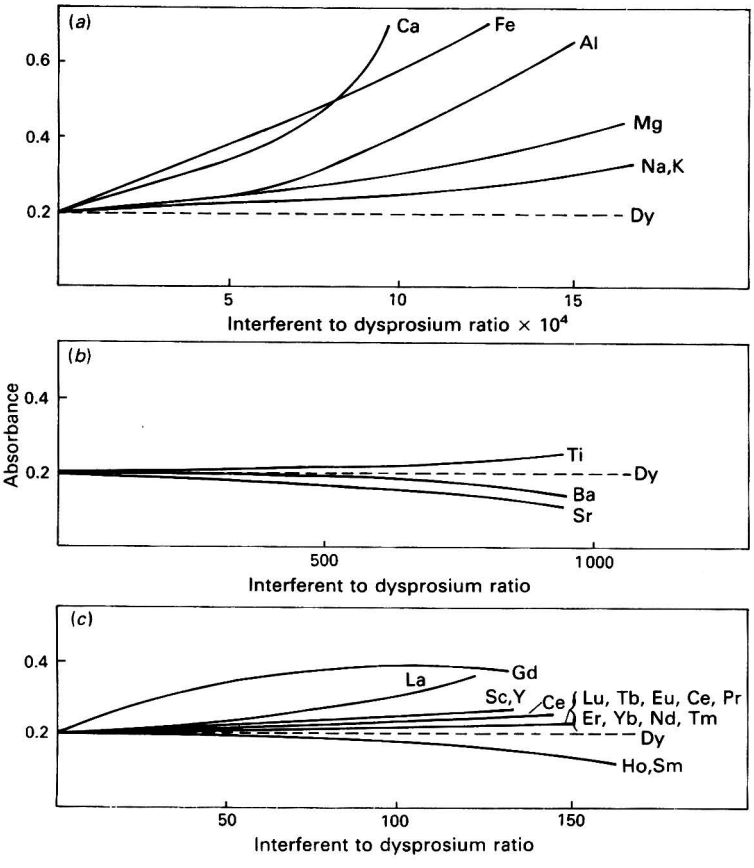

Fig. 1. Interferences of (*a*) major, (*b*) minor and (*c*) trace elements on the electrothermal AAS determination of dysprosium.

Apparatus

A Varian Techtron AA 775 atomic-absorption spectrophotometer, equipped with a Hewlett-Packard storage oscilloscope, Model 1223 A, as the recorder, was used.

The atomisations were carried out using a Varian Techtron CRA–63 carbon rod atomiser and the following parameters: 60 s at 90 °C in the first, drying stage; 20 s at 1450 °C in the second, ashing stage; and 3 s at 2800 °C in the third, atomisation stage. The translation of voltage setting into temperature setting was made using the charts published by Varian Techtron.[7] A flow of ultrapure nitrogen was used during the atomisations.

The aliquots of sample injected were of constant volume (5 μl).

The wavelengths used were 421.2 nm for dysprosium, 410.4 nm for holmium, 371.8 nm for thulium and 398.8 nm for ytterbium.

Results and Discussion

Dysprosium Interferences

Fig. 1 shows the interference caused by the presence of some major, minor and trace elements in the atomic-absorption spectrophotometric determination of dysprosium. As shown, all the major elements have enhancing effects and calcium appears as the strongest interfering element. These interferences, although having the same effect, are probably

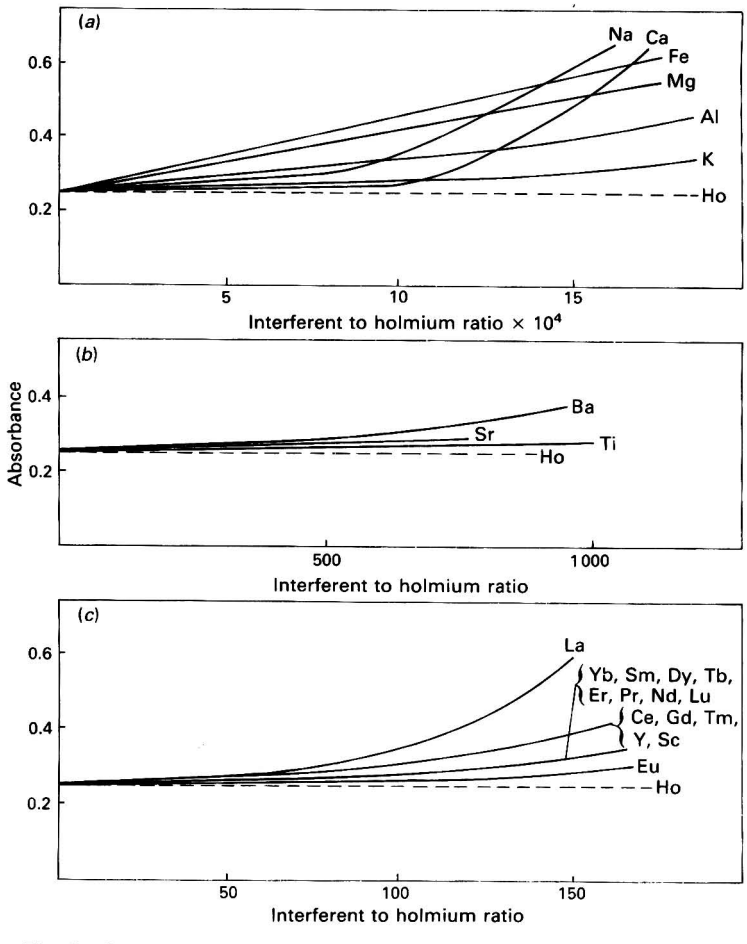

Fig. 2. Interferences of (*a*) major, (*b*) minor and (*c*) trace elements on the electrothermal AAS determination of holmium.

due to diverse causes. The enhancing effect of alkali metals may be postulated as being caused by some chemical or physical interference and may even be partially attributable to ionisation of metal atoms as shown by the absence of absorbance values when solutions containing only sodium or potassium were atomised at the dysprosium wavelength. However, the enhancing effect of the alkali elements disappears when the sample solution is ashed at a temperature of 1800 °C, when these elements are volatilised.

Minor elements have both depressing (barium and strontium) and enhancing effects (titanium); the former were tested at a common silicate rock ratio (about 1000) but the latter may also be present as a major element, with a subsequent increase in its interfering action.

The interferences due to the trace elements occur, for the most part, at ratios that are higher than those commonly present in silicates: only gadolinium shows a strong enhancing power but its concentration in silicates is of the same level as that of dysprosium.

Using non-treated graphite rods, dysprosium shows a very high tendency to form carbide compounds resulting in memory effects that disappear with subsequent firings without the sample: however, these effects are reproducible enough to allow a good evaluation of the interference effects due to the addition of foreign salts.

Holmium Interferences

As shown in Fig. 2 all the major elements have enhancing effects similar to those shown for dysprosium and discussed above, although less enhancement is caused by aluminium.

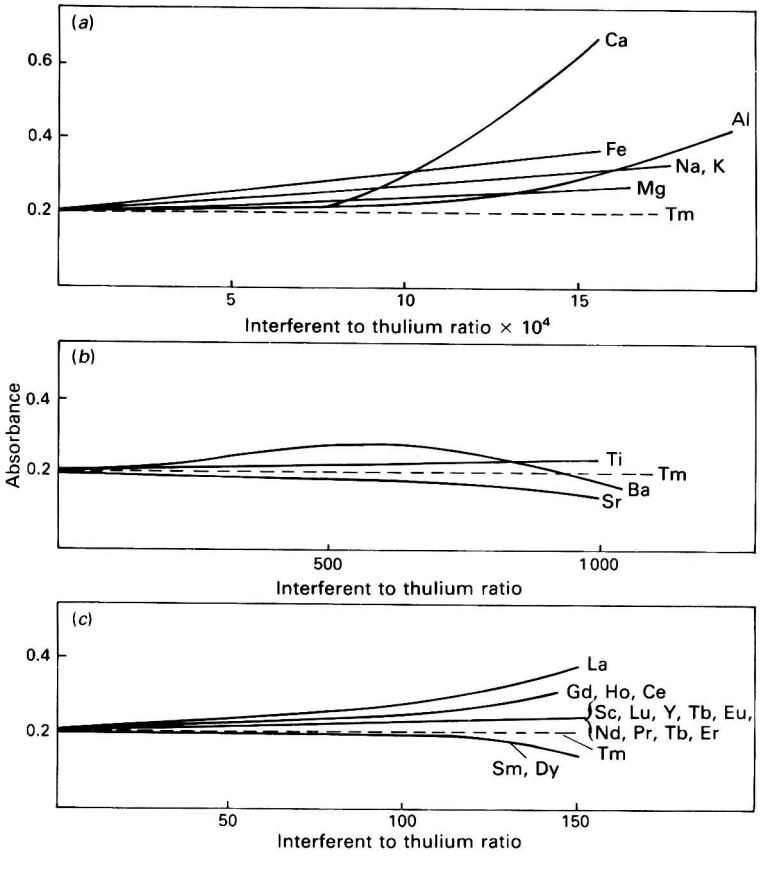

Fig. 3. Interferences of (*a*) major, (*b*) minor and (*c*) trace elements on the electrothermal AAS determination of thulium.

The greatest difference is in the behaviour of sodium atoms, which cause interference even when high ashing temperatures are used in an attempt to overcome the interferences, as was possible for dysprosium; under these conditions only the enhancement caused by potassium was avoided.

All the minor elements have enhancing effects showing (for barium and strontium) opposite trends to those observed for dysprosium.

The behaviour of trace element interactions is positive for all of the elements, with the disappearance of the strong interfering effect of the gadolinium.

Thulium Interferences

Fig. 3 shows that the general trends of the major element interferences are again similar to those shown for dysprosium, the only difference being in the intensities of the interaction effects. Also the minor element interferences investigated, such as those due to the presence of a few thousand parts per million of titanium, barium and strontium, are similar to those shown for dysprosium, except for that of barium, which causes enhancement in the ratio range 200–700 and then causes depression. The effects due to the presence of the other lanthanides fall between those trends shown for dysprosium and holmium, the depressing effect due to the presence of samarium and dysprosium being balanced by the disappearance of the strong enhancing effect of gadolinium.

Ytterbium Interferences

The interference effects of the major, minor and trace elements in the atomic-absorption spectrophotometric determination of ytterbium are particularly interesting because they are

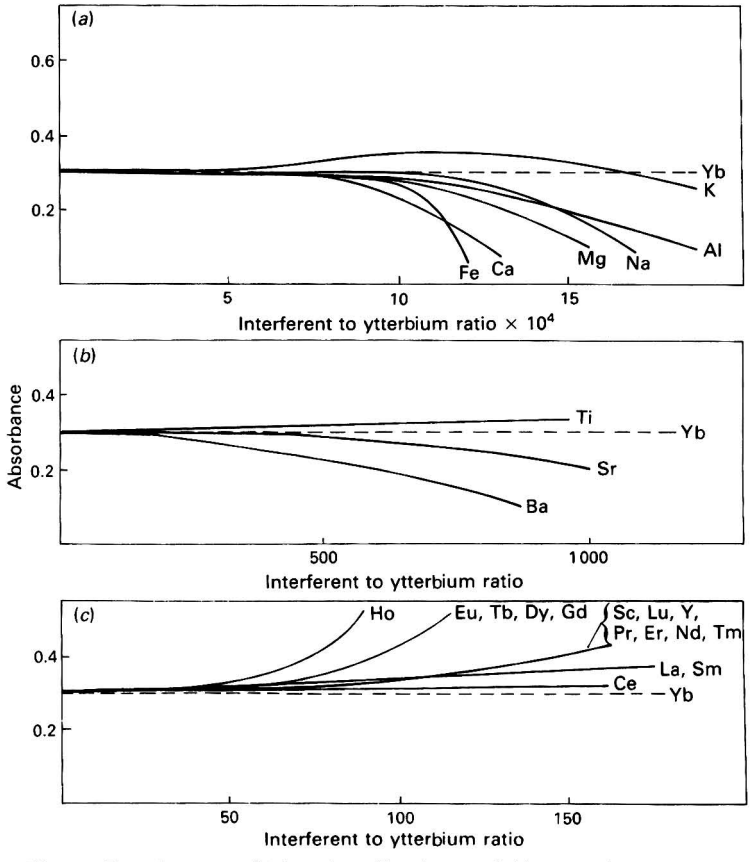

Fig. 4. Interferences of (*a*) major, (*b*) minor and (*c*) trace elements on the electrothermal AAS determination of ytterbium.

very different from those shown for the other lanthanides already mentioned. These trends are shown in Fig. 4. The main difference is in the strongly depressing effect caused by the addition of all major elements. Minor elements cause similar effects to those discussed previously, the only difference being in the absolute intensities. Holmium, dysprosium, ytterbium and gadolinium produced strong enhancing effects.

Conclusions

A general comparison of all the enhancing and depressing effects due to the interferences caused by the presence of major, minor and trace elements present in a common silicate matrix is shown in Table II, which includes the results obtained previously for europium. The most remarkable difference in the interference effects on the rare earth elements investigated is undoubtedly the strong depression in the determination of ytterbium caused by the presence of major elements.

Few of these effects may be explained by the usual spectral considerations such as spectral buffering or other general optical causes; we think that a possible way of investigating them may be to take into consideration all the chemical reactions that may occur at high temperatures in the presence of high amounts of carbon.[8–10]

TABLE II

TRENDS OF SOME INTERFERING MAJOR, MINOR AND TRACE ELEMENTS IN THE ELECTROTHERMAL ATOMIC-ABSORPTION SPECTROPHOTOMETRIC DETERMINATION OF DYSPROSIUM, EUROPIUM, HOLMIUM, THULIUM AND YTTERBIUM

++ = Strongly positive; + = positive; ± = slightly positive; ∓ = slightly negative; − = negative; −− = strongly negative; and +− or −+ = anomalous trend that starts positive (or negative) and becomes negative (or positive).

	Major elements						Minor elements				
	Na	K	Ca	Mg	Fe	Al	Ba	Sr	Ti	Y	Sc
Dysprosium	±	±	++	+	++	++	∓	∓	±	+	+
Europium	±	±	++	±	++	++	∓	∓		+	+
Holmium	++	±	++	+	+	±	±	±	±	+	+
Thulium	±	±	++	±	+	+	+−	∓	±	±	±
Ytterbium	−−	+−	−−	−−	−−	−−	−−	−	±	+	+

	Trace elements													
	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Dysprosium	++	+	±	±	∓	±	++	±		∓	±	±	±	±
Europium	−	∓	∓	∓	−		++	∓	∓	−	∓	∓	∓	+
Holmium	++	+	±	±	±	±	+	±	±		±	+	±	±
Thulium	++	+	±	±	∓	±	+	±	∓	+	±		±	±
Ytterbium	±	±	+	+	±	++	++	++	++	++	+	+		+

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SHORT PAPERS

Some Causes of Bias in the Measurement of Dissolved Oxygen Using Certain Modifications of the Winkler Method

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Keywords: Dissolved oxygen determination; Winkler modification; nitrite interference; iron(III) interference; phosphate modification

In some areas both iron and oxidised nitrogen concentrations in water may be high enough to interfere in the Winkler method for the determination of dissolved oxygen.[1] When this occurs, it is common practice to combine modifications to eliminate the problem, namely, the azide modification for nitrite[2–4] and replacement of sulphuric acid with 90% phosphoric acid for iron(III) interference.[3,4] Use of this combination has also been reported in semi-micro analysis for dissolved oxygen in the presence of high nitrate concentrations.[5] In this instance the phosphoric acid is used to remove the positive bias caused by the interaction of concentrated sulphuric acid and concentrated alkaline iodide. However, during a comparison of methods in a water in which both iron(III) and nitrite are present in relatively high concentrations, a negative bias was observed when the modifications were combined. This paper reports the results of a study of the validity of this bias and the conclusions that can be drawn from it.

Experimental

For ease of sampling, lake water on tap in the laboratory was used in most of this study. This water is abstracted from a bore hole on the side of Lake Windermere and the pH is adjusted by a calcium carbonate lining. For the sake of consistency only the results on the tap water are reported, but all the major steps were checked to ensure that water from other local lakes gave the same results.

Reproducible samples of water with the same dissolved oxygen concentration were obtained for each experiment by connecting a plastic tube to a tap, allowing the tap to run for several minutes to clear the pipework of stagnant water, inserting the tube into the bottom of a 100-ml glass bottle and, after adjusting the flow-rate to prevent entrainment of air bubbles, flushing the bottle with water for several seconds. The bottle was then stoppered with a ground-glass stopper. All the bottles were treated with the appropriate fixative[3] in random order and titrated in the same order, within a few minutes of iodine development.

Development of the iodine was carried out according to the standard texts[3,4] and 50 ml of the solution were titrated against 0.0125 N sodium thiosulphate solution delivered from a 5-ml piston burette using starch solution as indicator. The standard iodine solution was prepared from a vial (CVS solution, BDH Chemicals) and diluted before use to give a similar titration volume to the dissolved oxygen titrations.

Results and Discussion

Five replicate analyses of the dissolved oxygen content of water were carried out using (*a*) the normal Winkler method (no azide, concentrated sulphuric acid), (*b*) azide, concentrated sulphuric acid, (*c*) no azide, 90% phosphoric acid and (*d*) azide, 90% phosphoric acid. The means and standard deviations of the five replicates for each method are given in Table I.

The first three methods gave results that were not significantly different from each other at the 95% confidence level, but the combination of modifications in (*d*) gave a result with a mean difference of 0.42 mg l^{-1} ($\sigma = 0.24$) lower. Negative biases were observed over a range of dissolved oxygen concentrations from 5 to 13 mg l^{-1}.

TABLE I

COMPARISON OF METHODS FOR ELIMINATING INTERFERENCE

Method*	Acid added†	Azide added	Dissolved oxygen/ mg l^{-1}	Standard deviation/ mg l^{-1}
(*a*)	S	No	9.24	0.021
(*b*)	S	Yes	9.17	0.050
(*c*)	P	No	9.12	0.072
(*d*)	P	Yes	8.68	0.220

* See text.
† S = concentrated sulphuric acid; P = 90% phosphoric acid.

It was possible that this lower value obtained with method (*d*) was the real value and the higher values were caused by interferences, although the low iron and nitrite levels in tap water make this unlikely. This was tested by using the fluoride modification[3] instead of the phosphoric acid modification for removal of the iron(III) interference (this method tends to be less popular than the phosphoric acid step as it involves an extra addition of reagent). Replicate determinations using two methods, (*a*) azide, sulphuric acid and (*b*) azide, fluoride, sulphuric acid, were carried out. The means and standard deviations of the five replicates were (*a*) 10.20 $\pm$ 0.16 mg l^{-1} and (*b*) 10.28 $\pm$ 0.11 mg l^{-1}. The methods produced results without significant difference at the 95% confidence level and show that the bias is likely to be due to the combined effect of the two modifying reagents.

Azide and either sulphuric acid or 90% phosphoric acid were then added to five replicates each of the standard iodine solution. As a result, the final iodine concentrations in the solutions were the same as would be present after iodine development using the normal azide modification and the combined modification. The means and standard deviations of the five replicates were 8.48 $\pm$ 0.024 mg l^{-1} and 8.31 $\pm$ 0.076 mg l^{-1}, respectively, which are significantly different at the 95% confidence level. The lower concentration in the combined method implies that the azide - phosphoric acid combination probably interferes by reducing the iodine concentration in some way.

Rees and Hilton[6] reported that no change had taken place after 24 h in a stoppered bottle in which iodine had been developed using sulphuric acid and the azide modification. However, no mechanism could be envisaged by which azide - phosphoric acid could reduce iodine and azide - sulphuric acid would not. Using fresh reagents, a series of Winkler determinations were carried out on replicate samples and the titration step was delayed for different periods up to 24 h after iodine development. The results are shown in Fig. 1. As can be seen, there is a difference both in time of onset and rate of iodine consumption between the two methods. There appears to be an immediate onset of iodine reduction in the combined azide - phosphoric acid modification and therefore this combination is incompatible and should not be used. In analysis with larger sample volumes this can be achieved by using sulphuric acid with the fluoride modification. Unfortunately, with the semi-micro method there is apparently no way of removing nitrate interference while still retaining low blanks.

The standard methods[3,4] are not specific as to the maximum length of time that a developed bottle of iodine may be left before it must be titrated. However, this work suggests that normally developed Winkler bottles in which the azide modification is being used should be titrated within about 2 h or a negative bias will be obtained.

This result is contrary to that of Rees and Hilton.[6] A possible explanation may be that stored azide reagent was used in the latter work. This possibility was strengthened when the original comparison of methods used in this paper was attempted using 3-month-old azide reagent. No differences between the methods could be obtained although a repeat measurement using freshly prepared alkaline iodide azide reagent gave the expected difference. The standard texts give no indication of shelf-life and hence many users of this reagent make and store large amounts. This gradual decay of the azide is difficult to detect under normal circumstances but can be followed using the techniques discussed above. Five replicates of the normal azide modification and the azide - phosphoric acid modification were measured and compared over an extended period of time from the preparation of a fresh stock of alkaline iodide azide reagent.

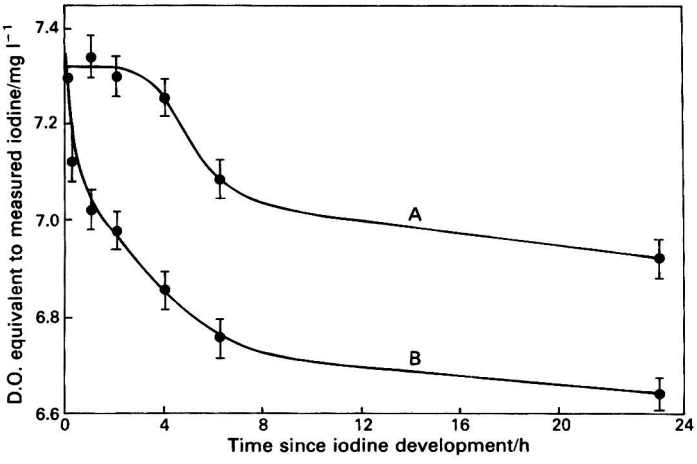

Fig. 1. Effect of time on iodine concentration in stoppered Winkler bottles. A, Azide and concentrated sulphuric acid present; B, azide and 90% phosphoric acid present. Error bars represent 1 standard deviation either side of the mean, obtained from replicate samples.

The results are shown in Fig. 2. As can be seen, the bias between methods is greatly reduced after the reagent has been stored for more than 1 month.

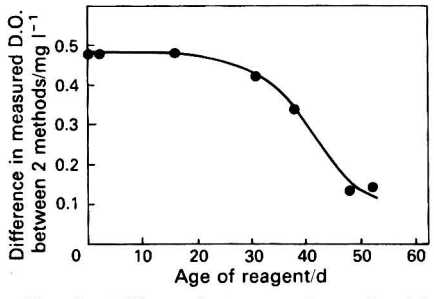

Fig. 2. Effect of storage time of azide reagent on the difference in dissolved oxygen measured by the two modifications. The negative bias is reduced with time.

Conclusions

In the Winkler determination of dissolved oxygen the following should be noted.

(*a*) If both nitrate - nitrite and iron(III) are present in sufficient concentrations to interfere with the Winkler method then the use of the azide modification with phosphoric acid will produce a negative bias. The fluoride modification should be used in place of the phosphoric acid modification in macro determinations. No procedure can be recommended for removing nitrate interference in semi-micro methods without introducing bias.

(*b*) When using the azide modification, the developed iodine must be determined within 2 h even if the vessel is sealed, otherwise negative bias will result.

(*c*) Alkaline iodide azide reagent should be stored for no longer than 1 month.

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Differential-pulse Polarographic Determination of the Coccidiostat Arprinocid in Feed Pre-mixes

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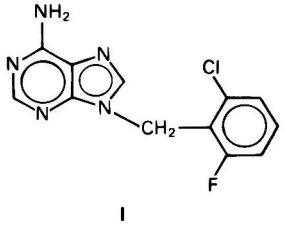
Keywords: *Arprinocid determination*; *differential-pulse polarography*; *feed pre-mixes*

Interest in applications of differential-pulse polarographic analytical techniques continues to increase, as evidenced by the number of recent reports, such as studies of many drugs in biological samples[1,2] and in formulations.[3–6] The 9-substituted adenine derivative arprinocid [9-(2-chloro-6-fluorophenylmethyl)-9*H*-purine-6-amine, **I**], a new anticoccidial agent,[7,8] has been determined in medicated feeds using methods based upon either spectrophotometric[9] or high-performance liquid chromatographic[10,11] measurements after appropriate isolation procedures.



I

The adenine nucleus of arprinocid, which is substituted at the 9-position in analogy with adenine nucleotides, is a well known electroactive moiety that engages in complex, non-reversible reductions at many different electrode surfaces, as well as simple, reversible electron-transfer reactions in biological systems. Indeed, even the biological mode of anticoccidial action of this drug depends on its adenine ring, as it probably functions via partial inhibition of hypoxanthine incorporation into purine nucleotides.[12] The commercial pharmaceutical formulation of this coccidiostat is a feed pre-mix containing 12% of arprinocid; for administration in the feed, 500 g of this pre-mix is blended into 1000 kg of feed to yield a final finished arprinocid feed concentration of 60 mg kg^{-1}. This paper describes a rapid analytical method for the determination of arprinocid in this product based on electrochemical measurement at the dropping-mercury electrode.

Experimental

Apparatus

Differential-pulse polarographic measurements were made using a Princeton Applied Research, Model 174A, polarographic system with a Hewlett-Packard 7040A *X* - *Y* recorder.

It was operated in a three-electrode configuration with a conventional dropping-mercury indicator electrode, a platinum wire auxiliary electrode and a silver - silver chloride reference electrode. A Princeton Applied Research, Model 174/170, drop knocker regulated the mercury drop times. The acidity was adjusted with concentrated hydrochloric acid and measured on an Orion, Model 801, digital pH meter equipped with an Orion micro-combination electrode. All solvents were of analytical-reagent grade and used without further purification.

The differential-pulse polarograms were measured over the range −0.90 to −1.20 V *versus* a silver - silver chloride electrode with a forced drop time of 1 s, a scan rate of 2 mV s^{-1} and a modulation amplitude of 25 mV. The current range was 2 μA full scale and temperature was maintained at 25.0 ± 0.1 °C.

Procedure

Accurately weigh 0.2 g of pre-mix and transfer it into a 50-ml centrifuge tube. Add, by pipette, 20.0 ml of chloroform and shake mechanically for 30 min. Filter approximately 10 ml of the chloroform extract through a Whatman No. 42 filter-paper. Transfer, by pipette, 2.0 ml of the chloroform extract into a 50-ml centrifuge tube. Place the tube in a water-bath at a temperature of about 50 °C and evaporate to dryness under nitrogen. Add, by pipette, 20.00 ml of 0.1 N hydrochloric acid and 5 ml of carbon tetrachloride to the tube and shake for 30 min in a mechanical shaker.

Transfer the complete two-phase analytical solution into the electrochemical cell, without separation. The carbon tetrachloride lower organic phase does not affect the electrochemical properties in the upper aqueous 0.1 N hydrochloric acid analytical solution. Record the differential-pulse polarogram from −0.90 to −1.2 V *versus* a silver - silver chloride electrode and measure the current peak height to the −0.90 V base line. Determine the arprinocid concentration from a previously prepared calibration graph or a concurrently run analytical standard.

Results and Discussion

The electrochemistry of adenine and its nucleotides and nucleosides has been extensively studied by Elving and co-workers.[13–18] In common with adenine and other electroactive 9-substituted adenines, differential-pulse polarography of arprinocid reveals an E_{peak} of about −1.13 V *versus* a silver - silver chloride electrode in an acidic aqueous solution (Fig. 1). This potential, which varies markedly with analyte concentration and with pH, can be

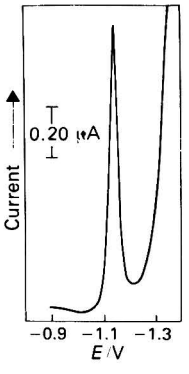


Fig. 1. Differential-pulse polarogram, *versus* a silver - silver chloride electrode, of arprinocid in 0.1 N hydrochloric acid obtained from the analysis of a pre-mix sample. PAR 174A polarographic analyser; drop time, 1 s; scan rate, 2 mV s^{-1}; modulation amplitude, 25 mV; and [arprinocid], about 1 × 10^{-3} M.

assigned as a single four-electron wave, which encompasses an initial two-electron reduction of the N(1)–C(6) bond electroactive site,[13,16,18] essentially unperturbed by the 2-chloro-6-fluorobenzyl substituent on N(9). Previous studies[14,16] have also shown that a sugar moiety on N(9), similarly, does not significantly affect the electrochemistry, which is generally that of the purine base. The pH and concentration effects on the reduction of arprinocid are shown in Figs. 2 and 3, which demonstrate that arprinocid becomes more difficult to reduce with increasing concentration and pH. Elving and Webb[17] have related the shift to a more

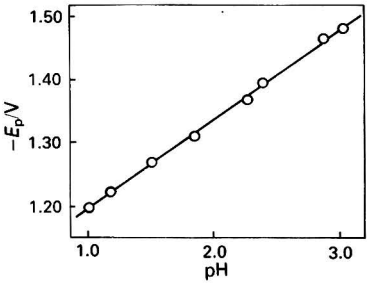


Fig. 2. Effect of pH on E_{peak} of arprinocid in aqueous solution determined by differential-pulse polarography. [Arprinocid] about 5×10^{-3} M.

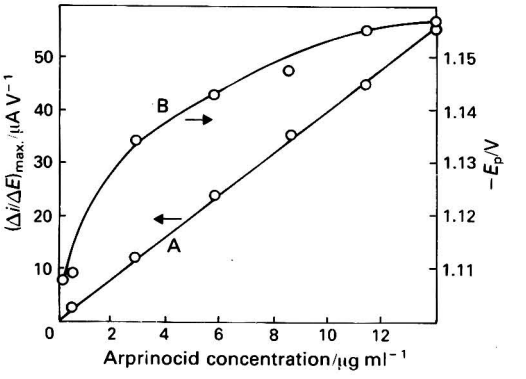


Fig. 3. Calibration line (A) and variation of E_{peak} (B), *versus* a silver - silver chloride electrode with concentration in differential-pulse polarography of arprinocid in 0.10 M hydrochloric acid.

TABLE I

REPLICATE DIFFERENTIAL-PULSE POLAROGRAPHIC DETERMINATIONS OF ARPRINOCID IN FEED PRE-MIXES

Arprinocid in pre-mix, %	Sample mass/g	Current/μA	Arprinocid found, %
10.2	0.1563	0.724	10.4
	0.1672	0.732	10.4
	0.2167	0.972	10.6
	0.1963	0.856	10.3
	0.1852	0.820	10.5
	0.2035	0.900	10.5
	Average		10.5
	Relative standard deviation ..		1.00
12.2	0.1655	0.892	12.5
	0.1967	1.040	12.3
	0.1926	1.020	12.3
	0.1705	0.926	12.6
	0.1863	1.000	12.4
	0.1967	1.028	12.1
	Average		12.4
	Relative standard deviation ..		1.42
14.3	0.1646	0.948	14.0
	0.1840	1.156	15.2
	0.1635	0.964	14.3
	0.1990	1.116	13.6
	0.1567	0.920	14.2
	0.1700	0.998	14.2
	Average		14.3
	Relative standard deviation ..		3.71

negative potential to the strong intermolecular association at higher concentrations. Temerk and Kamal[19] have reported similar potential shifts with pH in their studies of the adenine electroactive moiety. For analytical purposes, the data confirm that the absolute differential-pulse polarographic peak height (relative to the base line at −0.90 V *versus* a silver - silver chloride electrode) is a linear function of the drug concentration over the range 1.8×10^{-6} to 4×10^{-4} M arprinocid in 0.1 M hydrochloric acid. This medium was selected as the final analytical solution for pulse-polarographic measurement.

Accuracy and Precision

The accuracy and precision of this analytical method were determined with six replicate analyses of each of three complete formulated arprinocid pre-mixes. The formulated product is blended to contain 12% arprinocid on a carrier of rice hulls using 3.5% soybean oil as a binder and 0.5% ethoxyquin antioxidant stabiliser to inhibit the oxidative polymerisation of the oil. The pre-mixes examined here were formulated to contain 10.2, 12.2 and 14.3% arprinocid to evaluate the linearity of the method over a range of concentrations of ±15% relative to the commercial product. The results are presented in Table I. Over this concentration range, the method has an accuracy (mean error) and a precision (standard deviation) of less than ±2% relative.

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Communications

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

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

Proton Magnetic Resonance Shifts of the Trimethylsilyl Derivatives of Silicate Materials

Keywords: *Trimethylsilylation*; *silicate analysis*; *ASIS effect*; *proton magnetic resonance*

The analysis of silicate minerals by means of their trimethylsilyl derivatives was first proposed by Lentz.[1] Subsequently, a number of variations on this derivatisation technique have been suggested by Gotz and Masson,[2] Tamas[3] and Dent Glasser and Sharma[4]; the application of these methods to the analysis of glasses, cements as well as mineral silicates has recently been reviewed.[5]

In general, derivatisation is accomplished by acid leaching the silicate material to give a silicic acid, which is then converted in a suitable solvent system to a trimethylsilyl ester as follows:

$$^{-}O[(SiO^{-})_2]_nO^{-} + HCl/H_2O \xrightarrow{\text{cation removed}} HO[(SiOH)_2]_nOH$$

$$HO[(SiOH)_2]_nOH + [(CH_3)_3SiO]_2O \xrightarrow{\text{ester formation}} XO[(SiOX)_2]_nOX$$

$$X = Si(CH_3)_3$$

The characterisation of the trimethylsilyl esters formed then gives structural information on the molecular size distribution of silicate anions in the original silicate mineral. A new method of polyorganosiloxane analysis based on a nuclear magnetic resonance (NMR) technique is proposed, in contrast to the more usual methods which have included thin-layer chromatography,[6] gas - liquid chromatography (GLC)[7] and, more recently, gel permeation chromatography (GPC) and high-performance liquid chromatography (HPLC).[8]

Experimental

Sample Preparation

Sample A, containing hexamethyldisiloxane (MM), tetrakistrimethylsiloxysilane (QM_4) and hexakistrimethylsiloxydisiloxane (Q_2M_6), was prepared by trimethylsilylating olivine (300 mesh, 168 h) using the Lentz method. This sample on subsequent analysis was shown to contain also some higher relative molecular mass homologues.

Sample B was derived from suzorite, a mica mineral. It was used to show the application of the technique to QM_4 and Q_2M_6 analyses even in the presence of a broad molecular size distribution, as shown in Table I.

TABLE I

ASIS* EFFECT ON Q/M POLYORGANOSILOXANES (60 MHz)

Pyridine added/ $\mu l \times 10^2$	Chemical shift†/Hz			Chemical shift, p.p.m.		
	QM_4	Q_2M_6	$>Q_2M_6$	a‡	b‡	c‡
0	6.2	6.2	9.0	8.7	7.4	7.8
1	7.5	8.0	11.0	8.7	7.3	7.7
2	8.0	8.5	11.0	8.7	7.3	7.7
3	8.5	10.0	12.0	8.7	7.4	7.7
4	9.5	11.0	13.0	8.8	7.4	7.8
6	10.5	12.5	ppt	8.8	7.4	7.8
9	10.7	12.6	ppt	8.7	7.3	7.6
14	12.1	14.5	ppt	8.7	7.3	7.7
18	12.1	14.5	ppt	8.8	7.3	7.7

* ASIS = aromatic solvent-induced shift.
† Chemical shift relative to tetramethylsilane internal standard.
‡ Pyridine protons: a = *ortho*, b = *meta* and c = *para* to the nitrogen atom.

Analysis by GLC

A Pye Series 104 gas chromatograph equipped with a flame-ionisation detector (FID) was used to analyse the mixtures prepared above. A 9 ft 5% OV-1 column was used, temperature

programmed from 90 to 300 °C at 16 °C min^{-1}; the injection temperature was 130 °C, the detector temperature was 350 °C and 0.1-μl injections were used. A second method used a 5 ft 5% SE-30 column, temperature programmed from 110 to 300 °C at 16 °C min^{-1}; the injection temperature was 110 °C, the detector temperature was 350 °C and 0.1-μl injections were used. In both instances dodecane was used as an internal standard.

Analysis by GPC and HPLC

A portion of sample A (2.505 g) was added to the top of a column consisting of a 32 × 2.2 cm i.d. glass tube with a porous PTFE disc at the bottom, which had previously been wet-packed with a gel (Biobeads SX1). The Biobeads SX1 (200–45 mesh) had been allowed to swell in chloroform - propan-2-ol (3 + 1) for 40 h. The effluent was collected in fractions of about 2 ml and the eluent was allowed to evaporate at 60 °C for 24 h. The residues were weighed and analysed by HPLC.

Sample A was also analysed directly by HPLC without pre-separation by GPC. A Waters 501 instrument with a column bank of seven μStyragel columns with permeability ranges from 10 to 1000 nm was calibrated by using Waters Associates polystyrene standards, a range of poly-dimethylsiloxane fluids and also a range of QM polymers. For quantitative work *tert*-butyl-benzene was used as an internal standard.

Analysis by Proton Magnetic Resonance (PMR)

A portion of sample A (9.6 g) was dissolved in deuterochloroform (10 ml) and pyridine (120 ml) was added. The mixture was shaken and left to stand overnight. An aliquot of the solution was taken and the sample was analysed using a Perkin-Elmer R12B 60-MHz NMR spectrometer.

TABLE II

ASIS EFFECT ON Q/M POLYORGANOSILOXANES (400 MHz)

Benzene added/ $\mu l \times 10^2$	Chemical shift*/Hz		
	Q_2M_6	QM_4	MM
0.5	74.58	59.20	29.41
1.0	86.79	69.45	36.74
1.5	90.45	72.63	39.94
2.0	94.36	75.80	40.64
2.5	96.55	77.76	41.87
3.0	98.26	79.22	42.83
3.5	99.24	79.95	43.09
4.0	99.73	80.44	43.33
4.5	99.97	80.69	43.58
5.0	100.46	80.93	43.82
5.5	100.95	81.42	43.82
6.0	101.19	81.42	43.82
6.5	101.68	81.90	44.06
7.0	101.68	81.90	44.06

* Chemical shift relative to benzene internal standard.

A second sample of A (50 μl) was added to benzene (50 μl) and the spectrum was measured on a Brucker 400-MHz NMR spectrometer. The spectrum was re-measured after successive additions of benzene (50 μl) and the results are shown in Table II.

Results and Discussion

Chromatographic Methods of Analysis

The most commonly used method for the analysis of the trimethylsilyl derivatives of a silicate material has been GLC. Unfortunately, this technique will only show the presence of derivatives up to about Q_6M_{14} or in the relative molecular mass range up to 1500, whereas as we have shown elsewhere,[8] some of the derivatives from mica minerals reach 135000. However, in many instances it is the low relative molecular mass species, especially **I** and **II**, which are of most interest.

$[(CH_3)_3SiO]_4Si$ — **I** (QM_4)

$[(CH_3)_3SiO]_6Si_2O$ — **II** (Q_2M_6)

Even in this low relative molecular mass region we have found that GLC analysis is unsatisfactory for quantitative analysis. The SE-30 column is better than the OV-1 column, as the latter suffers from severe peak tailing. There is little reproducibility from repeated injections of QM_4 and Q_2M_6, possibly owing to build-up of silica on the FID.

Sample A (2.505 g) was analysed by GPC, each component being separated and weighed. The affinity of hexamethyldisiloxane for the column eluents made separation difficult and this method gives a hexamethyldisiloxane yield by difference (Table III). A second sample of A was analysed by quantitative HPLC (Table IV).

TABLE III

RESULTS FOR ANALYSIS OF SAMPLE A BY GPC

Compound	Mass/g	Yield, %
MM*	1.399	55.8
QM_4	0.801	32.0
Q_2M_6	0.296	11.8
Q_3M_8 (isomers)	0.002	0.4
Q_4M_{10} (isomers)	0.006	
White solid†	0.001	

* MM{hexamethyldisiloxane, $[CH_3)_3Si]_2O$} was calculated by difference.

† The white solid was in the relative molecular mass range 2.5×10^3–9.0×10^3.

TABLE IV

RESULTS FOR ANALYSIS OF SAMPLE A BY HPLC

Compound	Yield,* %	Standard deviation, %	Variance, %
MM	57.3	0.6	0.3
QM_4	30.8	0.5	0.2
Q_2M_6	11.3	0.3	0.1

* Average values based on a series of six repeated observations. The yields of compounds of relative molecular mass greater than Q_2M_6 were difficult to detect on the same sensitivity ranges.

These two chromatographic procedures give good agreement, especially when the volatile nature of the hexamethyldisiloxane is considered. These methods suffer from their time-consuming nature, which makes the investigation of kinetics, for example, rather difficult.

Nuclear Magnetic Resonance Experiments

The methyl protons on a trimethylsilyl group attached to a siloxane backbone would appear to be chemically equivalent. This is reflected in the singlet peak that is observed when polyorganosiloxanes derived from minerals are analysed by NMR spectroscopy. The higher relative molecular mass homologues do show a very small downfield shift, which may be due to the relative proportions of methyl groups to oxygen atoms in an individual molecule. Some preliminary work had shown that a number of shift reagents do not separate the various components of the olivine polymer. However, the addition of pyridine to a polymer sample B produced the effect shown in Table I. This table refers to a sample derived from a mica; the polyorganosiloxanes present as a result of the trimethylsilylation reaction are in the relative molecular mass range 384–135000. The NMR effect is such that even under these unfavourable conditions QM_4 and Q_2M_6 signals are moved, and can be quantitatively analysed. This effect was also observed using several aromatic solvents (benzene, benzaldehyde, chlorobenzene and methylnaphthalene) or even using a neutral solvent such as deuterochloroform and subsequently adding a solid aromatic species such as naphthalene.

The addition of known pure samples of MM, **I** and **II** to the NMR experiments allowed the easy identification of the three distinct peaks as MM, QM_4 and Q_2M_6.

The nature of this effect has been the subject of much debate and amongst the theories proposed two seem to have found most favour: either there is a 1 : 1 complex between the solvent and the solute or, as has been suggested more recently, the solvent clusters around the solute, changing the magnetic anisotropy.[9] Fragments such as Ph–C–CH_2–Si$(CH_3)_3$[10] and the trimethylsilyl derivatives of *o*-phenylphenol undergo an intermolecular ASIS effect as the plane of the benzene ring is allowed to interact with the methyl protons attached to the silicon atom.[11] Intramolecular effects have also been observed in the site of methoxy substitution in common trimethylsilyl ethers.[12]

The analysis of sample A by the use of an aromatic solvent shift reagent gave on integration the results shown in Table V.

TABLE V

RESULTS FOR ANALYSIS OF SAMPLE A USING AN AROMATIC SOLVENT SHIFT REAGENT

Compound	Yield, %	Standard deviation, %	Variance, %
MM	57.7	1.7	2.8
QM_4	30.6	1.5	2.3
Q_2M_6	11.7	0.4	0.1

These initial results from this new technique are very satisfactory, although they are less precise than those obtained with the two other methods. Improvements have already been made to peak shape and separation by running spectra at 400 MHz*; this will enable the subsequent analysis to be improved (see Table II). Analysis of the 400-MHz spectra gave QM_4 73.0% and Q_2M_6 27.1%, which compare favourably with the figures given in Table VI.

TABLE VI

COMPARISON OF RESULTS OBTAINED BY DIFFERENT METHODS

Compound	Concentration, %		
	GPC	HPLC	NMR (60 MHz)
MM	55.8	57.3	57.7
QM_4	32.0	30.8	30.6
Q_2M_6	11.8	11.3	11.7

The NMR method offers a quick and efficient method for the analysis of the low relative molecular mass homologues and this method will be developed to include Q_3M_8 and Q_4M_{10}. Future work will concentrate on the development of the technique and the use of external standards to explain further the ASIS phenomenon. In addition, it will be used to study the kinetics of formation and rearrangements of Q/M polymer systems, especially in the kinetics of Portland cement formation.

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Rapid Determination of Albumin-bound Zinc in Human Serum by Simple Affinity Chromatography and Atomic-absorption Spectrophotometry

Keywords: *Zinc*; *albumin*; *affinity chromatography*; *atomic-absorption spectrophotometry*; *electrothermal atomisation*

Measurements of the total concentration of zinc in plasma or serum are of limited value in the assessment of zinc deficiency in clinical medicine. The zinc content of plasma is almost entirely bound to proteins with approximately 50–60% bound to albumin and 30–40% to α_2-macroglobulins. The remainder may be present as low relative molecular mass species. As the concentrations of these carriers are themselves subject to change, the relevance of an abnormal total plasma zinc concentration requires consideration of the zinc content of the individual zinc-binding species. Various techniques have been proposed for the fractionation of zinc-binding proteins, including anion-exchange chromatography,[1] gel filtration,[1–4] sucrose density-gradient centrifugation,[5] salt fractionation[6] and electrophoresis.[7] Some of these procedures may disrupt the binding of zinc to protein whereas others are time consuming or require specialised equipment, limiting their application in clinical laboratories.

Affinity chromatography of human plasma on Reactive Blue 2-Sepharose (Cibacron Blue F3G-A immobilised on Sepharose CL6B) results in the removal of approximately 98% of the albumin.[8] This affinity has been exploited to isolate albumin-bound zinc from that bound to other plasma species in a procedure lasting no more than 30 min. The albumin-containing fraction and the albumin-depleted proteins are eluted separately and analysed for zinc by atomic-absorption spectrophotometry with electrothermal atomisation without further preparation. This provides a simple and rapid method for measuring the zinc concentrations of albumin and α_2-macroglobulin fractions which may be readily applied to clinical investigation.

Experimental

Apparatus

Affinity chromatography

Column. Analytical glass column, 250 × 6 mm i.d., Altex type 252–00 fitted with an adjustable plunger, Altex type 252–05 (Anachem Ltd., Luton).

Pump. Varioperpex II Pump 2120 (LKB Produkter AB, Bromma, Sweden).

Sample valve. Two four-way slider valves, Altex type 201–02, fitted with a by-pass and a laboratory-made 200-μl sample loop (Anachem Ltd.).

Tubing and connections. PTFE tube (0.3-mm), Altex type 200–34, and couplers (Anachem Ltd.). All sample tubes and glassware should be washed with dilute nitric acid (1 + 9) for 2 h and then rinsed six times with de-ionised water.

Atomic-absorption spectrophotometry

A Perkin-Elmer, Model 2380, atomic-absorption spectrophotometer fitted with an HGA-500 graphite furnace and an AS–40 autosampler was used, together with Perkin-Elmer pyrolytically coated tubes, type 2901766.

Reagents

Unless otherwise stated, reagents were of AnalaR or Aristar grade (BDH Chemicals Ltd., Poole).
Ammonium thiocyanate.
Disodium ethylenediaminetetraacetate.
Potassium chloride.
Sodium dihydrogen phosphate, $NaH_2PO_4.2H_2O$.
Disodium hydrogen phosphate, Na_2HPO_4.
Hydrochloric acid, 11 M.
Nitric acid, 15 M.
Zinc chloride solution for atomic-absorption spectrophotometry. Containing 5 mmol l^{-1} of zinc.
Ammonia solution, Primar grade, sp. gr. 0.88. Fisons Scientific Ltd., Loughborough.
Reactive Blue 2-Sepharose. Sigma London Chemical Co., Poole.
Chelex 100 (−400 *mesh*). Bio-Rad Laboratories, Watford.

Procedure

Affinity chromatography of serum

Prepare a starting buffer containing 0.1 M potassium chloride in 0.05 M phosphate buffer adjusted to pH 7.0 by dissolving 2.97 g of sodium dihydrogen phosphate, 4.34 g of disodium hydrogen phosphate and 7.46 g of potassium chloride in 1 l of de-ionised water. Similarly prepare an eluting buffer containing 0.2 M ammonium thiocyanate in 0.05 M phosphate buffer at pH 7.0 by dissolving the same amounts of sodium phosphate salts and 15.2 g of ammonium thiocyanate in 1 l of water. Purify all buffers used for the separation of protein fractions by passage down a column of Chelex 100 converted into the ammonium form.[9]

Swell 1 g of Reactive Blue 2-Sepharose in 50 ml of de-ionised water for 15 min and then wash over a sintered-glass filter with 50 ml of de-ionised water four times.

Decontaminate the buffer reservoirs, pump, column, ultraviolet monitor, sample valves and interconnecting tubing by flushing the system with dilute nitric acid (1 + 9) for 2 h followed by de-ionised water for 4 h. Pack the prepared Reactive Blue 2-Sepharose to a height of 6 cm in a 0.6 cm diameter column using the adjustable plunger to reduce the dead space to zero. Wash the packed resin with 10 bed volumes of 0.002 M disodium ethylenediaminetetraacetate and then equilibrate the resin with 10 bed volumes of starting buffer.

Load 200 µl of sample serum, prepared in trace metal-free tubes, into the sample loop. Introduce the sample to the column in starting buffer at a flow-rate of 0.35 ml min^{-1}. Collect the effluent from the column from the time of sample introduction until its absorbance at 280 nm falls to 0.05 absorbance unit. This first fraction contains serum proteins other than albumin. Replace the starting buffer with eluting buffer and collect the second fraction, containing albumin alone, until the absorbance value is again below 0.05 absorbance unit. Following the elution of albumin, the column may be re-equilibrated with 10 bed-volumes of starting buffer for immediate re-use. Columns used over twenty times have shown no deterioration in performance.

Determination of zinc by atomic-absorption spectrophotometry

Prepare a stock zinc standard solution containing zinc at 200 µmol l^{-1} with 0.5 M hydrochloric acid preservative by diluting 2 ml of zinc chloride standard solution (BDH Chemicals Ltd.) and 2.5 ml of concentrated hydrochloric acid to 50 ml with de-ionised water. From this stock solution prepare calibrating standard solutions containing zinc at 0, 0.2, 0.4, 0.6, 0.8, 1.2, 1.6 and 2.0 µmol l^{-1} in both starting and eluting buffers. Determine the zinc concentrations in the column fractions obtained using the instrumental conditions given in Table I and calibrating standards prepared in the appropriate buffer.

The albumin-bound zinc concentration and the non-albumin-bound zinc concentration in the original sample are easily obtained by:

Zinc concentration associated with protein species (µmol l^{-1}) =

$$\text{Zinc concentration in appropriate fraction } (\mu\text{mol } l^{-1}) \times \frac{\text{volume of fraction (ml)}}{0.2}$$

TABLE I

CONDITIONS FOR ATOMIC-ABSORPTION SPECTROPHOTOMETRY

Instrumental settings—
Wavelength, 213.9 nm; spectral band width, 0.7 nm; deuterium background correction; integration time, 3.0 s; and peak-height measurement, absorbance mode.

Furnace programme—

Stage	Temperature/°C	Ramp time/s	Hold time/s	Internal gas flow-rate/ml s^{-1}
Dry 1	90	10	30	5
Dry 2	100	10	10	5
Ash	500	20	20	5
Base line	500	1	3	1.7
Read	2100	1	5	1.7
Clean	2500	3	5	0.5

Sample volume 5 μl: alternate (wash) volume 6 μl of dilute (1 + 9) hydrochloric acid.

Results

Electrophoresis of samples of both protein fractions on cellulose acetate membranes indicated that the separation of albumin from other protein species was almost complete, supporting previously published observations of the removal of 98% of albumin with this resin.[8] The time required for analysis was 28 min for the chromatographic separation and 2 min atomic-absorption measurement.

The concentrations of zinc associated with albumin and the other protein species were determined in sera from 10 healthy volunteers. These data are given in Table II together with the total zinc concentrations in the sera, having been determined by flame atomic-absorption spectrophotometry.[10]

TABLE II

RESULTS FOR DETERMINATION OF ZINC IN SERA

Zinc concentration/μmol l^{-1}				
Albumin fraction	Non-albumin fraction	Albumin + non-albumin fractions	Total serum	Recovery, %
7.4	5.7	13.1	11.8	111
8.0	5.6	13.6	14.8	91
8.3	6.2	14.5	16.0	90
8.2	6.8	15.0	14.4	104
8.2	5.2	13.4	14.4	93
8.4	4.1	12.5	11.6	107
7.1	6.2	13.3	13.8	96
7.0	5.9	12.9	12.6	102
5.6	4.0	9.6	11.4	84
9.0	4.6	13.6	15.0	91

Discussion

The summation of the zinc concentrations in the albumin and non-albumin fractions gave a reasonably good agreement with the total zinc concentrations that were measured by flame atomic-absorption spectrophotometry (Table II). The total recovery of zinc in the two fractions given as the mean ± standard deviation was 96.9 ± 8.7% ($n = 10$) with a range of 84 — 111%. The percentage of the total serum zinc distributed in the two fractions, given as the mean ± standard deviation (number of observations) were: 58.4 ± 4.8% (10) for the albumin fraction and 41.6 ± 4.8% (10) for the albumin-depleted fraction. These results are in broad agreement with previous studies although the literature does indicate some difference of opinion regarding the relative distribution of zinc in normal serum. These differences may arise from exogenous zinc contamination, disruption of the protein - metal interaction and incomplete protein fractionation. The method presented here is capable of almost complete (98%) separation of albumin from serum and examination with cellulose acetate membrane electrophoresis revealed negligible contamination between fractions, although we intend to investigate this with quantitative immunoelectrophoresis. Disruption of the protein - metal complex is likely to be insignificant because we have found that Reactive Blue 2-Sepharose offers negligible affinity for zinc in the presence of 0.05 M phosphate buffer.

Our initial studies show that free zinc, and that bound to low relative molecular mass species,

may be expected to elute directly from the column in starting buffer. The non-albumin-bound zinc value we have obtained, therefore, represents zinc in these forms in addition to that bound by α_2-macroglobulin. We intend to investigate the relative contribution of the various zinc species in the albumin-depleted fraction shortly.

We thank Professor Barbara E. Clayton for her direction and encouragement. We are grateful to Miss L. Hinks and Mrs. S. Diaper for their technical assistance. Financial assistance from BDH Chemicals Ltd. is gratefully acknowledged. The atomic-absorption equipment was loaned by Perkin-Elmer Corporation, Norwalk, Conn., U.S.A. and Bodenseewerk, West Germany.

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Technique for Reducing the Cycle Time in Atomic-absorption Spectroscopy with Electrothermal Atomisation

Keywords: *Atomic-absorption spectroscopy*; *electrothermal atomisation*; *cycle time*

In a previous paper[1] we described a method for the separation of plasma proteins using ion-exchange chromatography with a constant-strength eluent in which copper and zinc in the separated fractions were determined by pulse nebulisation flame atomic-absorption spectroscopy. This procedure has been in regular use for some time in our laboratory and has generated valuable clinical information. Other workers[2] have used electrothermal atomisation for the determination of copper and zinc in fractions obtained by gel filtration techniques. The greater sensitivity of electrothermal systems facilitates the detection of lower concentrations of those elements, but the procedure is slow and this is a limiting factor when the large number of samples generated by the separation procedure is to be analysed; therefore, we sought some means of reducing the cycle time in furnace atomic-absorption spectroscopy.

Initially, alternative furnace configurations were examined. Encouraging results were obtained but the systems tended to be mechanically elaborate. Fortunately, a much simpler approach was found to be highly successful. In this new technique, the drying and ashing stages of conventional electrothermal systems are omitted and only the atomisation stage is retained. In conjunction with effective water cooling, the cycle time was reduced to less than 30 s.

The laboratory-built furnace atomic-absorption system used in this study incorporated a mini-Massmann type atomiser with modified furnace tube and support electrodes made from Ringsdorff spectroscopic carbon (Type RW003). The furnace was shielded with nitrogen (2 l min^{-1}) and driven by a laboratory-built power supply capable of providing up to 300 A at 11.5 V d.c. Flame atomic-absorption measurements were made using a Pye Unicam SP2900 instrument operated under the manufacturer's recommended conditions. Both instruments incorporated D_2-arc background correction.

The sample matrix was the buffer used to elute plasma proteins from a DEAE-Sepharose CL6B packed column and contained 0.05 M Tris - hydrochloric acid and 0.2 M sodium chloride. To evaluate the analytical performance of the system, known amounts of copper and zinc were added

to the buffer solution. Samples (2–10 μl) were dispensed into the furnace using an SMI Micro-Pettor. Experiments were carried out to compare the performance of the new technique with the established procedures of pulse nebulisation flame atomic-absorption spectroscopy and conventional furnace operation. The operating conditions of the furnace atomiser are summarised in Table I.

TABLE I

ATOMISER OPERATING CONDITIONS

Element	Parameter	Conventional operation: Dry	Conventional operation: Ash	Conventional operation: Atomise	Direct atomisation
Zn	Current/A	35	43	290	290
	Time/s	10	10	0.9	0.9
	Temperature/K	350	720	2090	2090
Cu	Current/A	37	50	300	300
	Time/s	10	10	1.1	1.1
	Temperature/K	350	920	2970	2970

The results were derived from absorbance peak-height measurements and are presented in Table II. The conventional and direct atomisation modes of furnace operation give comparable results. These results are 1–2 orders of magnitude more sensitive than the flame measurements and require less sample. The reproducibility of both flame and furnace methods is such that duplicate measurements are necessary to obtain the desired precision. The lack of reproducibility is principally attributable to inaccuracies in manual sampling and to the use of peak-height rather than peak-area measurement. The new technique is 3 times faster than the conventional furnace cycle but 2.5 times slower than pulse nebulisation. Preliminary studies indicate that interference effects are no more serious with direct atomisation than when the cycle of drying, pyrolysis and atomisation is followed.

TABLE II

ATOMISER PERFORMANCE DATA

Parameter	Flame: Cu	Flame: Zn	Furnace: Conventional: Cu	Furnace: Conventional: Zn	Furnace: Direct atomisation: Cu	Furnace: Direct atomisation: Zn
Sensitivity, p.p.m. per 1% absorption	5.5×10^{-2}	3.1×10^{-2}	4.5×10^{-3}	4×10^{-4}	4.8×10^{-3}	3.7×10^{-4}
Detection limit (DL), p.p.m. in buffer solution	6×10^{-3}	5×10^{-3}	10^{-3}	10^{-4}	10^{-3}	10^{-4}
Detection limit (absolute)/g	6×10^{-10}	5×10^{-10}	5×10^{-12}	2×10^{-13}	5×10^{-12}	2×10^{-13}
Coefficient of variation at 20 × DL, %	4	3.6	5.5	6.5	5.5	7.5
Sample volume/μl	100	100	5	2	5	2
Time interval between sample injections/s	12		90		30	

The success of this new direct atomisation approach was unexpected because in conventional systems great care is necessary in the drying stage if loss of sample through rapid boiling is to be avoided. It appears, therefore, that the direct atomisation technique is very different in its mode of operation from the conventional approach and thus merits further examination with a view to assessing its suitability for the analysis of other sample types. Preliminary experiments indicate that the mini-Massmann furnace configuration with an absorbent surface to the graphite may play an important part in the success of the direct atomisation technique.

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Book Reviews

FLUORIMETRIE. (In German.) By MAXIMILIAN ZANDER. *Anleitungen für die Chemische Laboratoriumspraxis. Band* XVII. Pp. viii + 127. Springer-Verlag. 1981. Price DM68; $32.40. ISBN 3 540 10512; 0 387 10512 3.

Fluorimetric analysis has advanced substantially in the last few years, both in the range of techniques and instrumentation available and in the breadth of its application, yet it is several years since a major book appeared in this field. Zander's monograph goes some way towards filling the gap, but at the same time its brevity (only 125 pages of excellently produced text) prevents the reviewer from endorsing it wholeheartedly as an essential work for a practising analyst or a student of analytical chemistry.

The first major chapter contains a good account of the theory of organic luminescence, while the second dicusses instrumentation and experimental methods. The next chapter introduces a number of specialised and recent techniques such as matrix-isolation methods, derivative spectroscopy, X-ray excited fluorescence, quenchofluorimetry etc. Again, the reader's appetite is whetted rather than satisfied; there are some other interesting topics that have been omitted, such as β- and Cerenkov-induced fluorescence and synchronous scanning spectrometry. The last chapter attempts the impossible in summarising the applications of fluorimetry in just 15 pages.

Despite, or perhaps because of, its brevity this book would provide an interesting and stimulating read for many workers in the field, and an English Edition would be most welcome.

J. N. MILLER

TABELLEN ZUR STRUKTURAUFKLÄRUNG ORGANISCHER VERBINDUNGEN MIT SPEKTROSKOPISCHEN METHODEN. ZWEITEN AUFLAGE. (In German.) By E. VON PRETSCH, T. CLERC, J. SEIBL and W. SIMON. Pp. vi + 316. Springer-Verlag. 1981. Price DM39.80; $19. ISBN 3 540 10556 5; 0 387 10556 5.

This book consists of compilations of molecular structure - spectroscopic data from carbon-13 NMR, proton NMR, infrared, ultraviolet - visible and mass spectroscopy. The data are presented in the form of tables and bar charts, without the detailed discussion of the respective spectroscopic methods, sample preparation, etc., found in most books in English on this subject. Consequently, this book is not an introduction to spectroscopic methods of organic structural analysis; however, it is a very useful compilation of data for the experienced molecular analyst.

The most detailed information is contained in chapters devoted to the separate spectroscopic techniques, for example, the NMR chapters contain empirical additivity rules and factors, chemical shifts, coupling constants and solvent spectra. A minor criticism of the infrared data is that it does not extend below 500 cm^{-1}, although many routine infrared spectrometers scan to lower frequencies than this.

The analyst is assisted in the identification of unknown substances by a section that presents a summary of the information from all the techniques under molecular structure function headings. This section also contains the more familiar Colthup type frequency - structure correlation charts for carbon-13 and proton NMR, infrared and ultraviolet - visible spectroscopy, and is also cross referenced to the detailed chapters.

The analyst who knows very little German should not automatically dismiss this book from consideration as a knowledge of the language is not essential for its use. It is, however, surprising that the publishers have not produced an English Edition in view of the minimum translation required, which would increase its attractiveness to a very much larger market.

C.P. RICHARDS

STANDARDS IN ABSORPTION SPECTROMETRY. ULTRAVIOLET SPECTROMETRY GROUP. Edited by C. BURGESS and A. KNOWLES. *Techniques in Visible and Ultraviolet Spectrometry, Volume* 1. Pp. xii + 142. Chapman and Hall. 1981. Price £9.50. ISBN 0 412 22470 4.

This is, in fact, the third book that the Ultraviolet Spectrometry Group has published. "Computers for Spectroscopists" appeared in 1974 and "Electronics for Spectroscopists" some years before that. This volume, which easily maintains the standard set by its predecessors, is the first

in a new series of three volumes; Volume 2, concerned with fluorescence spectrometry, is already at the printers and Volume 3, on instrumentation, is on the stocks.

The advertisement in the Ultraviolet Spectrometry Group's Bulletin states that "Standards in Absorption Spectrometry" is intended as a practical manual to be kept on the bench and that no spectroscopist should be without one. This claim is well justified on both counts for it contains a wealth of valuable practical information not previously, as far as this reviewer knows, put together conveniently in one volume.

Even though the 10 chapters are written by different authors (and even a party of authors) the style is consistently good throughout and there is virtually no duplication. One or two typographical errors were spotted in the main text (*e.g.*, first equation on page 3) but the editing is clearly of the highest quality.

Criticisms are very minor except possibly in Section 1.4.3, Choice of Absorbance. Here the derivation of the absorbance value of minimum error, 0.869 which is correct, is too quickly glossed over and a working absorbance range of 1–2, which does not include that value, is then recommended without explanation. The effects of stray light, which above certain levels would curtail the higher end of this range, are also too briefly mentioned in passing.

There are very many good points, however: the information on cell sizes, the excellent accounts of absorbance standards (particularly the explanation of the behaviour of dichromate solutions) and the valuable guidance to methods of wavelength calibration. Particularly welcome are the authors' suggestions for standard practice where no formal standard yet exits. There are examples in cell location (pp. 44–45) and beam location (p. 42). Practical tips too, such as the recommendation that solution absorbance standards should be prepared in one's own laboratory as commercially prepared ones offer no particular advantage, can save many times the cost of the book. The point that different wavelength standards are suitable for different ranges of spectral slit width is probably one of which many non-specialist users of this type of equipment have been unaware.

There is much good advice on cell handling and cleaning and the need or otherwise for using matched cells. The section on checking cell quality (path length and angular deviation) is extremely useful for those engaged on accurate work (even though the layout of Fig. 9.2 suggests that the operator needs to lie on his back under the bench).

It is usual these days in reviews to slate the price of books. At £9.50 this one is astonishingly good value.

W. J. PRICE

CHEMICAL DERIVATIZATION IN GAS CHROMATOGRAPHY. By J. DROZD. *Journal of Chromatography Library, Volume* 19. Pp. xiv + 232. Elsevier. 1981. Price $58.50; Dfl120. ISBN 0 444 41917 9 (Volume 19); 0 444 41616 1 (Series).

This is the most recent volume in the *Journal of Chromatography Library* and it is somewhat surprising that this topic has not appeared earlier in the list. However, the book is nonetheless welcome for all that as it gives reasons for using chemical derivatives and detailed sample preparation and derivatisation techniques.

Methods for the preparation of ester, ether, acyl, silyl, oxime, hydrazone and cyclic derivatives are described and (taking up most of the volume) derivatives of particular species of compounds mentioned include: pesticides, pharmaceuticals, alcohols, phenols, aldehydes and ketones, amines, anions and cations, sulphur compounds, carboxylic and amino acids, steroids, sugars, nucleosides and nucleotides.

Abbreviations are used throughout the text but a list of such abbreviations is given at the beginning of the book. The volume comprises 5 chapters with 881 references, a good subject index and a list of some suppliers of reagents and accessories. A useful Appendix is also to be found, giving methods of purification of chemicals and solvents likely to be used. In addition to the general contents listed, as is frequently found in books of this type there are more detailed contents lists at the beginning of the chapters.

Other useful features include flow diagrams for a few specific extraction procedures (*e.g.*, extraction of drugs from serum), a short account of quantitation, metal chelates (*e.g.*, beryllium in body fluids and in environmental analyses), and separation of trimethylsilyl derivatives of anions.

This is essentially a practical work probably of more use to a practising gas chromatographer than as an introductory text for a student. It is perhaps somewhat high in price.

D. SIMPSON

THE ANALYSIS OF EXPLOSIVES. By JEHUDA YINON and SHMUEL ZITRIN. *Pergamon Series in Analytical Chemistry, Volume* 3. Pp. xii + 310. Pergamon Press. 1981. Price $60; £25 (hardback), $22.50 £9.35 (softback). ISBN 0 08 023846 7 (hardback); 0 08 023845 9 (softback).

The purport of this relatively modest monograph is to serve as a reference book for chemists in analytical and forensic laboratories. The authors set out to achieve this aim by an effective blend of theoretical aspects, practical detail and a sizeable area of review material covering the period up to 1980. A large proportion of the references (of which there are some 450) are taken from the open literature published during the last decade. As suggested by the title, the book is concerned primarily with the analytical techniques commonly applied to explosive materials. A reader expecting to find details of methods for analysing explosives compositions could well be disappointed as the scope of the work is concerned almost exclusively with the identification and quantification of explosive substances either alone or in admixture with other (non-explosive) materials, such as stabilisers and plasticisers. The constituents of a limited number of commercial and military explosives and propellants are listed but nominal quantitative data are not given. Inevitably there are some omissions from a volume of this size. Important amongst these is the absence of any reference to that current favourite triaminotrinitrobenzene (TATB).

The format of the book is straightforward. The first chapter deals with the classification of explosives under the two main headings of pure compounds and mixtures. These categories are then further sub-divided into the groups primary explosives, high explosives and non-explosives; and high explosives and propellants, respectively. Within these subdivisions each individual explosive is presented by systematic and trivial names and structural formula. The usefulness of the monograph to the practising explosives chemist would be greatly enhanced had the authors taken the opportunity to include additional physical data such as melting-point, boiling-point, density and colour. Even some indication of the physical state would have been welcome.

The succeeding 12 chapters pursue the analysis of explosives by reference to specific analytical techniques. The first of these examines applications of available chemical methods mainly as qualitative "spot-tests." This section is followed by chapters on various chromatographic techniques (column, paper, TLC, GC and HPLC) and spectroscopic methods (ultraviolet, visible, infrared, mass spectrometry, NMR and ESR). There is nothing systematic about the presentation of this material although diligent use of the detailed index should enable the interested reader to select a technique suitable for application to the particular problem in hand. Each of the chapters dealing with instrumental analysis is introduced by a brief outline of the particular technique. Whilst this treatment has some advantage to the casual interested reader or student, the explosives specialist will find insufficient detail to merit inclusion of this material in a volume of this nature. The space could possibily have been used to greater advantage by inclusion of reference spectra (ultraviolet, infrared, NMR etc.) and a section describing the use of the polarising microscope in the identification of explosive species. The final two chapters deal with the detection and identification of residues and the detection of hidden explosives, respectively, and include a short dissertation on the use of X-ray diffraction and mass spectrometry with short descriptions of instruments based on electron-capture, gas and plasma chromatography. Recent biological detection systems described include enzymatic and animal olfaction. In the space available these topics gain little more than a passing mention.

Presentation of the monograph is good based as it is on photoreduction of the authors' original manuscripts. (The paperback version was reviewed.) The hand-drawn formulae are clear and informative with few significant errors observed. The reviewer found irritating the alphabetical listing of the authors' names rather than the more common practice of serial numbering in the text. The system employed makes the finding of a listed reference in the text an extremely tedious task. Within the limitations imposed by its length, the book achieves the aims expressed by the authors. In these days of rising publishing costs it represents excellent value and should find a treasured place on the bookshelves of the majority of its intended audience.

J. K. CORBETT

Determination of Trace Amounts of Nitrite by Derivatisation and Gas Chromatography

A gas-chromatographic method for the determination of nitrite is described. Nitrite is converted into substituted benzene derivatives by reaction with substituted anilines and hypophosphorous acid, and the resulting benzene derivatives are determined by gas chromatography. The derivatisation yields of nitrite were studied by using anilines with various substituents that have a high response to an electron-capture detector. Nitroanilines give high yields and *m*-nitroaniline is the most suitable derivatisation reagent for determining trace amounts of nitrite. By using *m*-nitroaniline and an electron-capture detector, the detection limit and determination range of nitrite are 0.5 ng ml^{-1} and up to 1.00 $\mu g\ ml^{-1}$, respectively, which are much lower than those of the widely used colorimetric method for the determination of nitrite (detection limit 16 ng ml^{-1}). River water samples containing nitrite were analysed by both this gas-chromatographic method and the colorimetric method.

Keywords: *Nitrite determination*; *trace analysis*; *gas chromatography*; *derivatisation*

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Analyst, 1982, **107**, 82–88.

Determination of Germanium in Coal Ashes by Hydride Generation and Flame Atomic-absorption Spectrophotometry

This paper describes the determination of germanium by atomic-absorption spectrophotometry with direct introduction into a dinitrogen oxide - acetylene flame of the germanium(IV) hydride generated by reducing with sodium tetrahydroborate(III) solution. A comparative study of the sensitivity of the germanium determination has been carried out and a study of interferences from lead(II), arsenic(III) and -(V), iron(II) and -(III), tellurium(IV), selenium(IV), antimony(III), tin(II), tartrates, oxalates and fluorides is described. The sensitivity of the proposed method is 0.012 $\mu g\ ml^{-1}$ and the detection limit is approximately 100 times better than obtained with silica tube atomisation at a wavelength of 265.14 nm. The method has been applied to the determination of germanium in lignite ashes with an average germanium recovery of 97.65% and a relative standard deviation of 2.87 %.

Keywords: *Hydride generation*; *dinitrogen oxide - acetylene flame*; *atomic-absorption spectrophotometry*; *germanium determination*; *coal ashes*

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Analyst, 1982, **107**, 89–95.

Determination of Mercury Vapour in Air Using Electrothermal Atomic-absorption Spectrometry with an Electrostatic Accumulation Furnace

The "electrostatic accumulation furnace for atomic-absorption spectrometry" technique has been tested for the determination of mercury vapour in the atmosphere. Even if the electrostatic capture of mercury vapour is much more difficult than for particles, an efficiency higher than 90% can be achieved. A calibration procedure is proposed. Under appropriate experimental conditions, a detection limit (signal to noise ratio = 3) of 50 ng m^{-3} was obtained.

Keywords: *Electrothermal atomic-absorption spectrometry*; *electrostatic precipitators*; *particulate matter analysis*; *mercury vapour determination*

G. TORSI, E. DESIMONI, F. PALMISANO and L. SABBATINI
Istituto di Chimica Analitica dell'Università Degli Studi, Via Amendola 173, Bari, Italy.

Analyst, 1982, **107**, 96–103.

Interactions of Major, Minor and Trace Elements on the Carbon Rod Atomic-absorption Spectrophotometric Determination of Micro-amounts of Ytterbium, Holmium, Dysprosium and Thulium

The electrothermal atomisation of trace amounts of some heavy rare earth elements has been investigated using a carbon rod atomiser. The interferences due to the components (major, minor and trace) of a common silicate matrix have been studied in order to obtain the best analytical conditions for future determinations. The detection limits (using uncoated graphite rods) are 5 pg of dysprosium, 3 pg of holmium and thulium and 0.4 pg of ytterbium.

Keywords: *Dysprosium, holmium, thulium and ytterbium determinations*; *silicate rocks*; *atomic-absorption spectrophotometry with electrothermal atomisation*; *interferences*

AMBROGIO MAZZUCOTELLI and MARIO GALLI
Istituto di Petrografia, Università di Genova, Genoa, Italy.

and ROBERTO FRACHE
Istituto di Chimica Generale ed Inorganica, Università di Genova, Genoa, Italy.

Analyst, 1982, **107**, 104–109.

Some Causes of Bias in the Measurement of Dissolved Oxygen Using Certain Modifications of the Winkler Method

Short Paper

Keywords: *Dissolved oxygen determination*; *Winkler modification*; *nitrite interference*; *iron(III) interference*; *phosphate modification*

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Analyst, 1982, **107**, 110–112.

Differential-pulse Polarographic Determination of the Coccidiostat Arprinocid in Feed Pre-mixes

Short Paper

Keywords: *Arprinocid determination*; *differential-pulse polarography*; *feed pre-mixes*

JOHN D. STONG and DAVID W. FINK
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Analyst, 1982, **107**, 113–116.

Proton Magnetic Resonance Shifts of the Trimethylsilyl Derivatives of Silicate Materials

Communication

Keywords: *Trimethylsilylation*; *silicate analysis*; *ASIS effect*; *proton magnetic resonance*

B. R. CURRELL, H. G. MIDGLEY, J. R. PARSONAGE and E. A. VIDGEON
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Analyst, 1982, **107**, 117–121.

Rapid Determination of Albumin-bound Zinc in Human Serum by Simple Affinity Chromatography and Atomic-absorption Spectrophotometry

Communication

Keywords: *Zinc*; *albumin*; *affinity chromatography*; *atomic-absorption spectrophotometry*; *electrothermal atomisation*

J. W. FOOTE and H. T. DELVES
University Department of Chemical Pathology and Human Metabolism, Southampton General Hospital, Southampton, SO9 4XY.

Analyst, 1982, **107**, 121–124.

Technique for Reducing the Cycle Time in Atomic-absorption Spectroscopy with Electrothermal Atomisation

Communication

Keywords: *Atomic-absorption spectroscopy*; *electrothermal atomisation*; *cycle time*

M. H. BAHREYNI-TOOSI, J. B. DAWSON and D. J. ELLIS
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Analyst, 1982, **107**, 124–125.

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