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Volume 108 No 1284 March 1983

Summaries of Papers in this Issue

Determination of Palladium in Nuclear-waste Samples by Inductively Coupled Plasma Emission - Fluorescence Spectrometry

The determination of palladium in sample solutions of nuclear waste by inductively coupled plasma (ICP) optical emission spectrometry with a 0.5-m monochromator is hindered by severe spectral interference that occurs at all the analytically useful wavelengths of palladium. Interferences from argon, yttrium, zirconium, samarium and neodymium lines have been experienced with the palladium lines at 363.47, 324.27, 340.45 and 360.95 nm. In order to overcome these interferences a spectrometer of high resolution is required. This precludes the use of a multi-channel spectrometer and indicates the application of a high-resolution scanning monochromator. In this paper we show that a simple and effective alternative approach to the problem is offered by combination of the ICP emission characteristics with the spectral selectivity of atomic-fluorescence spectrometry. Measurement of the palladium atomic fluorescence at 363.47 nm from a sample atomised in an argon-shielded air - acetylene flame, excited by the emission of a pure 5000 μg ml^{-1} solution of palladium fed into the ICP torch, allows for the precise and accurate determination of palladium, with no problems due to scattering. In addition, the palladium concentration in the samples is such that the ICP - resonance monochromator technique could be quickly and effectively used with no sample dilution, provided that sufficient sample is available and that radioactivity is at a tolerable level.

Keywords: *Palladium determination*; *inductively coupled plasma*; *atomic-fluorescence spectrometry*; *nuclear materials*

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Analyst, 1983, **108**, 297–304.

Determination of Tellurium by Atomic-absorption Spectroscopy with Electrothermal Atomisation after Pre-concentration by Hydride Generation and Trapping

A procedure for the determination of tellurium at sub-microgram levels by atomic-absorption spectroscopy with electrothermal atomisation after the evolution and trapping of hydrogen telluride is described. Interferences are observed in the presence of silver(I), copper(II), mercury(II) and selenium(IV) but can be overcome by suitable pre-treatment procedures. The detection limit (based on four times the standard deviation of six blank measurements) is 0.006 μg and the coefficient of variation is 4% at the 0.3-μg level.

Keywords: *Tellurium*; *hydride generation and trapping*; *atomic-absorption spectroscopy*; *electrothermal atomisation*; *interferences*

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Analyst, 1983, **108**, 305–309.

Determination of Trace Amounts of Molybdenum in Natural Waters by Solvent Extraction - Atomic-absorption Spectrometry, After Chelating Ion-exchange Pre-concentration

A method for the determination of molybdenum in natural waters in the micrograms per millilitre range is proposed. The method involves the pre-concentration of molybdenum on a Chelex-100 chelating resin and subsequent elution with ammonia solution, followed by extraction with a complexing reagent, 1,4-dihydroxyphthalimide dithiosemicarbazone dissolved in *NN*-dimethylformamide - isoamyl alcohol (1 + 4), and a final direct determination by atomic-absorption spectrometry. The addition of ascorbic acid prior to extraction eliminates the interfering effect of several ions at the concentration levels normally found in natural waters. The sensitivity of the method is 0.3 $\mu g\ l^{-1}$ for 1% absorption. The method has been applied to the determination of molybdenum in sea and surface waters.

Keywords: *Molybdenum determination*; *natural water analysis*; *chelating ion-exchange separation*; *atomic-absorption spectrometry*; *1,4-dihydroxyphthalimide dithiosemicarbazone*

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Analyst, 1983, **108**, 310–315.

Effect of pH on the Response of Glassy Carbon Electrodes

The use of glassy carbon as an electrode material engenders a number of practical problems owing to the presence of C–O functionalities on its surface. One such problem is the susceptibility of the electrode to pH changes. Highly surface-active glassy carbon electrodes having a high proportion of irreversible C–O groups are particularly prone to variations in pH compared with ones having mainly quinoidal species. This is reflected in the performance of glassy carbon in the cyclic voltammetry of hydroquinone.

Keywords: *Glassy carbon electrode*; *surface groups*; *pH*; *hydroquinone*

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Analyst, 1983, **108**, 316–321.

Determination of Polychloro-2-(chloromethylsulphonamido)-diphenyl Ether Insectproofing Agents on Wool Textiles and in Textile Liquors by High-performance Liquid Chromatography

Polychloro-2-(chloromethylsulphonamido)diphenyl ether insectproofing agents were extracted from wool textiles with methanol - ammonia solution in sealed ampoules and determined by normal bonded-phase high-performance liquid chromatography. Liquor samples were extracted with dichloromethane and determined similarly. Satisfactory recoveries of the insectproofing agents were obtained from spiked liquor and wool samples. Accurate and reproducible analyses of the formulations were obtained down to concentrations of 0.05% *m/m* on wool textiles and 0.5 mg l^{-1} in liquor samples. The short analysis time and simplicity of this method make it ideally suited for the routine determination of these insectproofing agents on wool textiles.

Keywords: *Insectproofing agents*; *polychloro-2-(chloromethylsulphonamido)-diphenyl ethers*; *wool textiles*; *textile liquors*; *high-performance liquid chromatography*

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Analyst, 1983, **108**, 322–328.

Quantitative Determination of Carbonyl Compounds in Rendering Emissions by Reversed-phase High-performance Liquid Chromatography of the 2,4-Dinitrophenylhydrazones

A simple and rapid method for the determination of volatile carbonyl compounds in air has been developed. The method is applied to the quantitation of C_1–C_9 aldehydes and acetone in rendering emissions.

Carbonyl compounds are sampled by absorption in a 2×10^{-4} M 2,4-dinitrophenylhydrazine solution at pH 1 and the resulting hydrazones are extracted with 2,2,4-trimethylpentane, concentrated and analysed by HPLC on a 10-μm RP-C_{18} column with a water - acetonitrile gradient as the eluent. The hydrazones are then spectrophotometrically detected at 356 nm. The micro-scale conversion of carbonyls into 2,4-dinitrophenylhydrazones is investigated, the separation of hydrazones is improved and the sampling conditions are tested in order to achieve quantitative sampling at an air flow rate of 1 l min^{-1}. Quantitation is possible for concentrations as low as 15 p.p.b. (formaldehyde) and 2 p.p.b. (nonanal). The over-all coefficient of variation (taken over sampling, conversion and analyses) is less than 10%.

Keywords: *Carbonyl quantitation*; *2,4-dinitrophenylhydrazine derivatisation*; *reversed-phase HPLC analysis*; *rendering plant emissions*

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Analyst, 1983, **108**, 329–334.

Determination of Sulphur in Oils by X-ray Fluorescence Using a Thin Layer Solidified with Wax

A rapid method has been developed for the determination of the sulphur content in a small amount (minimum 0.5 g) of oil using an X-ray fluorescence technique. The sample is mixed and solidified with paraffin wax, which permits presentation of the sample in the normal inverted mode as a thin film and without the need of a retaining plastic window, which is usually required for liquids. Other elements may also be determined.

Keywords: *Oil analysis*; *sulphur determination*; *X-ray fluorescence spectroscopy*; *thin film*

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Analyst, 1983, **108**, 335–339.

Application of Zirconium Molybdophosphate Gel for the Selective Separation of Thallium(I) Ions

Zirconium molybdophosphate has been prepared in the form of hard yellow granules. The product possesses an ion-exchange capacity of 0.42 mequiv. g^{-1} and shows selectivity for Ag^+, Tl^+, UO_2^{2+} and Th^{4+} ions. It has been possible to separate Tl^+ ions selectively on columns of this ion-exchanger material, but only Tl^+ ions can be eluted completely.

Keywords: *Zirconium molybdophosphate gel*; *thallium(I) separation*

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Analyst, 1983, **108**, 340–345.

Liquid - Liquid Extractive Separation of Molybdenum(VI) in Malonate Solutions with High Relative Molecular Mass Amines

Molybdenum can be quantitatively extracted with 0.08 M Amberlite LA-2 in xylene at pH 3.0 from 0.02 M malonic acid, stripped with 0.25 M ammonia solution and determined spectrophotometrically with Tiron at 390 nm. Four other liquid anion exchangers were examined as possible extractants and extraction of molybdenum was found to be quantitative over a limited pH range with two of these. Eight common solvents were tested as diluents; of these, hexane, cyclohexane, benzene and xylene were found to be satisfactory.

Molybdenum can be separated from elements that do not form complexes with malonic acid by prior stripping of the extract with water. The metal can be separated from elements forming weak complexes by selective stripping with acids. A novel feature of the method is the separation of molybdenum from multi-component mixtures.

The method has been applied to the determination of molybdenum in steel and soil.

Keywords: *Molybdenum separation*; *molybdenum determination*; *liquid - liquid extraction*; *steel analysis*; *soil analysis*

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Analyst, 1983, **108**, 346–352.

Determination of the Active-ingredient Content of Technical and Formulated DNOC and Dinoseb and Technical Dinobuton by Spectrophotometry. A Collaborative International Pesticides Analytical Council Study

A spectrophotometric method is described for the determination of the active-ingredient content of technical and formulated DNOC (2-methyl-4,6-dinitrophenol) and dinoseb (2-*sec*-butyl-4,6-dinitrophenol), and technical dinobuton (2-*sec*-butyl-4,6-dinitrophenyl isopropyl carbonate). The method involves a preliminary clean-up on Woelm neutral alumina (grade I). Impurities are eluted from the column while the active ingredient is retained at the top of the column. The active ingredient is then eluted as its butylammonium salt and determined spectrophotometrically. Dinobuton samples require a preliminary separation from free dinoseb, de-esterification on the column and determination using the dinoseb procedure. Results obtained from international collaborative studies are presented and discussed.

Keywords: *DNOC*; *dinoseb*; *dinobuton*; *spectrophotometry*; *collaborative studies*

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Analyst, 1983, **108**, 353–360.

Spectrophotometric Determination of Phosphate in River Waters with Molybdate and Malachite Green

On the basis of the coloration formed with molybdate and malachite green in aqueous solution, trace amounts of phosphate were determined. The molar absorptivity was 7.8×10^4 l mol^{-1} cm^{-1} at 650 nm. The absorbance of the reagent blank was about 0.02, and its relative standard deviation was less than 10%. The recommended concentration range of phosphorus was 0.1–5 μg and the limit of detection was 0.01 μg of phosphorus. The sample solution was acidified with sulphuric acid and heated in a water-bath above 90 °C for 40 min, and subsequently it was coloured with molybdate and malachite green. The colour was stabilised by adding poly(vinyl alcohol). The method was applied to the determination of parts per billion (10^9) amounts of phosphorus in river and tap waters; the relative standard deviation was less than 4% and the recovery was 95–101%.

Keywords: *Spectrophotometry*; *phosphate determination*; *molybdate*; *malachite green*; *river waters*

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Analyst, 1983, **108**, 361–367.

Spectrophotometric and Fluorimetric Determination of Boron in Soils, Plants and Waters by Extraction with 2-Methylpentane-2,4-diol in Isobutyl Methyl Ketone

Two methods for the determination of boron, by molecular absorption spectrophotometry with curcumin and by molecular fluorescence with dibenzoylmethane, after extraction of boron into isobutyl methyl ketone (IBMK) with 2-methylpentane-2,4-diol, are proposed. The development of the colour or the fluorescence is carried out in the organic phase used for extraction by addition of curcumin in glacial acetic acid or dibenzoylmethane in IBMK and phosphoric acid as dehydrating agent. The different conditions for both spectrophotometric and fluorimetric methods have been established. A study has been made of the influence in aqueous solution of several ions as potential interferents. The spectrophotometric method has been applied to the determination of boron in soils and plants and the fluorimetric method to plants and natural waters.

Keywords: *Boron determination*; *2-methylpentane-2,4-diol extraction*; *spectrophotometry*; *fluorimetry*

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Analyst, 1983, **108**, 368–373.

Monoethanolamine as an Absorbing Reagent for the Spectrophotometric Determination of Atmospheric Sulphur Dioxide

The possible use of monoethanolamine as an absorbing reagent for sulphur dioxide has been investigated. The proposed reagent has an absorption efficiency of about 100% and is superior to the sodium tetrachloromercurate method with respect to ease of manipulation, speed, stability of absorbed sulphur dioxide and the use of stable and readily available pure and non-toxic reagents. The effects of reagent concentration, flow-rate and temperature on the absorption of sulphur dioxide were studied. The absorbed sulphur dioxide was subsequently determined spectrophotometrically using *p*-aminoazobenzene - formaldehyde reagent in hydrochloric acid medium. The interference of nitrogen dioxide is eliminated by the use of sulphamic acid. The procedure is suitable for air pollution studies.

Keywords: *Atmospheric sulphur dioxide absorption; monoethanolamine; spectrophotometry*; p-*aminoazobenzene - formaldehyde reagent*

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Analyst, 1983, **108**, 374–379.

Spectrophotometric Method for the Determination of Dobutamine Hydrochloride

A rapid, accurate and simple method is proposed for the determination of dobutamine hydrochloride in the bulk drug and in vials. This method is based on measuring the intensity of the pink colour that develops when dobutamine hydrochloride is allowed to react with thiosemicarbazide in an alkaline - acetone medium. The colour is stable for at least 3 h and can be quantified spectrophotometrically at 510 nm ($\epsilon_{max.} = 1.7 \times 10^4$ l mol^{-1} cm^{-1}). Beer's law is obeyed in a concentration range 0.5–20 μg ml^{-1} in the final assay solution. The effects of reagent concentration, alkali concentration and solvent on colour formation were investigated. A Job's plot of absorbance *versus* the molar ratio of dobutamine to thiosemicarbazide indicates a 1 : 1 ratio. As the catecholic group with free adjacent positions is required for colour development, the method is highly specific.

Keywords: *Spectrophotometry; dobutamine hydrochloride determination; thiosemicarbazide*

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Analyst, 1983, **108**, 380–385.

March 1983 Vol. 108 No. 1284

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Determination of Palladium in Nuclear-waste Samples by Inductively Coupled Plasma Emission - Fluorescence Spectrometry

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The determination of palladium in sample solutions of nuclear waste by inductively coupled plasma (ICP) optical emission spectrometry with a 0.5-m monochromator is hindered by severe spectral interference that occurs at all the analytically useful wavelengths of palladium. Interferences from argon, yttrium, zirconium, samarium and neodymium lines have been experienced with the palladium lines at 363.47, 324.27, 340.45 and 360.95 nm. In order to overcome these interferences a spectrometer of high resolution is required. This precludes the use of a multi-channel spectrometer and indicates the application of a high-resolution scanning monochromator. In this paper we show that a simple and effective alternative approach to the problem is offered by combination of the ICP emission characteristics with the spectral selectivity of atomic-fluorescence spectrometry. Measurement of the palladium atomic fluorescence at 363.47 nm from a sample atomised in an argon-shielded air - acetylene flame, excited by the emission of a pure 5000 μg ml^{-1} solution of palladium fed into the ICP torch, allows for the precise and accurate determination of palladium, with no problems due to scattering. In addition, the palladium concentration in the samples is such that the ICP - resonance monochromator technique could be quickly and effectively used with no sample dilution, provided that sufficient sample is available and that radioactivity is at a tolerable level.

Keywords: *Palladium determination*; *inductively coupled plasma*; *atomic-fluorescence spectrometry*; *nuclear materials*

Studies on the chemical separation processes of nuclear-waste solutions require analytical methods that are suitable for monitoring a series of key elements over a wide range of concentration levels. The analytical characteristics of the inductively coupled plasma (ICP)[1–3] make it very attractive for this type of application. In particular, the absence of self-absorption and self-reversal, the marked freedom from chemical interferences and the very high detection power for a large number of elements represent the most desirable characteristics for the analysis of complex sample matrices. A typical composition of a nuclear-waste solution is presented in Table I.

TABLE I

APPROXIMATE COMPOSITION OF A TYPICAL SAMPLE OF NUCLEAR-WASTE SOLUTION

Concentration interval/ μg ml^{-1}	Elements
0.1–10	Ag, Al, As, Ge, In, Sb, Tb
10–100	Cd, Cr, Cu, Eu, Gd, Ni, Rb, Rh, Se, Sn, Y
100–500	Ba, La, Pd, Pr, Ru, Sn, Sr, Te
500–1000	Ce, Cs, Mo, Nd, U, Zr
>1000	Fe, Na

Although a multi-channel optical emission spectrometer equipped with an ICP (ICP-OES) source appears to be the first choice of combination to be investigated, it must be stressed that with very complicated matrices (as, for example, the one under examination) the high detection power for a large number of elements can represent a serious limitation for the determination of some elements at the pre-selected wavelengths, owing to the possibility of

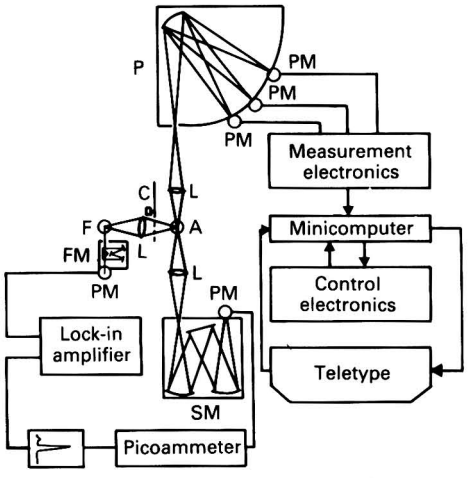

Fig. 1. Experimental arrangement: A, ICP torch; F, air - acetylene flame; L, quartz lenses; C, chopper; P, polychromator; SM, scanning monochromator; FM, fluorescence monochromator; and PM, photomultiplier.

spectral interferences. Further, channels corresponding to particular elements may be missing from the spectrometer, thus negating the determination of these elements. In these instances, sequential-scanning spectrometers offer a greater flexibility at the expense of the speed of analysis, the most serious limitation to a multi-element analysis being represented by the size of sample available. The 30-channel ICP-optical emission spectrometer available in our laboratory does not include the element palladium, whose determination is important in one of the several schemes available for the separation of the actinides.[4] Attempts to determine this element by coupling a medium-resolution scanning monochromator to the ICP source failed for reasons that will be discussed later.

The aim of this paper is to describe the practical application of the combined use of the ICP and a separate atomiser (flame) for determining palladium in solutions of nuclear waste, either by conventional atomic fluorescence, or by the resonance monochromator technique.[5–8]

TABLE II

EXPERIMENTAL CONDITIONS

Component	Details
ICP	Emission Quantometer (ARL, Lausanne, Switzerland), Model 3400, and a 3000 PGT/27 HF generator (Henry Radio, Los Angeles, CA). Argon flow-rates: 1, 0.8 and 10.5 l min^{-1} for carrier, internal and plasma gas, respectively
Nebuliser	Concentric glass nebuliser, T 2001 A4 (Meinhard Ass., Santa Anna, CA)
Emission monochromator and electronics	Jarrell-Ash 0.5-m Ebert grating monochromator, *f*/8.6, a 30000-grooves per inch grating, 25-μm matched slit widths. EMI 6256 PMT, operated at 1300 V; Keithley, Model 414, picoammeter; Perkin-Elmer, Model 56, strip-chart recorder
Fluorescence monochromator and electronics	Jobin Yvon H-10 grating monochromator, *f*/3.5, 1-mm slit widths. RCA 1 P28 PMT, Model 382A, chopper (Ithaco, Ithaca, NJ). Keithley 427 current to voltage converter and Dynatrac 391A lock-in amplifier (Ithaco, Ithaca, NJ)
Lenses	Quartz spherical lenses, 3 cm diameter, 12.5-cm focal length for emission work. No lenses were used for fluorescence work
Flame	Stoicheiometric argon sheathed air - acetylene flame

In the first instance, the sample, atomised in an air - acetylene flame, is excited by the emission from a solution of pure palladium fed into the ICP torch, and in the second approach, a solution of pure palladium atomised in the air - acetylene flame constitutes the resonance monochromator for the spectral frequencies emitted by the sample introduced into the ICP torch.

The basic principles and theory underlying these two approaches, their respective merits, analytical potentials and practical applications have been described in the literature[5–8] and will not be discussed further here.

Experimental

The ICP torch, energised by a 3000 PGC/27HF generator (Henry Radio, Los Angeles, CA, USA) and fitted on a 30-channel 34000 ARL Quantometer (ARL, Lausanne, Switzerland), was used for the study of the spectral characteristics of palladium, under medium resolution conditions. The torch was fed by a concentric glass nebuliser, Model T 200L A4 (Meinhard Ass., St. Anna, CA, USA) and the plasma plume was focused on to the entrance slit of a 0.5-m Ebert monochromator (Jarrell-Ash, Waltham, MA, USA). The same torch assembly was utilised for the fluorescence measurements by focusing the plasma plume on an argon-separated air - acetylene flame supported by a home-made five-slot circular burner. Fluorescence emission was monitored with a 0.1-m monochromator (Jobin Yvon, Longjumeau, France). The experimental apparatus is represented schematically in Fig. 1.

In considering the radioactive nature of the waste solutions, the entire study was carried out using synthetic solutions made from analytical-reagent grade compounds (Merck, Darmstadt, West Germany and Carlo Erba, Milan, Italy), closely matching the composition reported in Table I. The experimental parameters chosen in the course of the study are given in Table II.

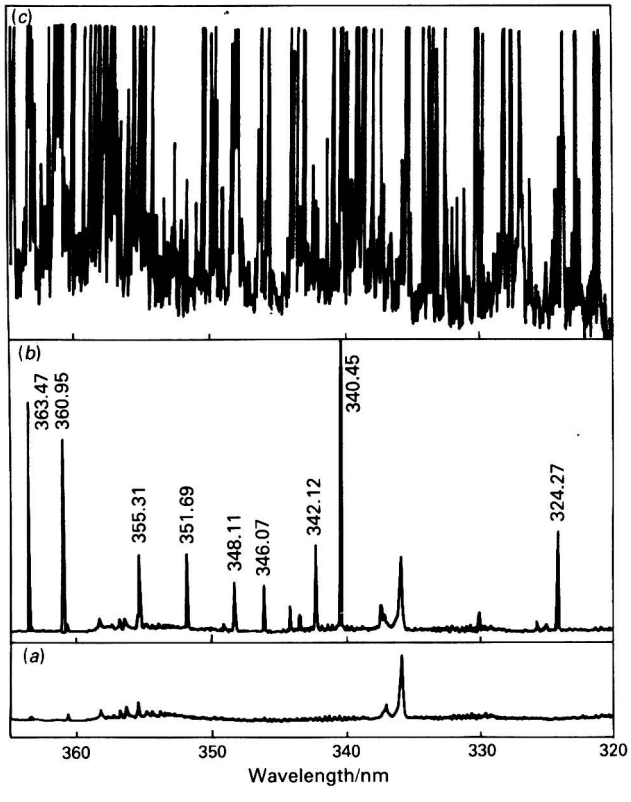

Fig. 2. Spectral profile of the wavelength region 320–365 nm. (*a*) Water; (*b*) pure Pd solution, 125 μg ml^{-1}; and (*c*) sample diluted 1 + 1.

Results and Discussion

The spectral lines of palladium emitted by an ICP source have been listed by Winge *et al.*[9] From this list it appears that a number of palladium lines should be suitable for analytical applications. From these, the lines at 340.45, 363.47, 360.95, 324.27 and 342.12 nm were considered to be the most promising. However, whenever each of these lines was used for quantitative measurements of palladium in synthetic solutions using the medium-resolution monochromator, concentration values significantly higher than the established ones were found in all instances. Examination of the spectrum obtained by scanning the wavelength region from 320.00 to 365.00 nm revealed the presence of a very complex structure, which was likely to be the cause of spectral interferences. The wavelength region from 220.0 to 250.0 nm appeared to be more attractive owing to the reduced number of intense lines in the spectrum. However, none of the nine palladium lines that were identified in this region showed sufficient sensitivity for analytical application.

The spectral emission profiles of the sample for these two wavelength intervals are presented in Figs. 2 and 3, respectively, and are compared with the corresponding spectra recorded while aspirating distilled water and a solution of pure palladium into the torch. It should be noted that the concentration of the palladium was chosen so as to correspond with that expected in the sample. A closer examination at the best obtainable resolution of the lines at 363.47, 360.95, 342.12, 340.45 and 324.27 nm showed, in all instances, the occurrence of a strong spectral interference from a concomitant in the solution.

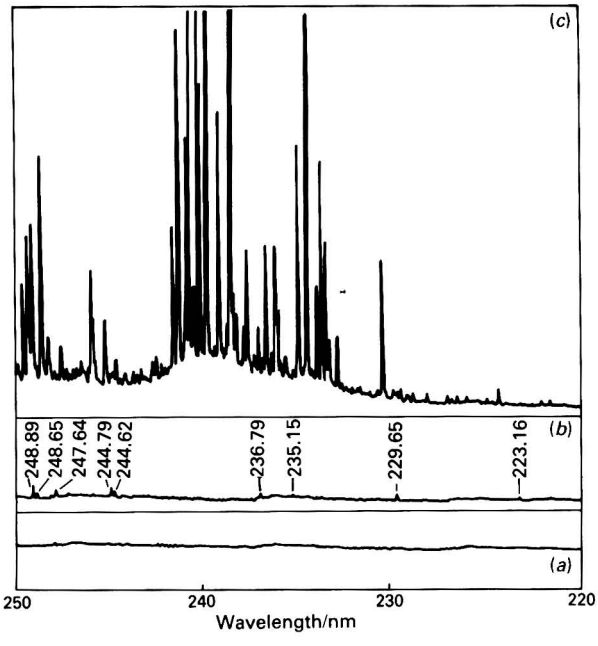

Fig. 3. Spectral profile of the wavelength region 220–250 nm. (*a*) Water; (*b*) pure Pd solution, 125 μg ml^{-1}; and (*c*) sample diluted 1 + 1.

The comparison of the tracings obtained from a 125 μg ml^{-1} solution of palladium with those exhibited by the synthetic solution of nuclear waste (diluted 1 + 1) at these spectral frequencies is shown in Fig. 4. The nature of these interferences has been investigated further by comparing the spectral profiles of a pure solution of palladium at each of the above mentioned wavelengths with those of the same solution with one of the concomitants added. The chosen concomitant was suspected of interfering both because of its concentration level and because of its spectral signature. In all of the examples considered, the palladium to concomitant concentration ratio was equal to that existing in the synthetic waste solution.

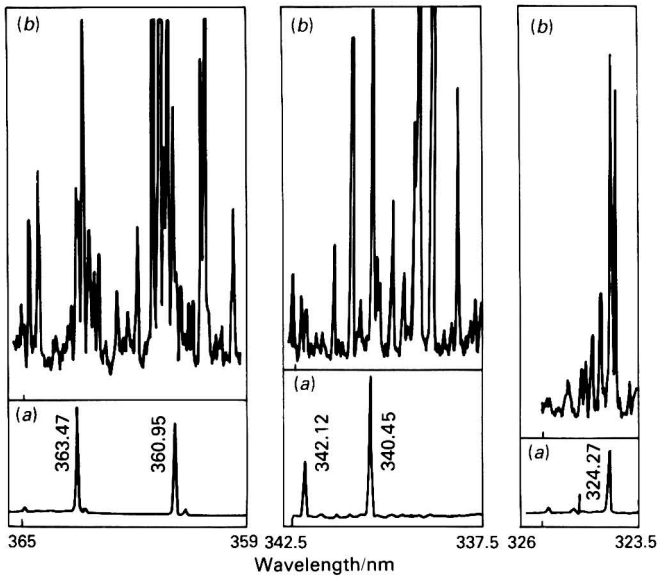

Fig. 4. Spectral profiles at indicated wavelengths as obtained with (*a*) pure Pd solution (125 μg ml^{-1}); and (*b*) synthetic nuclear-waste solution sample (diluted 1 + 1).

As a result of these investigations, the following conclusions were drawn: (*i*) the yttrium line at 324.23 nm interferes strongly with the palladium line at 324.27 nm, as is shown clearly by the spectral profiles in Fig. 5; (*ii*) the use of the palladium line at 340.45 nm is hindered by the complex interference resulting from the concurrence of the iron (340.44 nm), neodymium (340.47 nm), samarium (340.48 nm) and zirconium (340.48 nm) lines, while the palladium line at 342.12 nm is of no practical use, owing to its low intensity under the selected experimental conditions, as can be observed from the spectral tracing in Fig. 6; (*iii*) the argon line at 363.44 nm is almost coincident with the palladium line at 363.47 nm, thus prohibiting the use of this line for analytical applications; (*iv*) the palladium line at 360.95 nm is affected by a severe interference from the samarium and neodymium lines at the same wavelength (360.949 and 360.945 nm, respectively); the spectral profiles related to these interferences are shown in Fig. 7.

Thus, no useful lines for the direct determination of palladium in the sample by ICP-OES under the experimental conditions described could be identified, apart from the line at 229.65 nm, which is affected by a moderate background interference, but its intensity is too low for practical purposes, as is shown clearly in Fig. 8.

The use of a monochromator having a higher resolving power, such as those conventionally coupled to the ICP source (with a focal length of more than 1 m), is likely to make the majority of the palladium lines discussed useful for accurate determinations, without any interferences. However, it should be stressed that even when the necessary resolution is not available, one can still take advantage of the ICP source through the simple instrumental approach offered by the fluorescence technique or by the resonance monochromator technique. In the analysis of samples of nuclear waste however, only a limited amount (about 1–2 ml) of sample will be available; this fact and the radioactivity hazard suggest that the samples should be handled after a reasonable dilution. Therefore, the ICP - resonance monochromator technique would probably not be applicable to this matrix. Nevertheless, it was considered worthwhile to perform some measurements by this technique for comparative purposes, taking into account its greater versatility with respect to conventional atomic fluorescence and the fact that scattering problems can be completely overcome.[7]

Conventional atomic-fluorescence measurements have been performed by introducing a 5000 μg ml^{-1} solution of palladium into the ICP torch while the sample solution (diluted

1 + 9) was nebulised into the argon-shielded air - acetylene flame. A quartz lens was used to focus the plasma plume on to the flame. Conversely, no collecting optics were used to monitor the fluorescence at 363.47 nm with the 0.1-m focal length monochromator, whose entrance slit was located 5 cm away from the flame at right-angles to the main optical axis.

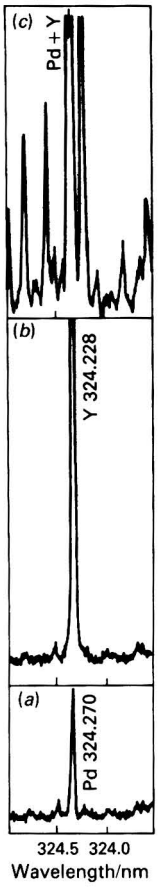

Fig. 5. Spectral interference of Y at 324.27 nm. (*a*) Pure Pd solution (5 μg ml^{-1}); (*b*) pure Y solution (1.5 μg ml^{-1}); and (*c*) synthetic nuclear-waste solution sample (diluted 1 + 49).

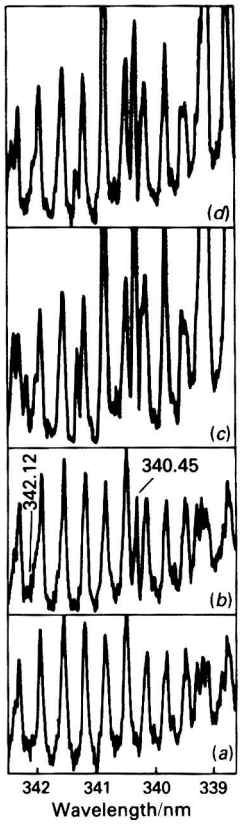

Fig. 6. Combined interference of Fe, Nd, Sm and Zr at 340.46 nm. (*a*) Water; (*b*) pure Pd solution (1 μg ml^{-1}); (*c*) synthetic nuclear-waste solution sample (diluted 1 + 199); and (*d*) Pd solution (1 μg ml^{-1}) + 3 μg ml^{-1} Zr solution.

Quantitation was achieved by aspirating standard palladium solutions into the flame, at concentration levels above and below that of the sample. No significant contribution to the scattering of the measured signal was found. Almost coincident analytical results were obtained by the ICP - resonance monochromator technique. In this instance the sample (undiluted) was aspirated into the ICP torch, the resulting emission being analysed by a resonance monochromator made by the air - acetylene flame fed with a 500 μg ml^{-1} solution of palladium. The excellent matching of the analytical data from the two techniques constitutes a further indirect check of the negligible influence of the scattering with conventional atomic-fluorescence measurements. The analytical data, as compared with atomic-absorption values, are given in Table III.

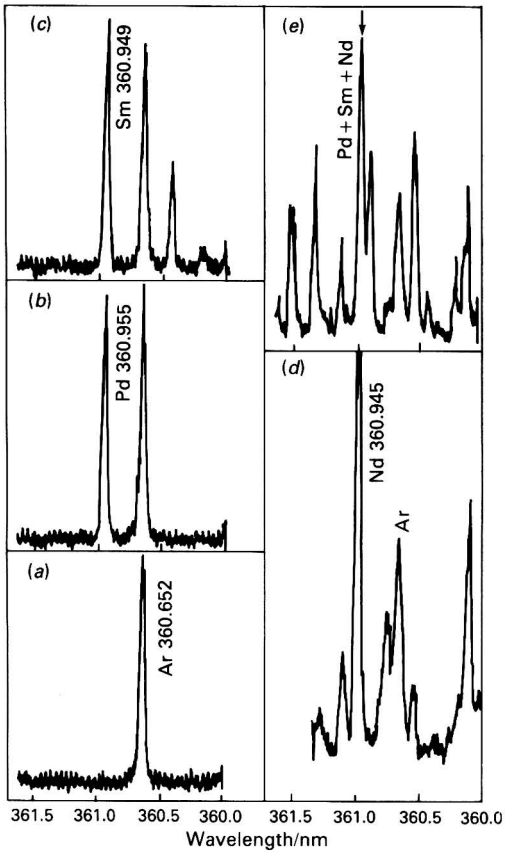

Fig. 7. Sm and Nd interference at 360.95 nm. (*a*) Water; (*b*) pure Pd solution (5 μg ml^{-1}); (*c*) pure Sm solution (3 μg ml^{-1}); (*d*) pure Nd solution (15 μg ml^{-1}); and (*e*) synthetic nuclear-waste solution sample (diluted 1 + 49).

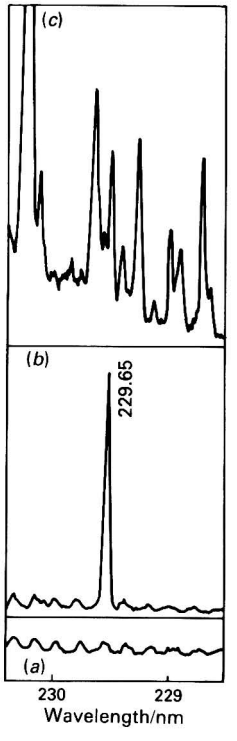

Fig. 8. Traces at 229.65 nm Pd wavelength. (*a*) Water; (*b*) pure Pd solution (250 μg ml^{-1}); and (*c*) synthetic nuclear-waste solution sample.

Conclusions

It has been shown that with very complex matrices, ICP-OES can suffer from severe problems arising from spectral interference, which are likely to hinder, or make impossible, analysis by simultaneous multi-channel spectrometers. In these circumstances scanning monochromators represent the obvious alternative way to solve the problem, provided that adequate resolving power is available. With the determination of palladium in solutions of

TABLE III

ANALYTICAL RESULTS

The reproducibility of the measurements was always of the order of 2%.

Method of analysis	Palladium concentration/ μg ml^{-1}	Analytical line/nm
Expected (synthetic solutions)	260	
ICP - excited fluorescence	250	363.5
ICP - emission resonance monochromator ..	250	363.5
Flame atomic absorption	265	247.6

nuclear waste, a simpler approach to removing spectral interferences from concomitants in the solution is offered by the combination of the ICP source with the spectral selectivity of atomic-fluorescence spectrometry.

The simplicity of the experimental set-up does not need to be stressed, the general configuration of the ICP-emission spectrometer, both simultaneous or sequential, being in no way altered by the addition of a supplementary atomiser.

As already discussed[7] the use of an ICP to excite fluorescence in an external atomiser must not be regarded as a replacement for the conventional application of this source for analytical emission spectrometry. However, by the techniques described the versatility and flexibility of the ICP can be further exploited and some troublesome analytical problems solved.

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Determination of Tellurium by Atomic-absorption Spectroscopy with Electrothermal Atomisation after Pre-concentration by Hydride Generation and Trapping

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A procedure for the determination of tellurium at sub-microgram levels by atomic-absorption spectroscopy with electrothermal atomisation after the evolution and trapping of hydrogen telluride is described. Interferences are observed in the presence of silver(I), copper(II), mercury(II) and selenium(IV) but can be overcome by suitable pre-treatment procedures. The detection limit (based on four times the standard deviation of six blank measurements) is 0.006 μg and the coefficient of variation is 4% at the 0.3-μg level.

Keywords: Tellurium; hydride generation and trapping; atomic-absorption spectroscopy; electrothermal atomisation; interferences

Tellurium is used extensively in the electronics industry and is a potentially toxic environmental pollutant.[1] Hence, sensitive methods of determination are required for monitoring tellurium concentrations in the environment and to allow the biochemical role and toxicological effects of tellurium to be assessed.

Many techniques are available for the determination of tellurium.[2–6] A widely reported technique has been hydride generation - atomic-absorption spectroscopy. The prior conversion of tellurium into hydrogen telluride is used to increase sensitivity and the hydride has been flushed directly into a flame[7–9] and a variety of heated silica tubes.[10,11] At present, methods incorporating hydride generation have mainly been used to analyse standard solutions[7–10] with limited application to complex or environmental samples.[8,11]

In this paper, the optimisation of a hydride generation and trapping system for the isolation and concentration of tellurium, prior to atomic-absorption spectroscopy with electrothermal atomisation, is described. The advantage of using a trapping system is in the elimination of the effects of interferents causing variable rates of hydride evolution. Interferences have been investigated and procedures developed for the removal of severe interferences.

Experimental

Equipment

The hydride generation and trapping system used is illustrated in Fig. 1. Nitrogen was used to flush hydrogen telluride from the generator into the centrifuge tube. Sodium tetrahydroborate(III) solution was injected using a plastic syringe connected to a small length of narrow-bore plastic tubing leading to the bottom of the flask. All atomic-absorption measurements were made with a Varian Techtron AA5 background-corrected atomic-absorption spectrometer fitted with a Perkin-Elmer HGA 72 carbon furnace. The following spectrometer conditions were used throughout the work: lamp current, 8 mA; wavelength, 214.3 nm; and spectral band pass, 0.2 nm.

Reagents and Glassware

All chemicals were of analytical-reagent grade. Glassware was soaked in sulphuric acid (2 + 8), rinsed with distilled water and dried before use.

Tellurium(IV) standard solution, 1000 μg ml^{-1}. Dissolve 0.1264 g of tellurium(IV) oxide in 100 ml of 3 M hydrochloric acid.

Tellurium(VI) standard solution, 1000 μg ml^{-1}. Dissolve 0.1802 g of ammonium tellurate in 100 ml of 3 M hydrochloric acid.

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Sodium tetrahydroborate(III) solution, 10% *m/V*. Dissolve 2.5 g of sodium tetrahydroborate(III) in 25 ml of 0.1 M sodium hydroxide solution. This solution is filtered through a 0.4-μm membrane filter to remove inhomogeneities and increase solution stability to at least 5 h.

Potassium iodide (0.8% *m/V*) - *iodine* (0.5% *m/V*) *solution*. Prepare a trapping solution by dissolving 0.8 g of potassium iodide and 0.5 g of iodine in 100 ml of distilled water.

Lanthanum(III) solution, 5% *m/V*. Dissolve 5 g of lanthanum(III) chloride in 100 ml of distilled water.

2,3-*Diaminonaphthalene* (*DAN*) *solution*, 0.2% *m/V*. Dissolve 0.2 g of DAN in 100 ml of 0.1 M hydrochloric acid containing 0.5 g of hydroxylammonium chloride. This solution is purified, after heating at 50 °C for 25 min, by extraction with cyclohexane.

EDTA - hydroxylamine solution, 10% *m/V*. Prepare by dissolving 5 g of disodium ethylenediaminetetraacetate (EDTA) and 5 g of hydroxylammonium chloride in 50 ml of distilled water.

Diphenylthiourea reagent, 1% *m/V*. Dissolve 0.5 g of diphenylthiourea in 50 ml of chloroform.

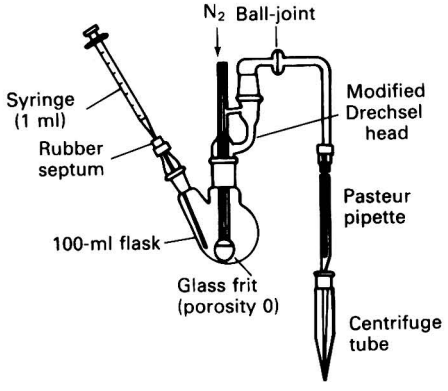

Fig. 1. Hydride generation and trapping system.

Procedure

Solutions are initially diluted to 50 ml with hydrochloric acid (final concentration 6 M) and heated at 100 °C for 40 min to reduce any tellurium(VI) present to tellurium(IV). A hydrochloric acid solution is transferred into the hydride-generation flask and the apparatus assembled as in Fig. 1, with 2 ml of potassium iodide - iodine solution in the centrifuge tube. The nitrogen flow-rate is adjusted to 300 ml min^{-1}, 1 ml of sodium tetrahydroborate(III) solution is injected and the hydrogen telluride that evolves is collected over a 4-min period.

Aliquots (20 μl) of the potassium iodide - iodine solution are injected into the carbon furnace and the tellurium atomic absorption is measured using the optimum conditions established for the removal of the potassium iodide - iodine solution (Table I).

TABLE I

OPTIMUM CARBON FURNACE PROGRAMME FOR THE DETERMINATION OF TELLURIUM BY ATOMIC-ABSORPTION SPECTROSCOPY WITH ELECTROTHERMAL ATOMISATION

	Stage			
Parameter	Dry	Ash 1	Ash 2	Atomise*
Temperature setting	18	35	90	600
Temperature/°C	39	98	370	2213
Time/s	40	60	10	5

* Atomisation temperatures quoted are manufacturer's temperatures.

Results

Optimisation of Hydride Generation and Trapping

To optimise the conditions for hydride generation and trapping, the effects of sodium tetrahydroborate(III) concentration, hydrochloric acid concentration, gas flow-rate and trapping-solution composition on the evolution and trapping of hydrogen telluride were investigated. Sample solutions containing 0.75 μg of tellurium(IV) in 50 ml of 5 M hydrochloric acid and 2 ml of a 1% *m/V* potassium iodide - iodine solution were used for the optimisation of flow-rate and sodium tetrahydroborate(III) concentration. The optimum gas flow-rate was 300 ml min^{-1} and solutions containing 10% *m/V* sodium tetrahydroborate(III) in 0.1 M sodium hydroxide quantitatively reduced up to 0.75 μg of tellurium to hydrogen telluride. Variation of the hydrochloric acid concentration was found to have a pronounced effect on hydride generation (Table II), the optimum hydrochloric acid concentration being 6 M. Under these optimum conditions the collection of hydrogen telluride was complete within 4 min. A trapping solution containing 0.8% *m/V* of potassium iodide and 0.5% *m/V* of iodine, quantitatively trapped up to 0.75 μg of tellurium as the hydride.

TABLE II

EFFECT OF HYDROCHLORIC ACID CONCENTRATION ON HYDRIDE GENERATION AND TRAPPING

The conditions were as follows: trapping solution composition, 1% *m/V* potassium iodide, 1% *m/V* iodine; flow-rate, 300 ml min^{-1}; collection time, 10 min; and all solutions contained 0.75 μg of tellurium(IV) and were injected with 1 ml of 10% *m/V* sodium tetrahydroborate(III) solution.

Hydrochloric acid concentration/M	2.4	3.6	4.8	6.0	7.2	8.4
Tellurium recovered, %	33	78	94	100	97	84

Preliminary experiments showed that only tellurium(IV) will form hydrogen telluride. Therefore any tellurium(VI) present, initially in extracts or produced during preparation of extracts, must be reduced to tellurium(IV). The method selected to reduce tellurium was to heat extracts with hydrochloric acid, *i.e.*, $Te(VI) + 2Cl^- \xrightarrow{H^+} Te(IV) + Cl_2$. The optimum heating time was 40 min at 100 °C when a hydrochloric acid concentration of 6 M was used (Table III).

TABLE III

EFFECT OF HEATING TIME ON THE REDUCTION OF TELLURIUM(VI) TO TELLURIUM(IV)

All solutions contained 0.75 μg of tellurium(VI) in 50 ml of 6 M hydrochloric acid; a temperature of 100 °C and optimised hydride generation trapping conditions were used.

Time of heating/min	10	20	30	40	50	60
Reduction, %	45	87	98	100	100	100

Precision and Detection Limit

The precision was estimated from replicate analyses of a 0.3-μg tellurium(VI) standard carried through the entire procedure. The relative standard deviation at this concentration was 4% (five determinations). The detection limit corresponding to four times the standard deviation of the blank analyses was 0.006 μg (six determinations).

Interferences

Identification

Possible interference by other elements was investigated by measuring the hydrogen telluride generated and trapped in the presence of elevated concentrations of other elements.

The concentrations at which certain elements interfere are shown in Table IV. Various other elements [Al(III), B(III), Ca(II), Cr(VI), K(I), Li(I), Mg(II), Mn(II), Na(I), Pb(II), S^{2-}, Si(IV), Zn(II)] showed no significant interference up to the 5000 μg (100 μg ml^{-1}) level.

TABLE IV

EFFECT OF INORGANIC IONS ON THE GENERATION AND TRAPPING OF HYDROGEN TELLURIDE

All solutions contained 0.5 μg of tellurium(IV) in 50 ml of 6 M hydrochloric acid and optimised hydride generation and trapping conditions were used.

Species	Concentration/μg per 50 ml	Tellurium recovered, %
Ag(I)	10	0
	1	100
As(III)	100	52
	50	100
Cd(II)	1000	100
Co(III)	2500	75
	1000	90
	500	100
Cu(II)	100	71
	50	100
Fe(III)	2500	87
	1000	93
	500	100
Hg(II)	10	0
	1	100
Mo(VI)	500	67
	250	105
	100	100
Ni(II)	1000	76
	500	100
Sb(III)	50	19
	10	98
	1	100
Se(IV)	50	18
	0.1	90
Sn(IV)	250	66
	100	100
V(V)	1000	100

Suppression and elimination

Although several elements cause significant interference when present at the 1000 μg (20 μg ml^{-1}) level only silver(I), copper(II), mercury(II) and selenium(IV) are likely to be found at concentrations in environmental materials (excluding sediments) that will cause significant interference.

Initially four complexing agents, thiosemicarbazide, 1,10-phenanthroline, 8-hydroxyquinoline and disodium ethylenediaminetetraacetate, were tested in an attempt to suppress interferences by complexation before generation of hydrogen telluride. These reagents were not effective in suppressing interferences. Interferences were removed by a combination of co-precipitation and sequential extraction.

Copper(II) and mercury(II). After reduction of tellurium(VI) to tellurium(IV) 1 ml of lanthanum(III) chloride solution and one drop of phenolphthalein solution were added with stirring, followed by the addition of 25% *V/V* ammonia solution until a pink colouration occurred (pH 9–10). The lanthanum precipitate containing tellurium but not copper(II) and mercury(II) was separated by centrifugation and washed twice with 20 ml of 3 M ammonia solution.

Selenium(IV). The precipitated lanthanum hydroxide was dissolved in 10 ml of DAN solution, 1 ml of EDTA - hydroxylamine solution was added and the mixture was heated in a water-bath at 50 °C for 50 min. The piazselenol formed was extracted into cyclohexane (2 × 10 ml) and discarded. Tellurium remained in the aqueous phase.

Silver(I). The previous solution was extracted with 20 ml of diphenylthiourea reagent and the chloroform phase was discarded.

The efficiency of the co-precipitation and sequential extraction procedure to remove interferences was assessed by the analysis of solutions containing 0.5 μg of tellurium(IV) together with 1000 μg of copper(III) and mercury(II) and 10 μg of silver(I) and selenium(IV). The percentage deviation from interference-free determinations of tellurium was less than 7%. Average recoveries of 93 ± 4% (five determinations) were achieved.

Discussion and Conclusion

A simple system for the generation and trapping of hydrogen telluride prior to the determination of tellurium by atomic-absorption spectroscopy with electrothermal atomisation has been developed and optimised.

Interferences from copper(II), mercury(II), silver(I) and selenium(IV) have been identified. Suppression of these interferences by complexing agents was unsuccessful, probably owing to the instability of complexes in concentrated acid solution. However, the use of a co-precipitation - sequential extraction procedure prior to hydride generation overcomes all identified severe interferences.

The technique should allow the sensitive measurement of tellurium concentrations in extracts. Investigation of interferences to the method also demonstrated that the use of hydrogen telluride generation to isolate and concentrate tellurium from extracts is in general of limited use unless potential interfering elements are identified and removed.

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Determination of Trace Amounts of Molybdenum in Natural Waters by Solvent Extraction - Atomic-absorption Spectrometry, After Chelating Ion-exchange Pre-concentration

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A method for the determination of molybdenum in natural waters in the micrograms per litre range is proposed. The method involves the pre-concentration of molybdenum on a Chelex-100 chelating resin and subsequent elution with ammonia solution, followed by extraction with a complexing reagent, 1,4-dihydroxyphthalimide dithiosemicarbazone dissolved in *NN*-dimethylformamide - isoamyl alcohol (1 + 4), and a final direct determination by atomic-absorption spectrometry. The addition of ascorbic acid prior to extraction eliminates the interfering effect of several ions at the concentration levels normally found in natural waters. The sensitivity of the method is 0.3 $\mu g\ l^{-1}$ for 1% absorption. The method has been applied to the determination of molybdenum in sea and surface waters.

Keywords: *Molybdenum determination*; *natural water analysis*; *chelating ion-exchange separation*; *atomic-absorption spectrometry*; *1,4-dihydroxyphthalimide dithiosemicarbazone*

The determination of certain trace elements has become significant in studies of geochemical, biochemical and industrial processes in natural waters. An investigation of this problem is being carried out in our laboratories and this paper describes the development of a method for the determination of molybdenum in the micrograms per litre range.

In order to detect the low levels of molybdenum that occur naturally[1] (from less than 1 to 10 $\mu g\ l^{-1}$ and even larger amounts in sea water), pre-concentration methods are usually necessary. For this purpose coprecipitation,[2–5] cocrystallisation,[6,7] extraction,[8–11] ion exchange[12–15] and activated charcoal[16] have been used, the determination being completed by either spectrophotometry or atomic-absorption spectrometry (AAS) or sometimes by other procedures (emission spectroscopy, neutron activation analysis). In recent years, many papers have described the direct determination of molybdenum by the use of modern techniques, for example graphite furnace AAS,[17] anodic-stripping voltammetry,[18] electron paramagnetic resonance spectrometry[19] and oscillography.[20] However, such techniques are not available to all laboratories and may also introduce other problems in achieving the desired accuracy of analysis, *e.g.*, the prevalence of matrix interference effects in electrothermal atomisation AAS.

This paper reports a sensitive and selective method based on pre-concentration of molybdenum on a Chelex-100 chelating resin,[21–22] followed by extraction with 1,4-dihydroxyphthalimide dithiosemicarbazone (OH-PDT) in isoamyl alcohol and a final direct determination by AAS. The use of OH-PDT as a complexing reagent for molybdenum has been reported previously[23] and applied to the determination of microamounts of molybdenum by an extraction - spectrophotometric method. The proposed method in this paper, with an AAS finish, gives a greater sensitivity and eliminates most of the interfering cations.

In recent years several systems based on solvent extraction of molybdenum complexes with subsequent AAS determination in the organic phase have been used for enhancement of the sensitivity of molybdenum absorption and for concentrating the samples and eliminating interfering cations. Of these, quinolin-8-ol - isobutyl methyl ketone (IBMK),[8] quinolin-8-ol - *n*-amyl methyl ketone,[24] ammonium tetramethylene dithiocarbamate - IBMK,[25] dithiol - IBMK[26] and thiocyanate - IBMK[11] are generally used for the determination of molybdenum in waters, usually with a preliminary concentration step. However, limitations with regard to sensitivity, simplicity and freedom from interferences (especially iron) are observed. The proposed method, with pre-concentration and separation on Chelex-100 resin, is relatively simple and sufficiently versatile for the analysis of sea and surface waters.

Experimental

Reagents

All reagents and solvents were of analytical-reagent grade unless specified otherwise. Distilled, de-ionised water was used.

Chelating resin. Digest Chelex-100 (Bio-Rad Laboratories, 100–200 mesh) with 2 N nitric acid and fill 2 cm diameter ion-exchange columns to a depth of 3 cm with it. Wash the columns with 20 ml of 2 N nitric acid and then with water until the pH of the eluate is 5–6.

1,4-Dihydroxyphthalimide dithiosemicarbazone solution. Prepare a 0.05% *m/V* solution by dissolving 0.05 g of the reagent in 20 ml of *NN*-dimethylformamide and dilute to 100 ml with isoamyl alcohol. This solution is stable for 1 week. The synthesis of OH-PDT has been described previously.[27]

Standard molybdenum solution, 1.000 g l^{-1}. Dissolve 1.500 g of molybdenum(VI) oxide in the minimum volume of 0.1 M sodium hydroxide solution, dilute with water, make slightly acidic (pH 3–4) with 0.1 M hydrochloric acid and dilute to 1 l with water. Prepare a working solution, containing 10 μg ml^{-1} of molybdenum, from this stock solution by appropriate dilution.

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 water.

Ascorbic acid solution, 1% *m/V*. Dissolve 1 g of ascorbic acid in water and dilute to 100 ml with additional water.

Nitric acid, 0.1–4 N. Prepare from analytical-reagent grade concentrated nitric acid by appropriate dilution.

Apparatus

Spectrophotometer. A Perkin-Elmer 103 atomic-absorption spectrophotometer, equipped with a dinitrogen oxide - acetylene burner head and a multi-element (cobalt, copper, iron, manganese, molybdenum) hollow-cathode lamp was used. The operating conditions used are summarised in Table I.

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

TABLE I

ATOMIC-ABSORPTION CONDITIONS FOR MOLYBDENUM DETERMINATION

Wavelength	313.3 nm
Slit width	0.7 nm
Lamp current	10 mA
Acetylene pressure	10 p.s.i.g.
Acetylene flow-rate	Setting 12 (approximately 8 l min^{-1})
Dinitrogen oxide pressure	40 p.s.i.g.
Dinitrogen oxide flow-rate	Setting 13 (approximately 16 l min^{-1})
Aspiration rate	3 ml min^{-1}
Burner height	Adjust for optimum reading

Procedure

Filter the sample, as soon as possible after collection, through a 0.5-μm membrane filter. Take 1–5 l of the filtered water, and adjust its pH to 5–5.5 by cautious addition of 0.1 N nitric acid. Allow the sample to flow through the Chelex-100 column at a rate not exceeding 5 ml min^{-1}. Wash the resin with 200 ml of water and elute molybdenum with 20 ml of 4 N ammonia solution. Collect the eluate in a 25-ml beaker and adjust its pH approximately to neutrality with appropriate dilute nitric acid (0.1–4 N). Transfer into a separating funnel, add 5 ml of pH 2.6 buffer solution and 5 ml of ascorbic solution. After mixing, add 10 ml of OH-PDT solution and shake vigorously for 1 min. Allow the phases to separate and draw off the aqueous layer. Transfer the organic phase into a glass-stoppered tube containing anhydrous sodium sulphate and aspirate directly into the dinitrogen oxide - acetylene flame. Determine the absorbance at 313.3 nm, using isoamyl alcohol as a blank, under the specified conditions.

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

Results and Discussion

Study of the Pre-concentration of Molybdenum on Chelex-100 Chelating Resin

The retention of molybdenum by Chelex-100 resin is dependent on pH. This effect was studied using a series of dilute standard solutions of molybdenum in the pH range 1–9. Quantitative retentions were observed in the pH range 5–6. A pH between 5 and 5.5 was selected for the analysis. These results are in agreement with those reported for retention of molybdenum from sea waters.[14]

The nature and concentration of the eluting agent was studied under the conditions described under Experimental, using a 2 × 3 cm column of Chelex-100. Ammonia solution, sodium hydroxide solution and nitric, sulphuric and perchloric acids were investigated. Molybdenum was removed from resin only by ammonia solution and sodium hydroxide solution. Fig. 1 shows the elution diagrams at several concentrations. These diagrams were constructed by elution with successive 5-ml volumes of eluting agent. The recovery of molybdenum was assessed by the recommended procedure. A 20-ml volume of 4 N ammonia solution is recommended as a suitable amount of eluting agent.

In order to establish the possibility of determining trace amounts of molybdenum in waters, the recovery from large volumes of sample was studied by the recommended procedure. Quantitative recoveries were obtained for sample volumes of 50 ml–5 l.

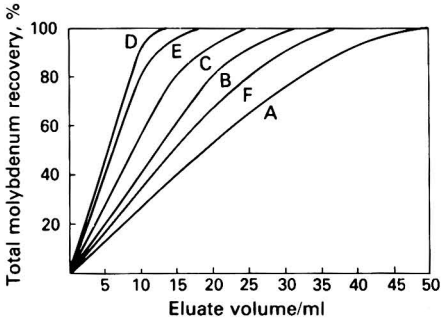

Fig. 1. Elution diagrams of molybdenum from Chelex-100 resin with ammonia solution and sodium hydroxide solution: A, B, C, D and E with 1, 2, 3, 4 and 5 N ammonia solution, respectively; F, with 2 N sodium hydroxide solution; amount of molybdenum added, 50 μg; successive volumes of eluate collected, 5 ml.

Extraction and Atomic-absorption Spectrometry of Molybdenum

When a solution of OH-PDT dissolved in *NN*-dimethylformamide - isoamyl alcohol is shaken with an aqueous acidic solution of molybdenum(VI), a yellow complex is formed immediately in the organic phase. This system has been used for the spectrophotometric determination of molybdenum.[23] However, it is unsuitable for water analysis because of interferences from certain ions [iron(II), iron(III), copper(II), cobalt(II) and vanadium(V)] at the levels commonly found in natural waters.

The extraction of molybdenum with subsequent AAS determination is dependent on pH. The most favourable pH range is 2–4, identical with that reported for the spectrophotometric determination. A chloroacetic acid - sodium hydroxide buffer solution is added for control of the pH of the extraction.

The effect of OH-PDT concentration in *NN*-dimethylformamide - isoamyl alcohol (1 + 4) was investigated in the range 0.002–0.1% *m*/*V*. A 0.006% solution was necessary in order to obtain maximum absorbance at 313.3 nm; the latter remained constant with increasing concentration. A 0.05% solution is recommended as a suitable concentration of reagent.

The influence of the phase-volume ratio (aqueous to organic phase) was studied. The volume of the organic phase was kept constant at 10 ml, varying the volume of the aqueous phase. When the phase-volume ratio was higher than 4 the absorbance increased because of

the appreciable solubility of the organic solvent in water. The absorbance remained constant when smaller ratios were employed. It is concluded that the volume of the aqueous phase should be smaller than 40 ml if a 10-ml volume of organic phase is utilised.

The ionic strength of the aqueous phase does not affect the atomic absorption of the extracted complex. Salts such as sodium sulphate, potassium perchlorate, potassium chloride and potassium nitrate do not affect the absorbance signal, even at a concentration of 2%.

In order to establish the suitability of this system for the determination of molybdenum, the results of a preliminary interference study with an AAS or spectrophotometric finish are reported in Table II. It is concluded that the selectivity with AAS is greater than that with spectrophotometry.

TABLE II

TOLERANCE LIMITS FOR THE DETERMINATION OF MOLYBDENUM WITH THE OH-PDT - ISOAMYL ALCOHOL SYSTEM BY AAS AND BY SPECTROPHOTOMETRY

Amount of molybdenum present: 50 μg.

Ions added	Tolerance limit (mass excess relative to Mo)	
	Spectrophotometry	AAS
Al(III), Ni(II), Mn(II)	15	>100
Zn(II), Cd(II), Ti(IV)		
La(III)		
Bi(III), Cr(III)	15	50
W(VI)	10	15
Pb(II)	1	50
Co(II)	2	15
Hg(II), V(V)	<1	2
Cu(II), Fe(III)	<1	2
Fe(II)	<1	5

Determination of Molybdenum in Waters

Calibration graph, sensitivity and precision

A calibration graph was prepared by using dilute standard solutions treated in the same way as the samples. A linear calibration graph was obtained up to 12 mg l^{-1} of molybdenum with respect to the organic phase and 24 μg l^{-1} with respect to water samples when a 5-l volume was utilised.

The sensitivity was 0.15 mg l^{-1} for 1% absorption in the organic phase and 0.3 μg l^{-1} with respect to water samples when a 5-l volume was utilised. The sensitivity obtained for the determination of molybdenum by AAS in the organic phase was about 3.3 times greater than that for aqueous solutions (0.50 mg l^{-1}).

The coefficient of variation, calculated from ten replicate analyses of dilute standard solutions containing 25 μg of molybdenum, was 3.0%.

Interference study

The recommended procedure was used to analyse standard molybdenum solutions in the presence of the major constituents of natural waters and of several trace elements that interfere in AAS (tolerance limits smaller than 10^2 mass excess relative to molybdenum) (see Extraction and Atomic-absorption Spectrometry of Molybdenum and Table II). Determinations in the presence of the major constituents were carried out at the levels normally present in sea water.[1] The results for the determination of 50 μg of molybdenum are shown in Table III.

From these results, it is concluded that the presence of the main constituents and of most of the trace elements did not affect the recovery of molybdenum at levels that occur naturally.

The tolerance limits for bismuth(III), cobalt(II), copper(II), iron(II), mercury(II), chromium(III) and lead(II) are greater than those obtained previously (Table II) because of their smaller retention on the resin. Tungstate(VI) does not interfere in amounts up to a 20-fold excess.

Vanadium(V) and iron(III) depress the molybdenum absorption markedly, probably because of preferential extraction of their OH-PDT complexes or flame interferences.[28] The

addition of ascorbic acid was found to prevent these interferences. When 5 ml of 1% ascorbic acid solution are added to the sample before extraction, up to 5 mg of iron(III) and 500 μg of vanadium(V) could be tolerated. The above limits are not likely to be exceeded in analyses of natural waters.

TABLE III

RECOVERY OF MOLYBDENUM IN THE PRESENCE OF THE MAJOR CONSTITUENTS OF WATER AND SEVERAL TRACE ELEMENTS THAT INTERFERE IN AAS

Amount of molybdenum present: 50 μg.

Ions added	Mass excess relative Mo	Amount of Mo recovered/μg
Na(I)	2.1×10^5	50.0
K(I)	7.6×10^3	50.0
Ca(II)	8.0×10^3	50.0
Mg(II)	2.7×10^4	50.0
Cl^-	3.8×10^5	50.0
SO_4^{2-}	1.7×10^4	50.0
Br^-	1.3×10^3	50.0
CO_3^{2-}	5.6×10^2	50.0
Bi(III), Co(II), Cu(II), Fe(II), Hg(II)	10^2	50.0
Cr(III), Pb(II)	10^2	33.5
	75	50.0
W(VI)	10^2	20.5
	20	50.0
Fe(III)	10^2	0.0
	10^{2*}	50.0
V(V)	10^2	10.5
	10*	50.0

* With addition of 5 ml of 1% ascorbic acid solution before extraction.

Analyses of natural waters

The proposed method was applied successfully to the determination of trace amounts of molybdenum in sea and surface water samples (Table IV).

The accuracy of the determinations was checked by carrying out replicate analyses of samples spiked with known amounts of molybdenum. The molybdenum recovery was calculated by comparing the results obtained before and after the addition of molybdenum standard solutions. The results showed that the recovery of molybdenum was satisfactory (Table IV).

TABLE IV

DETERMINATION OF MOLYBDENUM IN NATURAL WATERS

Type	Location of sampling	Mo found*/μg l^{-1} Mean	Mo found*/μg l^{-1} Range	Recovery, %
Bottled mineral water	Granada	0.3	0.2–0.3	100.0
Public water supply	Seville	0.9	0.8–1.0	99.5
River water	Guadalquivir river, Seville	2.7	2.7–2.8	99.5
Dock's river water	Guadalquivir's dock, Seville	6.3	6.2–6.4	98.9
Sea water A	Atlantic Ocean, Huelva	7.5	7.3–7.6	99.0
Sea water B	Atlantic Ocean, Huelva	7.2	7.1–7.4	99.2
Estuary water†	Huelva	9.9	9.8–10.0	98.5

* Average of three separate determinations.

† This water is affected directly by drainage of waste water from an industrial area.

Conclusion

The suitability of the proposed method for determining small amounts of molybdenum in waters in the range 0.3–24 $\mu g\ l^{-1}$ has been demonstrated. The use of a chelating resin in a pre-concentration and separation step separates molybdenum from the major components and eliminates the matrix effect on the atomic-absorption signal. The combination of solvent extraction with AAS provides greater sensitivity and eliminates most interfering cations. Finally, the method offers significant advantages with respect to relative rapidity, simplicity and versatility.

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Effect of pH on the Response of Glassy Carbon Electrodes

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The use of glassy carbon as an electrode material engenders a number of practical problems owing to the presence of C–O functionalities on its surface. One such problem is the susceptibility of the electrode to pH changes. Highly surface-active glassy carbon electrodes having a high proportion of irreversible C–O groups are particularly prone to variations in pH compared with ones having mainly quinoidal species. This is reflected in the performance of glassy carbon in the cyclic voltammetry of hydroquinone.

Keywords: *Glassy carbon electrode*; *surface groups*; *pH*; *hydroquinone*

For routine analytical applications it is often desirable to have an electrode material that is relatively insensitive to the effects of pH. In practice, however, electrodes show a marked change in response. The pH response of electrodes can be traced to acid - base reactions taking place at the surface. These reactions often involve surface oxides. For example, the rest potentials of a number of metal and semiconductor electrodes have been reported to show a Nernstian response to pH with the rest potential changing by about 59 mV per pH unit.[1–4] Such behaviour has been attributed to electrochemically reversible redox reactions involving the surface oxides and $H^+_{(aq.)}$.

The effect of pH on carbon electrodes has been previously reported.[5–11] The effect has mainly been thought to be a consequence of surface C–O functionalities, in particular quinoidal groups formed on free valence carbon sites by the chemisorption of oxygen. It has been observed that the equilibrium potential of glassy carbon electrodes varied by about 59 mV per pH unit.[10] The hypothesis put forward to explain this apparent Nernstian behaviour is that the quinoidal redox couple is reversible with respect to reactions involving $H^+_{(aq.)}$.

An important consideration with respect to the effect of pH is its influence on electrode reactions. There is the direct effect on the species (undergoing electrolysis) itself; this has been the subject of considerable research. What is less clear is the indirect influence on electrode reactions as a result of changes in the electrode surface with pH. Change in pH could occur in the bulk solution or, more subtly, in the region in the immediate vicinity of the electrode where the actual electrode processes take place. In the latter instance, depletion or enhancement of hydrogen ions (or hydroxyl species) could be the result of the electrode reaction itself.

In a previous paper[5] it was reported that glassy carbon showed varying surface characteristics depending on the degree of compactness of its bulk structure: the more compact the structure, the fewer the free valence carbon sites available on the surface and, hence, the fewer the C–O functionalities formed. Here, and in the previous work, the glassy carbon used was of two types: Tokai glassy carbon, characterised by low surface activity (mainly quinoidal); and Plessy glassy carbon, having a higher surface activity, including irreversibly formed functionalities.

This paper considers the effect of pH on the performance of glassy carbon electrodes in the pH range 0.3–8.4. At higher pH values a distinct difference in behaviour was observed. Differences in the electrochemical behaviour of glassy carbon at high and low pH have been noted by other workers.[6–8] Our own findings in this respect will be the subject of a separate paper.[9]

Experimental

The fabrication of glassy carbon electrodes has been described elsewhere.[5] Gold and platinum electrodes were similarly made using 3-mm discs encased in a Kel-F (3M, USA) body. The Plessy glassy carbons are classified as before, namely, GC1 and GC2.

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Glassy carbon electrodes were polished to a mirror finish with a 1-μm diamond paste, rinsed with ethanol and distilled water, and then soaked overnight in distilled, de-ionised water. After this treatment the chronopotentiometric and voltammetric experiments, described below, were performed with no further polishing. Electrodes were rinsed with distilled water prior to each analysis that required change of buffer solution. The major reason for not polishing the electrode between analyses was the likelihood of drastically changing the physicochemical characteristics of the carbon surface, which would have defeated the purpose of this study. Precautions were taken to keep potential limits at which electrodes were operated, to within the range -0.5 to $+1.5$ V *versus* S.C.E., which ensured that significant alteration of the carbon surface did not take place. Background cyclic voltammograms were routinely run in 0.5 M sulphuric acid between experiments to check the state of the surface. From these voltammograms it appeared that both Plessy and Tokai glassy carbon surfaces remained reasonably constant throughout the entire course of the experiments. This conclusion was based on the background current as well as the background-peak potentials of the voltammograms. The former is indicative of surface area as well as the concentration of surface functionalities. It should also be mentioned that no sign of adsorption was seen for the cyclic voltammetric studies of hydroquinone.

Gold and platinum electrodes were cleaned with concentrated nitric acid, polished with 1-μm diamond paste and then rinsed with ethanol and distilled, de-ionised water. The electrodes were soaked overnight in de-ionised water. The following buffer solutions were used in this work: sulphuric acid, pH 0.3–0.8; citrate buffer (citric acid - sodium citrate), pH 2.2–5.5; and phosphate buffer (Na_2HPO_4 - NaH_2PO_4), pH 5.8–8.4.

Open-circuit potentials were measured with a Corning EEL, Model 112, pH meter. Chronopotentiometric studies were carried out with a Model PAR 173 potentiostat - galvanostat (Princeton Applied Research). Cyclic voltammograms were obtained with a Model PAR 174 polarograph. Results were plotted on a Servoscribe plotter. Purified nitrogen was used to de-aerate the solutions.

Results and Discussion

Open Circuit Potential *versus* pH

As already mentioned, GC1 is more compact than GC2. Typical cyclic voltammograms of the two carbons obtained for 0.5 M sulphuric acid, given in Fig. 1, show the relative differences in surface activity. The cyclic voltammograms were obtained immediately prior to the open-

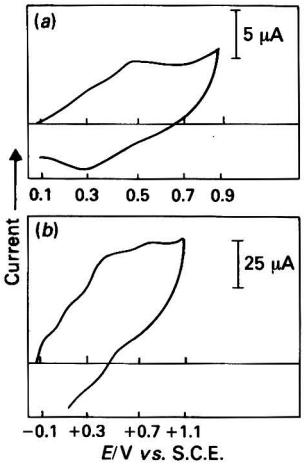

Fig. 1. Background of (*a*) GC1 and (*b*) GC2 in 0.5 M sulphuric acid. Scan rate, 20 mV s^{-1}.

circuit potential - pH measurements described below. The cyclic voltammogram of GC1, on the basis of the earlier reasoning, shows the dominance of the quinoidal redox couple whereas GC2 shows evidence of irreversible functionalities.[12]

Fig. 2 shows graphs of open-circuit potential *versus* pH, obtained for GC1 and GC2. Measurements were made 2 min after immersion to ensure that the electrode had approached its equilibrium-potential value. As can be seen, the graph for GC1 shows a near Nernstian behaviour with a slope of about 60 mV per pH unit. This slope is consistent for a $2e/2H^+$ process, which would be expected for the surface quinoidal redox couple; the slope for GC2 has a significantly lower value. The difference in the slopes could be explained on the basis that the acid - base redox reaction at the surface of GC2 is irreversible, a consequence of the irreversible C–O functionalities, dominant on the surface of this carbon.

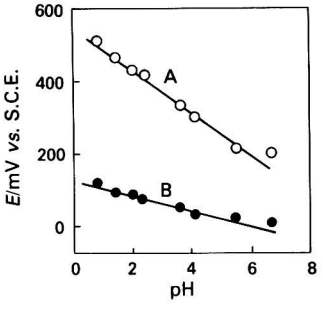

Fig. 2. Open-circuit *versus* pH plots for (A) GC1 and (B) GC2.

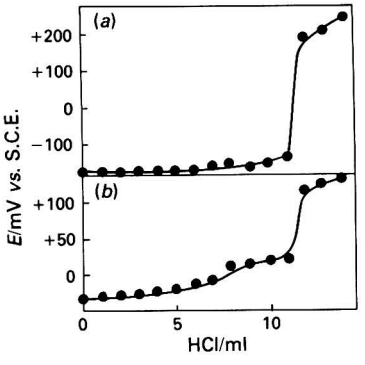

Fig. 3. Use of glassy carbon as an indicator electrode in acid - base titration: (*a*) GC1; and (*b*) GC2.

Acid - Base Titration

The use of carbon electrodes as indicators in acid - base titrations has been described and affords an interesting demonstration of the differing response of GC1 and GC2. Fig. 3 shows the titration of sodium hydroxide by hydrochloric acid as monitored by the two carbon electrodes. Each measurement was made 2 min after addition of the acid. As can be seen, GC2 shows a poorer response at the end-point; again, this is consistent with the irreversible nature of the C–O groups found on the surface of this carbon.

Tokai glassy carbon, previously oxidised at +1.5 V, showed a behaviour similar to GC1 with respect to the open-circuit potential *versus* pH graphs. This could be expected as the surface C–O functionalities of this carbon are mainly of the quinoidal type.

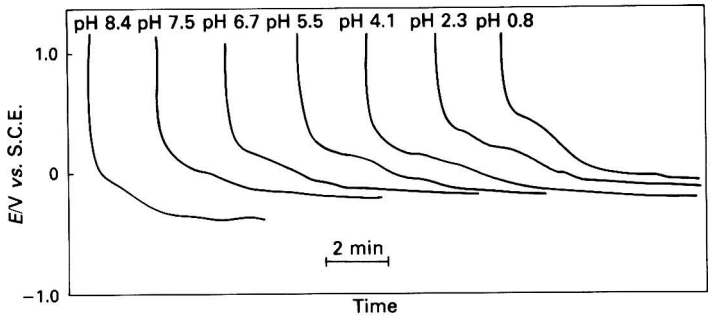

Fig. 4. Effect of pH on cathodic charging curves of Plessy glassy carbon. Charging current = −10 μA.

Chronopotentiometric Studies

According to Vetter,[13] the anodic and cathodic charging curves for platinum are the same regardless of pH and the only apparent change was a displacement of the charging curves by 59.2 mV per pH unit. This result is indicative of the reversible nature of the acid - base redox reactions involving adsorbed oxides on platinum surfaces. With glassy carbon, the effect of pH on the charging curves is more complex. Fig. 4 shows the cathodic-charging curves obtained for GC2 between a pH of 0.8 and 8.4. It can be seen that as pH increases, the charging curve becomes broader having less defined arrests. If corresponding points for each potential *versus* time charging curve are plotted against pH, interesting trends are found, as shown in Fig. 5. Plot (A), representing corresponding points of the cathodic charging curves 5 min

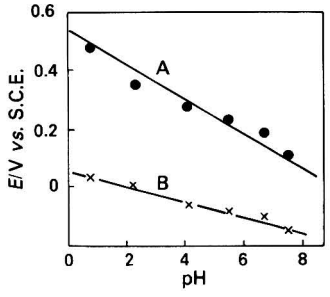

Fig. 5. Corresponding potential *versus* pH plots: (A) points taken 1 min after start of cathodic charging curve; and (B) points taken 5 min after start.

after the start of each curve, has a slope of about 60 mV per pH unit. This value reflects the response of the reversible quinoidal couple. A similar observation was reported by Evans and Kuwana,[11] for oxygen plasma treated pyrolytic graphite, though here the "surface quinone" potential was evaluated by cyclic voltammetry and differential pulse voltammetry. Plot (B), representing points 1 min after the start of the cathodic charging curves, has a significantly lower slope that is close to 25 mV per pH unit. As the points are from the extreme negative region of the charging curves, it is plausible to surmise that the small slope is the result of reactions involving irreversible C–O functionalities and hydrogen adsorption. Hence, hydrogen adsorption appears to be an irreversible process on glassy carbon.

Comparable trends were observed for anodic charging curves of GC2 as shown in Fig. 6. With increase in pH, arrests appeared to become broader and less well defined than with the cathodic charging curves.

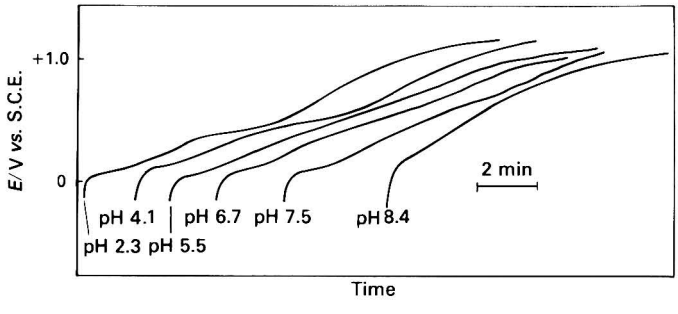

Fig. 6. Effect of pH on anodic charging curve of Plessy glassy carbon. Charging current = -10 μA.

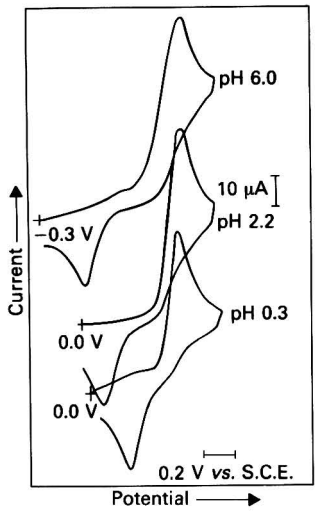

Fig. 7. Cyclic voltammograms of hydroquinone for platinum electrode.

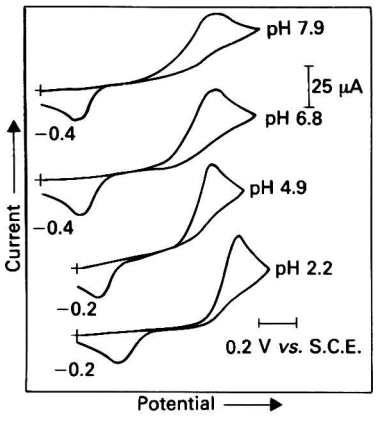

Fig. 8. Cyclic voltammograms of hydroquinone for gold electrode.

Cyclic Voltammetry of Hydroquinone

According to Adams,[14] the cyclic voltammetry of hydroquinone on solid electrodes suggests a high degree of irreversibility. The over-all reaction involves a two-electron transfer and is highly sensitive to pH. Vetter[15] showed that the reaction involved consecutive one-electron transfers. It led to the conclusion that the reaction mechanism varies with pH; one at low pH and the other at high pH. Hydroquinone was chosen for our investigations on account of its well studied electrochemistry and because the response of the quinhydrone redox couple to pH should parallel that of surface quinoidal species on glassy carbon.

Figs. 7–10 show cyclic voltammograms of 5 mM hydroquinone at different pH for platinum, gold, Tokai glassy carbon and GC2 electrodes. The scan rate for all voltammograms is 20 mV s^{-1}. It can be seen that for the Tokai carbon, platinum and gold electrodes, as pH is increased, the shape of the cyclic voltammogram does not change as significantly as it does with

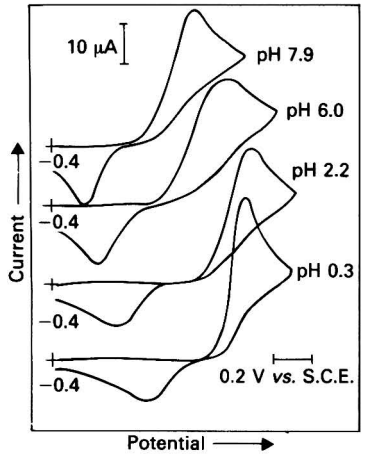

Fig. 9. Cyclic voltammograms of hydroquinone for Tokai glassy carbon.

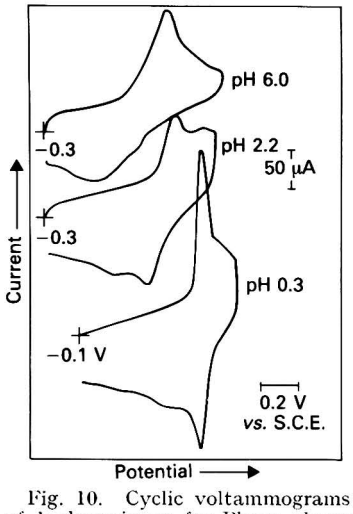

Fig. 10. Cyclic voltammograms of hydroquinone for Plessy glassy carbon (GC2).

GC2. Also, the peak current decreases (as pH increases) most appreciably for GC2. Tokai glassy carbon in fact shows the least susceptibility to pH change in the way of peak shape as well as peak current. A plot of peak potential *versus* pH, given in Fig. 11, shows that anodic and cathodic peak potentials become more negative with increase in pH. This has been described by Adams.[14] The line drawn through the plotted points marks the average potential *versus* pH change in the low pH range; the slope of this line for GC2 is significantly closer to the Nernstian value of 60 mV per unit than for the other electrodes. This is indicative of the greater reversibility of the hydroquinone electrode reaction in the GC2 electrode; a point further substantiated by the fact that the separation of the cathodic and anodic peak potentials, as shown in the cyclic voltammograms of Fig. 10, are less for this carbon. The deviation at pH 6 for all the electrodes, seen in Fig. 11, could be ascribed to an anion effect caused as a result of changing from citrate to phosphate buffers. Another reason could be the difference in the quinhydrone redox process at high and low pH, as mentioned before.

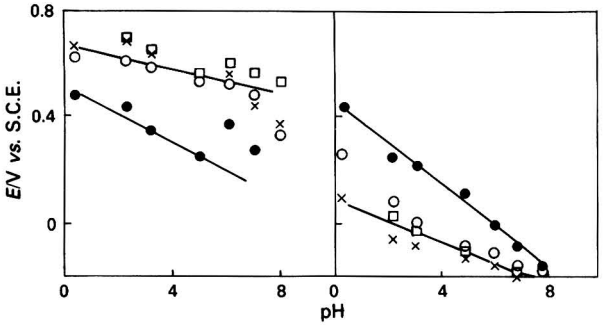

Fig. 11. Variation of anodic (left) and cathodic (right) peak potentials with pH.

Conclusion

The results presented in this paper confirm the assertion previously made that the greater the surface activity of glassy carbon electrodes, the greater the susceptibility to pH. Plessy glassy carbon of the GC2 type, having a significantly greater proportion of irreversible C–O functionalities at its surface, appears to be more susceptible in this respect. It may, therefore, be desirable, from the analytical point of view, to use a less surface active carbon such as the Tokai type, in favour of a more active one such as GC2. The criterion would be electrocatalytic performance (that is, sensitivity) *versus* reproducibility (in this example with change in pH). Moreover, Tokai carbon affords some measure of predictability in regard to its response.

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Determination of Polychloro-2-(chloromethylsulphonamido)diphenyl Ether Insectproofing Agents on Wool Textiles and in Textile Liquors by High-performance Liquid Chromatography

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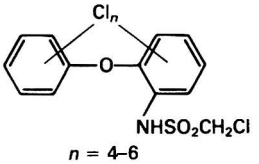
Polychloro-2-(chloromethylsulphonamido)diphenyl ether insectproofing agents were extracted from wool textiles with methanol - ammonia solution in sealed ampoules and determined by normal bonded-phase high-performance liquid chromatography. Liquor samples were extracted with dichloromethane and determined similarly. Satisfactory recoveries of the insectproofing agents were obtained from spiked liquor and wool samples. Accurate and reproducible analyses of the formulations were obtained down to concentrations of 0.05% *m/m* on wool textiles and 0.5 mg l^{-1} in liquor samples. The short analysis time and simplicity of this method make it ideally suited for the routine determination of these insectproofing agents on wool textiles.

Keywords: *Insectproofing agents; polychloro-2-(chloromethylsulphonamido)-diphenyl ethers; wool textiles; textile liquors; high-performance liquid chromatography*

Polychloro-2-(chloromethylsulphonamido)diphenyl ethers (PCSDs), **I**, are the main active constituents of several commercial insectproofing formulations used to impart durable insect resistance to wool and wool-blend textiles. Eulan WA New (Bayer), Eulan U33 (Bayer) and Mitin LP (Ciba-Geigy) are the major products now in use worldwide for industrial application to wool. The Eulan formulations are based entirely on PCSD[1,2] and differ only in their concentration of active constituents. Mitin LP contains PCSDs as the major component in admixture with 4,4′-dichloro-3,3′-bis(trifluoromethyl)diphenylurea (flucofuron).[3] Molantin P (Chemapol) is also based on PCSDs and is considered to be similar to Eulan U33.[4]



n = 4–6

I

Thin-layer chromatography and gas chromatography have been used to determine Eulan WA New in textile waste liquors.[5] A standard method[6] reported for determining PCSDs on wool textiles required Soxhlet extraction of the insectproofing agent over a 2-h period with 2-methoxyethanol followed by gas-chromatographic determination. Another gas-chromatographic method has been described[7] for determining Eulan U33 on wool textiles in which the wool is first degraded with sodium hydroxide solution and the insectproofing agent is then extracted with diethyl ether. Wells[2] has recently reported that PCSDs undergo thermal rearrangement at the injection port at temperatures exceeding 230 °C during gas chromatography. This places some doubt on the accuracy and reproducibility of gas-chromatographic methods for PCSD determinations.

Normal-phase high-performance liquid chromatography (HPLC) has been used to determine PCSDs on wool and in textile liquors.[8] Peak shape and resolution were better than those obtained by gas chromatography and analysis time was shorter. More recently, Wells and Johnstone[9] have described a method for extracting low levels of PCSDs from natural water with silicone oil coated polyurethane-foam plugs and determination by reversed-phase

HPLC. Their work included a study of the effects of solvent composition, pH, column packing and detector wavelength on the HPLC determination of PCSDs, but did not include the determination of PCSDs extracted from wool textiles.

In our search for a suitable HPLC method for determining PCSDs extracted from wool and textile liquors, the normal-phase mode was found to be superior to the reversed-phase mode. The use of a normal bonded-phase column was later found to provide additional benefits. We report here the normal bonded-phase HPLC analysis of PCSDs together with the simple and small-scale techniques developed for extracting them from wool textiles and textile liquors.

Experimental

Materials

The wool used was a commercial, woollen-spun 3-fold carpet yarn made from 33-μm wool and a soap-scoured plain-weave fabric of 135 g m^{-2}, made from 22-μm Merino wool. The polyamide flocks (20 decitex) were T-6 (Allied Chemicals) and 6-6 (Du Pont). The insect-proofing agents used and their manufacturers were given earlier. Albegal A (Ciba-Geigy) was used as a levelling agent where stated. PCSDs were isolated from Eulan WA New by treating a solution of the formulation (10 g) in water (200 ml) with 1 M hydrochloric acid (15 ml) and filtering the precipitate after thorough stirring. The collected solid was washed with several portions of water and dried. *N*-Benzoyl-4-butylaniline was prepared from benzoyl chloride and 4-butylaniline (Aldrich). The crude product was recrystallised three times from methanol. All solvents and chemicals were of analytical-reagent grade.

Reagents

Methanol - ammonia solution (3% *V/V*). Add concentrated ammonia solution (28–30%, 3 ml) to methanol (97 ml).

Hydrochloric acid. Approximately 1 M.

Internal standard solution. Dissolve *N*-benzoyl-4-butylaniline (0.040 g) in dichloromethane (500 ml), add 2,2,4-trimethylpentane (500 ml) and mix thoroughly.

Apparatus

The ampoules (10 ml capacity, Australian Consolidated Industries) were made of Pyrex glass and were 130 mm in length (including the neck) and 17 mm in diameter. The neck was scored at the bottom and had a 13-mm opening, which narrowed to 7 mm.

The 14-ml screw-cap vials were from Pierce. A Techne SB-4 shaking water-bath was used for shaking the sealed ampoules. Dye-bath treatments were performed in an Ahiba Turbomat laboratory dyeing machine. The HPLC system was equipped with an Altex, Model 100, pump, a Hitachi, Model 100–10, variable-wavelength detector and an Altex 155–00 flow cell. Injections were made through a 30-μl sample loop attached to a Valco AH60 pneumatic valve and Varian 8055 Autosampler. A stainless-steel column (250 $\times$ 4.6 mm) packed with RSIL–CN (10 μm, Alltech Associates) was used with a mobile phase of 50% *V/V* dichloromethane in 2,2,4-trimethylpentane. The flow-rate was set at 1.5 ml min^{-1} and the detector wavelength at 242 nm. Peak areas were determined by a Varian CDS IIIC data system.

Method

Extraction of textile samples

Condition the samples at 65 $\pm$ 2% relative humidity and 20 $\pm$ 2 °C for at least 4 h. Cut the sample into pieces that can be introduced easily into the ampoule, weigh (to the nearest milligram) about 0.2 g of the samples, place in ampoules and pipette in the methanol - ammonia solution (5 ml). Seal the ampoules at ambient temperature using a glassblowing torch, and place in a shaking water-bath at 80 °C for 2 h.

Caution—Wear safety spectacles when entering or removing the ampoules from the water-bath in case an ampoule should explode. The water-bath should be fitted with a lid.

Remove the ampoules, open them after cooling and transfer aliquots (2.5 ml) by pipette into screw-cap vials (14 ml) containing 1 M hydrochloric acid (7 ml) and the internal standard solution (2.5 ml). Cap the vials using PTFE-lined septa as seals and shake thoroughly. Facilitate phase separation by centrifuging the vials for a few minutes. Introduce the organic phase (upper layer) into the sample loop of the high-performance liquid chromatograph.

Extraction of liquors

To 100 ml of the liquors in 250-ml conical flasks add anhydrous sodium sulphate (15 g), 1 M hydrochloric acid (2 ml) and dichloromethane (50 ml). Stopper the flask and stir vigorously on a magnetic stirrer for 1 h. Pour the contents into a separating funnel and collect the dichloromethane phase. Shake the aqueous layer in the funnel with fresh dichloromethane (25 ml), allow it to separate and then combine it with the first extract. Evaporate the extract to dryness on a rotary evaporator, dissolve the residue in the internal standard solution (2.5 ml) and introduce this into the sample loop of the high-performance liquid chromatograph.

Standards

Prepare calibration standard solutions of the insectproofing agents in methanol in the concentration range 20–400 mg l^{-1}. Pipette aliquots (2.5 ml) of these standards into screw-cap vials containing 1 M hydrochloric acid (7 ml) and the internal standard solution (2.5 ml) and proceed as described for the textile samples. Include samples of fabric or carpet yarns (treated with known amounts of the appropriate insect-proofing agent by the dye-bath procedure described) in each set of analyses as standards to check on reproducibility and extraction efficiency.

Calibration

For determining the area of peak A, the data system was programmed to project the base line at the onset of peak A to the completion of peak B, and to drop a perpendicular to the projected base line at the valley between these peaks.

Determine the peak-area ratios (peak A to peak C, Fig. 2) for the appropriate standard solutions and plot these against the concentrations to produce a linear calibration graph. Similarly, determine the peak-area ratios for the unknown samples and obtain the concentration of insectproofing agent from the calibration graph. Calculate the results as follows:

$$\text{Insectproofing agent (\% } m/m\text{) on a textile sample} = C/2m$$
$$\text{Insectproofing agent (mg } l^{-1}\text{) in liquors} = C/40$$

the where C is the concentration (in milligrams per litre) from the calibration graph and m is the mass (in milligrams) of sample.

Application of Insectproofing Agents to Wool

Dye-bath method

The wool fabric (25 g) or carpet yarn (15 g) was pre-wetted in a 0.02% m/V solution of a non-ionic surfactant in water, rinsed and placed in the dye-bath (liquor to wool ratio, 20 + 1) which was adjusted to 40 °C and contained ammonium sulphate (2 g l^{-1}) and formic acid (90% m/m, 2 g l^{-1}). The liquor was circulated through the wool for 10 min and then the appropriate amount of insectproofing agent, dissolved in water (10 ml), was added to the bath. The temperature of the bath was raised to 100 °C at a rate of 2 °C min^{-1} and maintained at this temperature for 30 min. The fabric was removed from the bath, placed in cold water (500 ml) for 5 min, hydroextracted and air dried for 48 h.

Solvent application

Solutions (1 g l^{-1}) of the insectproofing formulations in acetone were prepared. Aliquots (1 and 2 ml) were pipetted on to suspended wool fabric (0.2 g) and the acetone was removed by a stream of warm air to give applications of 0.5 and 1.0% m/m.

Results and Discussion

Extraction of Textiles

In normal practice, insectproofing agents are applied to wool in an acidic dye-bath (pH 3–6) at or near the boil. Under these conditions they migrate into the swollen fibres to provide a treatment with good durability.

Efficient extraction of the insectproofing formulations used from wool treated in a dye-bath was accomplished in sealed glass ampoules or screw-cap vials with 2-methoxyethanol, dimethylformamide or methanol - ammonia solution. However, only methanol - ammonia solution proved satisfactory, the other solvents giving rise to interfering peaks in subsequent normal-phase HPLC analysis of the extracted PCSDs.

The optimum conditions for methanol - ammonia solution extraction of the insectproofing agents from wool fabric and carpet yarn treated at the 0.6% *m/m* level were found by varying the temperature (40–80 °C) and time (0.5–4 h) of extraction. Maximum recoveries from wool fabric were achieved after 1 h at 60 °C or after 0.5 h at 80 °C. The coarser carpet wool fibres required at least 1 h at 80 °C. Prolonging the extraction time to 4 h at 80 °C slightly reduced the recoveries, presumably owing to some chemical degradation of the PCSDs. An extraction time of 2 h at 80 °C was adopted for all types of wool textiles.

Sealed glass ampoules were found to be more reliable than screw-cap vials for extraction of the wool. The high vapour pressure of the methanol - ammonia solution at 80 °C required good sealing to prevent leakages. No problems have been experienced with several thousand extractions that have now been performed in glass ampoules. However, as a precautionary measure against injury in the event of an ampoule exploding, the shaking water-bath should be fitted with a stainless-steel lid and safety glasses should be worn when entering or removing ampoules from the water-bath.

Good recoveries (Table I) of PCSDs were obtained from wool fabric treated with known amounts of the different insectproofing agents applied from an organic solvent. An exact amount of formulation can be applied in this manner giving a surface deposit on the fibres that is easily extracted.

TABLE I

RECOVERY OF INSECTPROOFING AGENTS FROM WOOL BY EXTRACTION WITH METHANOL - AMMONIA SOLUTION AT 80 °C FOR 2 h

	Solvent application		Dye-bath application*			
			Yarn		Fabric	
Formulation	Amount applied, % *m/m*	Recovery, %	Amount applied, % *m/m*	Recovery, %	Amount applied, % *m/m*	Recovery, %
Eulan WA New	0.5	98	0.6	90	0.3	93
	1.0	96	1.2	91	0.6	90
Eulan U33	0.5	104	0.6	92	0.2	90
	1.0	102	—		—	
Mitin LP	0.5	95	0.6	85	0.3	86
	1.0	98	1.2	88	0.6	88
Molantin P	0.5	100	0.7	87	0.5	90
	1.0	100	1.0	85	—	

* These recoveries do not include small amounts of the insectproofing agents (2–4% of Eulan WA New, Eulan U33 and Mitin LP and 4–6% of Molantin P), which remained in the dye-bath liquors.

Lower recoveries of the PCSDs were found for wool fabric and carpet yarn treated with known amounts of the insectproofing agents in a dye-bath at 100 °C (Table I). This method of treatment allows migration of the insectproofing agent into the swollen fibre, making its extraction more difficult. However, a second methanol - ammonia solution extraction performed on the higher level treatments (1.0–1.2% *m/m*) failed to yield additional detectable amounts of PCSDs, indicating that incomplete extraction was not the cause of the lower recoveries. Quantitative transfer of the insectproofing agent on to the wool from boiling dye-bath liquors is much more complex than by solvent application, and some losses invari-

ably occur.[10] For example, the dye-bath liquors from the applications in Table I contained 2–4% of the applied amount of Eulan WA New, Eulan U33 and Mitin LP and 4–6% of the applied amount of Molantin P.

The determination of insectproofing agents on wool - polyamide blend carpets, treated during blend dyeing, is frequently required. PCSDs partition in favour of the polyamide by as much as 10:1 when applied to the blend in a dye-bath at or near 100 °C. Methanol - ammonia solution extraction of two types of polyamide used in carpets, and treated with 0.6 and 1.8% *m/m* of Eulan WA New and Mitin LP in the dye-bath, afforded recoveries of 92–94%, indicating the extraction procedure to be applicable to both polyamide and wool.

Extraction of Liquors

Acidification to a pH of about 2 followed by a single extraction with dichloromethane was adequate for determining PCSDs in dye-bath liquors down to 0.5 mg l^{-1}. A 40-fold concentration step was necessary for liquors containing less than 2 mg l^{-1} of the formulations, and a 20-fold concentration sufficed for more concentrated liquor samples.

Recoveries are given for the extraction of the insectproofing formulations from three typical dye-bath liquors (Table II) spiked with a range of concentrations (0.5, 1, 5 and 20 mg l^{-1}) normally encountered in practice. The extraction efficiency was generally good with some variability occurring at the lowest concentration examined (0.5 mg l^{-1}), which is near the detection limit. The levelling agent Albegal A, which is a surfactant, did not hinder the extraction.

TABLE II

RECOVERY OF KNOWN CONCENTRATIONS OF INSECTPROOFING AGENTS FROM DYE-BATH LIQUORS

Dye-bath liquor	Amount Added/ mg l^{-1}	Recovery of insectproofing agent, %			
		Eulan WA New	Eulan U33	Mitin LP	Molantin P
Ammonium sulphate (2 g l^{-1}) + glacial acetic acid (0.25 g l^{-1})	0.5	86	80	84	70
	1.0	86	—	94	—
	5.0	90	98	88	98
	20.0	92	93	91	97
Ammonium sulphate (2 g l^{-1}) + formic acid (90%, 1.25 g l^{-1})	0.5	84	80	74	60
	1.0	88	—	87	—
	5.0	92	98	86	98
	20.0	92	97	91	97
Ammonium sulphate (2 g l^{-1}) + glacial acetic acid (0.25 g l^{-1}) + Albegal A (0.5 g l^{-1})	0.5	90	90	68	90
	1.0	88	—	87	—
	5.0	90	96	90	101
	20.0	92	94	90	96

HPLC Analysis

Reversed-phase HPLC was examined briefly for the determination of PCSDs extracted from wool but was found, on some occasions, to suffer from interfering peaks, presumably from materials (dyes and textile auxiliaries) co-extracted with the PCSDs. These interferences were not encountered with normal-phase HPLC, and on this basis normal phase was selected as the preferred method of analysis. Silica particles, initially used as the column packing, gave good peak shapes and resolution but long equilibration periods were required following slight changes in the mobile phase to obtain reproducible retention times. This problem was overcome by using silica particles with a cyanopropyl bonded phase.

Transfer of the PCSDs in the methanol - ammonia solution extract to a less polar solvent, as required for normal-phase HPLC, was achieved by the addition of dilute hydrochloric acid to the extract and partitioning of the PCSDs into 2,2,4-trimethylpentane - dichloro-

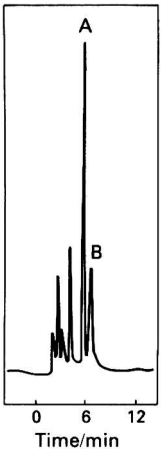


Fig. 1. HPLC chromatogram of the PCSDs (40 mg l^{-1} in 1 + 1 2,2,4-trimethylpentane - dichloromethane) isolated from Eulan WA New.

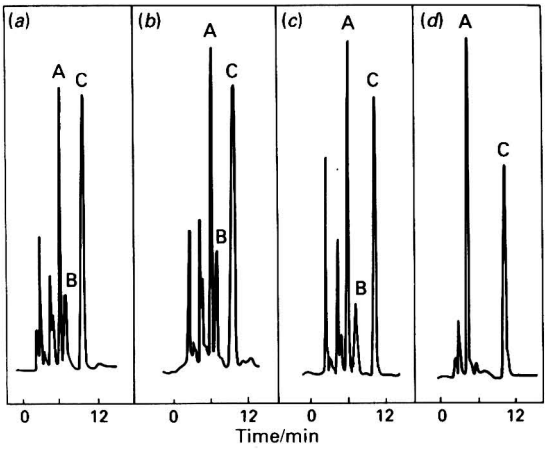


Fig. 2. HPLC chromatograms obtained from extracts of wool treated with (*a*) 0.6% *m/m* Eulan WA New; (*b*) 1.2% *m/m* Mitin LP; (*c*) 0.4% *m/m* Eulan U33; and (*d*) 0.4% *m/m* Molantin P. Peaks A and B are PCSDs and peak C the internal standard.

methane. When using solutions of 400, 200 and 100 mg l^{-1} of Eulan U33 in methanol - ammonia solution, at least 98% of the PCSDs were found to partition into the 2,2,4-trimethylpentane - dichloromethane phase in this step.

The chromatogram (Fig. 1) obtained for PCSDs isolated from Eulan WA New contained two peaks (A and B) with capacity factors (k) of 1.69 and 2.19, respectively. Chromatograms (Fig. 2) obtained on analysis of wool treated with Eulan U33 and Mitin LP afforded values of k identical with those obtained for the PCSDs isolated from Eulan WA New. However, Molantin P showed only one major peak (A) with a much smaller value of k (1.03) and clearly contained PCSDs, which were of a different composition to that in the Eulan and Mitin formulations.

Peak C in the chromatograms in Fig. 2 is due to *N*-benzoyl-4-butylaniline used as an internal standard. The HPLC analysis time was 12 min for each sample. Some other internal standards found to be suitable were the benzoyl derivatives of 2,4-dimethylaniline and *p*-toluidine, but their retention times were longer.

PCSDs exhibited an ultraviolet absorption maximum at 242 nm and this wavelength was used for detection to obtain the best sensitivity. With a 30-μl injection volume this enabled the formulations used to be determined down to 0.05% *m/m* on wool textiles and 0.5 mg l^{-1} in spent textile liquors, well below the concentrations normally encountered in practice.

The peak-area ratio of peak A to peak C was used for quantification of the insectproofing agents. Calibration graphs of peak-area ratio *versus* concentration, over the range 0–400 mg l^{-1}, for the formulations used were linear (correlation coefficients 0.9991–1.0000). This concentration range corresponds to the treatment levels normally found on wool textiles. Standard fabric samples containing known amounts (0.3 and 0.6% *m/m*) of the insectproofing agent being determined are incorporated in each analytical run as a check on the extraction efficiency and reproducibility.

Details of the repeatability (within a batch of analyses) and the reproducibility (between batches of analyses) of the method, as applied to the determination of Eulan WA New on wool fabric, carpet yarns and a dye-bath liquor, are given in Table III. The repeatability was very good (coefficient of variation 0.5–4.6%) for all samples except the industrial sample A. In this sample, the poor repeatability was probably due to an unlevel application of insectproofing agent. The reproducibility of the analyses was very good in every instance (coefficient of variation 1.5–3.3%).

TABLE III

REPEATABILITY AND REPRODUCIBILITY OF METHOD APPLIED TO THE DETERMINATION OF EULAN WA NEW

Sample and treatment level	Batch No.*	Mean, % *m/m*	Standard deviation, % *m/m*	Coefficient of variation, %
Standard wool fabric (0.6% *m/m*)	1	0.566	0.006	1.0
	2	0.567	0.004	0.74
	3	0.592	0.003	0.56
Overall		0.575	0.015	2.6
Standard wool carpet yarn (0.6% *m/m*) ..	1	0.574	0.007	1.3
	2	0.562	0.007	1.2
	3	0.578	0.003	0.50
Overall		0.571	0.008	1.5
Industrial carpet yarn A (application level unknown)	1	0.634	0.053	8.4
	2	0.616	0.094	15
	3	0.616	0.109	18
Overall		0.622	0.010	1.6
Industrial carpet yarn B (application level unknown)	1	0.097	0.003	3.1
	2	0.098	0.003	3.3
	3	0.092	0.003	3.3
Overall		0.096	0.003	3.3
Dye-bath liquor sample (spiked with 1 mg l^{-1}) ..	1	1.00	0.046	4.6

* Each batch consisted of 5 replicate analyses.

Conclusions

Insectproofing agents based on PCSDs are extracted efficiently from wool and polyamide with methanol - ammonia solution and from spent dye-bath liquors with dichloromethane, and determined by normal bonded-phase HPLC. The limits of detection for the commercial formulations on textile samples and in dye-bath liquors were 0.05% *m/m* and 0.5 mg l^{-1}, respectively, well below the levels normally encountered in practice. Recoveries obtained from textile samples and dye-bath liquors containing known amounts of the insectproofing formulations were very good, as were the repeatability and reproducibility of the method. The simple extraction procedure and short analysis time make this method ideally suited to the routine analysis of textile samples for these insectproofing formulations. More than 3000 samples have already been determined in these laboratories by this method.

The technical assistance of Mrs. Wendy Jackson is gratefully acknowledged.

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Quantitative Determination of Carbonyl Compounds in Rendering Emissions by Reversed-phase High-performance Liquid Chromatography of the 2,4-Dinitrophenylhydrazones

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A simple and rapid method for the determination of volatile carbonyl compounds in air has been developed. The method is applied to the quantitation of C_1–C_9 aldehydes and acetone in rendering emissions.

Carbonyl compounds are sampled by absorption in a 2×10^{-4} M 2,4-dinitrophenylhydrazine solution at pH 1 and the resulting hydrazones are extracted with 2,2,4-trimethylpentane, concentrated and analysed by HPLC on a 10-μm RP-C_{18} column with a water - acetonitrile gradient as the eluent. The hydrazones are then spectrophotometrically detected at 356 nm. The micro-scale conversion of carbonyls into 2,4-dinitrophenylhydrazones is investigated, the separation of hydrazones is improved and the sampling conditions are tested in order to achieve quantitative sampling at an air flow-rate of 1 l min^{-1}. Quantitation is possible for concentrations as low as 15 p.p.b. (formaldehyde) and 2 p.p.b. (nonanal). The over-all coefficient of variation (taken over sampling, conversion and analyses) is less than 10%.

Keywords: *Carbonyl quantitation*; *2,4-dinitrophenylhydrazine derivatisation*; *reversed-phase HPLC analysis*; *rendering plant emissions*

The lower aliphatic aldehydes, formaldehyde, acetaldehyde and acrylaldehyde, are well known in atmospheric pollution chemistry as important intermediates in photochemical smog formation. Higher aldehydes, on the other hand, are more associated with flavour chemistry because they are secondary oxidation products of fatty acids. Aliphatic aldehydes as a whole are emitted by animal rendering plants and these compounds, together with lower aliphatic acids, amines and sulphur compounds, contribute to the odour nuisance commonly accompanying rendering activities. In order to evaluate the relative importance of different groups of malodorants a method for the quantitation of aldehydes at odour threshold levels (parts per 10^9, p.p.b.) has been developed.

The derivatisation of aldehydes into 2,4-dinitrophenylhydrazones, followed by either gas chromatographic or high-performance liquid chromatographic (HPLC) analysis of the derivatives, is a widely proposed method.[1–6] Derivatives of isomeric carbonyl compounds have been separated by gas chromatography on capillary columns coated with OV-17, OV-101 and SF 96.[1] However, double peaks due to *syn*- and *anti*-isomeric forms may hamper the determination and quantitation of compounds in unknown mixtures by altering the retention data.[1,2]

Kuwata *et al.*[3] reviewed the advantages of HPLC for hydrazone separation. These workers summarised different normal and reversed-phase HPLC analyses of hydrazones that were in use up to 1979. They also reported the separation of 2,4-dinitrophenylhydrazones of carbonyls up to C_6 by reversed-phase HPLC with a LiChrosorb RP-18 column and acetonitrile - water as the mobile phase. Other reversed-phase separations have been reported with either isocratic[4] or linear solvent programming elution.[5] The latter technique allows the separation of C_3–C_{10} linear aliphatic aldehydes within 10 min.

The micro-scale conversion of propanal with 2,4-dinitrophenylhydrazine was investigated by Selim.[6] According to this worker a two-phase system, aqueous reagent - 2,2,4-trimethylpentane, was needed to achieve quantitative conversion.

In this paper, results of studying the micro-scale reactivity of C_1–C_9 carbonyls and 2,4-dinitrophenylhydrazine (2,4-DNPH), the trapping efficiency of aldehydes in 2,4-DNPH reagent and analyses of the derivatives obtained by HPLC are described. Results of the quantitation of carbonyl compounds in rendering emissons are also reported.

Experimental

Reagents

The carbonyl compounds investigated were obtained from various commercial sources. Owing to trimer formation, the purification of the aldehydes is essential if reliable results are to be obtained. Formaldehyde, acetaldehyde and propanal were purified by distillation. Higher aldehydes were purified by preparative gas chromatography. The 2,4-dinitrophenylhydrazine reagent consists of 0.125 g of 2,4-DNPH in 100 ml of 6 N hydrochloric acid. The reagent was extracted twice with 50 ml of carbonyl-free 2,2,4-trimethylpentane and the purified reagent was kept covered with a layer of the same solvent. The 2,2,4-trimethylpentane (1 l) was refluxed with 0.5 g of 2,4-DNPH and 50 ml of 6 N hydrochloric acid for 2 h and was then distilled from the reaction mixture. Hydrazone standards were prepared and purified by use of standard methods.[7]

Apparatus and Chromatographic Conditions

A Varian 8500 liquid chromatograph and a Varian spectrophotometer (UV-VIS Model 635) operating at a wavelength of 356 nm were used to perform the analyses. Samples were injected by use of an injection valve (Valco CV-6-UVPa N60) together with a 10-μl loop. The column used was a pre-packed, 25 cm $\times$ 4 mm i.d., 10-μm LiChrosorb RP-18 column (E. Merck, Darmstadt, F.R. Germany). The column temperature was held at 40 °C with a water-jacket. The mobile phase was acetonitrile - water at a flow-rate of 1.5 ml min^{-1}. Solvent concentrations are indicated on the chromatograms.

Micro-scale Reaction

The micro-scale conversion of C_1–C_9 linear aliphatic aldehydes into 2,4-dinitrophenylhydrazones was performed in an aqueous reagent (25 ml of distilled water and 0.8 ml of 2,4-DNPH reagent) and in a two-phase system (aqueous reagent and 15 ml of 2,2,4-trimethylpentane). The aldehyde concentrations ranged from 8.0×10^{-4} M for formaldehyde to 1.8×10^{-5} M for nonanal. After being stirred for 1 h, reaction mixtures were extracted with 2,2,4-trimethylpentane (2 $\times$ 15 ml). The solvent was then evaporated, the hydrazones were re-dissolved in 1 ml of acetonitrile and analysed by HPLC. In a second experiment, propanal, hexanal and nonanal standards were diluted 1 + 2, 1 + 4 and 1 + 9 and the reaction was carried out in the aqueous reagents.

Sampling Efficiency

In order to determine the sampling efficiency, air concentrations of 0.36 p.p.m. (mol/mol) of formaldehyde, 0.93 p.p.m. of propanal, 0.56 p.p.m. of hexanal and 0.39 p.p.m. of nonanal were generated with a motor-driven syringe. Two bubblers containing different amounts of 2×10^{-4} M 2,4-DNPH solution were connected in series and the polluted air was sampled at a rate of 1 l min^{-1} for 30 min. After sampling the reagent solutions were combined and stirred with a magnetic stirrer for 1 h. The hydrazones were next extracted with 50 ml of carbonyl-free 2,2,4-trimethylpentane, while the bubblers were rinsed with a further 15 ml of carbonyl-free 2,2,4-trimethylpentane. The solvent fractions were then combined and the analysis was completed as described above.

Rendering Emission Sampling

Samples were taken in a Belgian rendering plant with an annual capacity of 10000 t of raw material. The material is processed in batch cookers under vacuum. Gases and vapours released during cooking pass consecutively through a grease trap, a surface condenser and two water scrubbers. Samples were taken at the last scrubber outlet. The air sampling flow-rate was 1 l min^{-1}; samples were taken during 15-min sampling periods.

Results and Discussion

HPLC Separation and Calibration of the 2,4-Dinitrophenylhydrazones

The separation of 2,4-dinitrophenylhydrazones of the linear aliphatic C_1–C_9 aldehydes, 2-methylpropanal, 2-methylbutanal, 3-methylbutanal and acetone were investigated. Fig. 1(*a*) and (*b*) show the separation obtained with 10- and 5-μm columns, respectively. In both instances the derivatives of linear aliphatic aldehydes and acetone are completely separated.

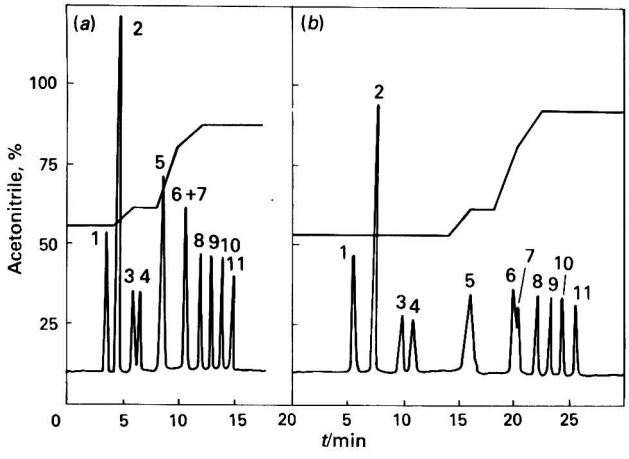

Fig. 1. Separation of 2,4-dinitrophenylhydrazones by reversed-phase HPLC, using (*a*) a 10-μm and (*b*) a 5-μm RP-C_{18} column. The water - acetonitrile gradient is indicated as percentage of acetonitrile. The compounds in the mixture are 2,4-dinitrophenylhydrazones of: 1, formaldehyde; 2, acetaldehyde; 3, acetone; 4, propanal; 5, butanal and 2-methylpropanal; 6, 2-methylbutanal and 3-methylbutanal; 7, pentanal; 8, hexanal; 9, heptanal; 10, octanal; and 11, nonanal.

On the 10-μm column, the same relative molecular mass derivatives of isomeric C_4 and C_5 carbonyl compounds overlapped. As is shown in Fig. 1(*b*), partial separation of pentanal from 2-methylbutanal and 3-methylbutanal could be achieved. Different solvent programmes with initial acetonitrile concentrations ranging from 40 to 60% were tested with no better result. On analysing standard hydrazone solutions of the different C_4 and C_5 aldehydes it was found that equal amounts (0.5 μg of derivative) of equal relative molecular mass derivatives gave equal peak heights, the ratio of peak heights of derivatives of different aldehydes being as follows: 2-methylpropanal to butanal, 1.00; 2-methylbutanal to pentanal, 1.02; 3-methylbutanal to pentanal, 1.02. Therefore, 2-methylpropanal and butanal were determined as the C_4 aldehyde group, 2-methylbutanal, 3-methylbutanal and pentanal as the C_5 aldehyde group. Derivatives of butanal and pentanal were the standards for the C_4 and C_5 aldehyde group.

Calibration of the method was carried out by plotting peak heights (in millimetres) *versus* the amounts of aldehydes injected. Five-point calibration graphs ranging from 15 to 1.5 μg gave correlation coefficients greater than 0.999 for all of the aldehydes tested. Assuming an air sampling volume of 25 l (at 25 °C) and 200 μl of acetonitrile in order to re-dissolve hydrazones, quantitation of aldehydes can be performed from as low as 15 p.p.b. (formaldehyde) to 2 p.p.b. (nonanal).

TABLE I

CONVERSION OF LINEAR ALIPHATIC ALDEHYDES INTO 2,4-DINITROPHENYLHYDRAZONES

Conversion efficiency is expressed as a percentage relative to standards (n = 5).

Aldehyde	Injected/ nmol	One-phase system	Two-phase system
Formaldehyde	20.9	70.95 ± 2.6	74.7 ± 7.6
Acetaldehyde	14.25	101 ± 3.5	99 ± 4.2
Propanal	11.12	101 ± 4.9	105 ± 5.2
Butanal	9.1	93 ± 5.1	102 ± 2.4
Pentanal	7.6	89 ± 4.7	95 ± 3.6
Hexanal	6.7	104 ± 2.8	93 ± 3.7
Heptanal	6.0	96 ± 3.6	55 ± 2.8
Octanal	5.1	94 ± 3.5	32 ± 6.2
Nonanal	4.7	101 ± 2.5	6 ± 5.1

Micro-scale Conversion of Aldehydes Into 2,4-Dinitrophenylhydrazones

Table I shows that a quantitative conversion of linear C_2–C_9 aldehydes is obtained in the one-phase system. Formaldehyde shows a conversion of 75%. In the two-phase system heptanal and higher aldehydes show a decreasing conversion due to the hydrophobic character of these compounds. Table II shows that no decrease in conversion efficiency is ascertained by lowering aldehyde concentrations. From this it can be concluded that C_2–C_9 aldehydes can be quantitatively converted in the aqueous reagent system. The efficiency of conversion for formaldehyde is 75%.

TABLE II

CONVERSION OF PROPANAL, HEXANAL AND NONANAL INTO THEIR 2,4-DINITROPHENYLHYDRAZONES AT LOWER ALDEHYDE CONCENTRATIONS

Conversion efficiency is expressed as percentage relative to standards.

Aldehyde	Initial aldehyde concentration/M	Conversion, (%) at dilution 1 + 2	1 + 4	1 + 9
Propanal ..	.. 44×10^{-6}	100	103	90
Hexanal ..	.. 26.5×10^{-6}	100	111	104
Nonanal ..	.. 18.6×10^{-6}	105	103	110

Sampling Efficiency

The sampling efficiency of formaldehyde, propanal, hexanal and nonanal in the 2,4-dinitrophenylhydrazine solution was tested, using increasing amounts of reagent. Table III shows the best sampling results. It can be seen that a sampling unit of two bubblers, containing 100 ml of reagent each, was efficient for formaldehyde, propanal and hexanal; nonanal, however, was not quantitatively sampled. Quantitative sampling of all four aldehydes was achieved by using two bubblers containing 200 ml of reagent each. Therefore, the latter conditions were used during rendering emission sampling.

TABLE III

SAMPLING EFFICIENCY OF PROPANAL, HEXANAL AND NONANAL IN THE 2,4-DINITROPHENYLHYDRAZINE REAGENT, AT A SAMPLING FLOW-RATE OF 1 l min^{-1}

Conversion efficiency is expressed as percentage relative to standards ($n = 5$).

Aldehyde	Sampling efficiency, using two bubblers containing 100 ml of reagent each	200 ml of reagent each
Formaldehyde	97 ± 6.4	104 ± 5.4
Propanal	100 ± 5.4	100 ± 8.7
Hexanal	103 ± 2.8	104 ± 7.0
Nonanal	90 ± 2.3	103 ± 7.5

Quantitation of Carbonyls in Rendering Emissions

Table IV shows the results of the quantitative determination of aldehydes in rendering emissions, taking into account the 75% efficiency of conversion of formaldehyde. Fig. 2 shows a typical chromatogram. Samples were taken on 13.2.1981 between 4 p.m. and 8 p.m. The sampling time was 15 min and it took another 15 min to prepare for sampling. During sampling 10 cookers were in use. Each cooker contains 1500–3000 kg of raw material, which is heated for 1–4 h. Malodorants are liberated by the thermal breakdown of cell structures and the chemical decomposition of animal matter. Amounts of malodorants vary during the heating process and depend on the nature and freshness of the raw material, which results in a typical emission pattern for each batch process. The normal activity of the plant involves processing different materials at the same time. Therefore, the concentrations mentioned in Table IV do not show the evolution of amounts of carbonyls emitted during the processing of

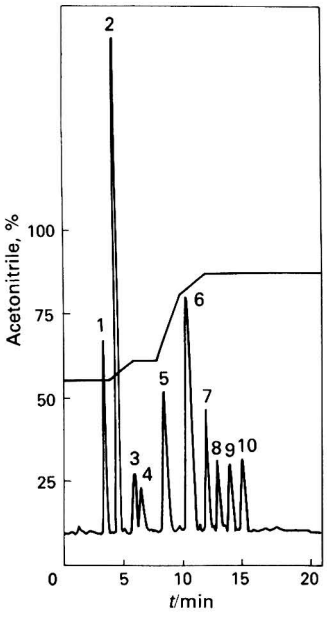

Fig. 2. Chromatogram of the analyses of 2,4-dinitrophenylhydrazones of carbonyl compounds sampled at the rendering plant. The water - acetonitrile gradient is indicated as percentage of acetonitrile. Compounds are 2,4-dinitrophenylhydrazones of: 1, formaldehyde; 2, acetaldehyde; 3, acetone; 4, propanal; 5, C_4-aldehydes; 6, C_5-aldehydes; 7, hexanal; 8, heptanal; 9, octanal; 10, nonanal.

one material. The variations in the carbonyl concentrations of the rendering emissions shown in Table IV are caused by the coincidence of malodorant producing stages in the different batches. This may explain the relatively large concentration differences between different samples.

In order to find out if there is some relationship between the individual carbonyl concentrations the Spearman rank correlation coefficients[8] for all aldehydes except formaldehyde, which shows little variation, were calculated. The results are shown in Table V. Hexanal, heptanal

Table IV

Quantitative determination of carbonyl compounds in rendering emission

Concentrations are in p.p.m.

	Sample number						
Aldehyde	1	2	3	4	5	6	7
Formaldehyde ..	0.2	0.2	0.19	0.19	0.21	0.19	0.19
Acetaldehyde ..	2	1.87	2	2.27	3.8	2.13	1.92
Acetone	0.16	0.16	0.14	0.08	0.22	0.2	0.13
Propanal ..	0.21	0.25	0.16	0.22	0.35	0.24	0.29
C_4-aldehydes ..	0.53	0.96	0.90	1.22	1.45	0.82	0.64
C_5-aldehydes ..	0.94	1.79	1.75	2.3	2.7	1.49	1.28
Hexanal	0.44	0.48	0.34	0.56	0.69	0.57	0.34
Heptanal ..	0.19	0.21	0.16	0.25	0.30	0.32	0.16
Octanal	0.17	0.21	0.16	0.23	0.27	0.36	0.14
Nonanal	0.28	0.38	0.33	0.41	0.48	0.44	0.27

octanal and nonanal correlate significantly at the 1% level. At the 5% level these aldehydes, also correlate with propanal. The C_4-aldehyde group correlates with the C_5-aldehyde group at the 1% level. Neither the C_4- nor the C_5-group correlate with propanal, hexanal, heptanal or octanal. An explanation for the correlations may possibly be found in the reactions that lead to aldehyde formation. Linear aliphatic aldehydes are formed by the dismutation of fatty acid hydroperoxides, with or without migration of double bonds.[9] During the heating of animal matter amino acids may react with α-dicarbonyl compounds to form an aldehyde having one carbon atom less than the original amino acid. This reaction, called Strecker degradation, will give rise to the branched aldehydes 2-methylpropanal, 2-methylbutanal and 3-methylbutanal from valine, isoleucine and leucine, respectively.[10]

TABLE V

SPEARMAN RANK CORRELATION COEFFICIENTS BETWEEN THE DIFFERENT CARBONYL CONCENTRATIONS

1, Acetaldehyde; 2, acetone; 3, propanal; 4, C_4-aldehydes; 5, C_5-aldehydes; 6, hexanal; 7, heptanal; 8, octanal; 9, nonanal.

	2	3	4	5	6	7	8	9
1	0.210	0.331	0.501	0.501	0.690	0.610	0.690	0.828†
2		0.652	0.104	0.104	0.580	0.540	0.576	0.557
3			0.607	0.607	0.879*	0.803*	0.786*	0.786*
4				1.000†	0.595	0.444	0.500	0.750*
5					0.595	0.444	0.500	0.750*
6						0.960†	0.954†	0.949†
7							0.992†	0.898†
8								0.929†

* Significant at 5% level ($n = 7$).
† Significant at 1% level ($n = 7$).

Taking into account these two reactions generating aldehydes, the Spearman correlation coefficients seem to indicate that the C_4- and C_5-aldehyde groups mainly consist of branched aldehydes, otherwise a correlation of these groups with aldehydes formed by fatty acid oxidation would be expected.

The literature results for odour detection values of individual aldehydes are not very consistent[11] in that the thresholds for aldehydes seem to vary between 1 and 50 p.p.b.[10,12] In contrast, the reported threshhold for acetone varies between 20 and 32 p.p.m.[13,14] Comparing these data with the concentrations found in rendering emissions, it can be concluded that acetone does not contribute to the odour problem. All of the aldehydes that were determined are present in supra-threshold concentrations. Therefore, these compounds are at least partially responsible for the rendering odours.

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Determination of Sulphur in Oils by X-ray Fluorescence Using a Thin Layer Solidified with Wax

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A rapid method has been developed for the determination of the sulphur content in a small amount (minimum 0.5 g) of oil using an X-ray fluorescence technique. The sample is mixed and solidified with paraffin wax, which permits presentation of the sample in the normal inverted mode as a thin film and without the need of a retaining plastic window, which is usually required for liquids. Other elements may also be determined.

Keywords: *Oil analysis*; *sulphur determination*; *X-ray fluorescence spectroscopy*; *thin film*

The increasing stringency of specifications for maximum sulphur content in petroleum products, because of both environmental considerations and also because of the corrosive nature of sulphur compounds produced during combustion of oils, has led to an increasing interest in rapid, accurate methods for the determination of sulphur. X-ray fluorescence methods for sulphur determination have been developed[1–8] that usually require relatively large samples and the use of cells with plastic film windows such as Mylar, to contain the oil for presentation in the instrument.

Some years ago we developed a method for producing a thin layer of oil of reproducible thickness, solidified with paraffin wax, for the determination of metals in oil (present in additives or arising from wear). This technique had the following advantages: no plastic film window was required; the use of a "solidified" oil gave physical stability to the sample, *e.g.*, no bowing of a retaining film window; high sensitivity, particularly for lighter elements, was obtainable as the sample is exposed directly and does not suffer attenuation by a retaining window; no interaction could occur between the oil and any plastic retaining window; no precipitation of constituents could occur on to the window under the influence of X-rays; and the small sample thickness (approximately 100 μm) greatly minimised inter-elemental effects.

The method now proposed requires the dissolution of a fixed proportion of paraffin wax in the oil by warming and the transfer of the resultant solution to a special thin-layer cell where the mixture solidifies. The total preparation and measuring time for a sulphur determination can be as little as 4 min per sample and further analysis for other elements can also be carried out on the same cell if required.

Experimental

Apparatus

The following apparatus was used: a Philips PW 1410 X-ray fluorescence spectrometer; a multi-purpose sample holder, type PW 1427/10, 46 mm i.d., fitted with an aluminium mask with a central, 25-mm aperture; glass specimen screw-neck vials (67 × 17 mm) with cap; a thermostatically controlled heating block, designed to hold 12 vials [Dri-Block DB-1, Techne (Cambridge) Ltd., Cambridge]; a razor blade, single-edged; and a thin-layer cell, made by cutting a section 4 mm long from a 30 mm diameter aluminium bar (preferably Grade 1A of BS 1476). A central recess, 26 mm in diameter and 0.1 mm deep, is either cut out or formed by a die in one face of the section, leaving a raised annulus that is then finished flat (Fig. 1) (if a number of matched cells are required the use of a die is recommended).

Reagents

Paraffin wax. BDH Chemicals, clearing point 65–71 °C.
Liquid Paraffin BP (white oil).
Dibenzyl disulphide. BDH Chemicals, organic analytical-standard grade.

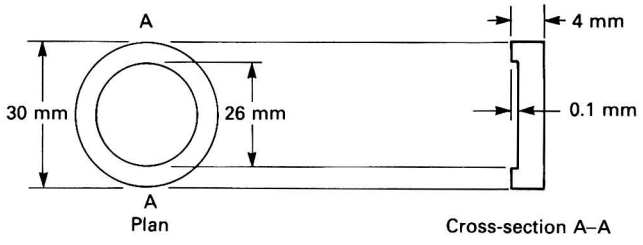

Fig. 1. Design of thin-layer cell.

Procedure

The sample of oil (about 1–2 g) is weighed accurately into a capped glass specimen tube and wax is added to give an oil to wax ratio of 5:1 (for different oils and calibrations the ratio may be varied, *e.g.*, 4:1 for fuel oil). The tube is placed in the Dri-Block (held at 90 °C) with the cap slightly loosened until the wax is completely dissolved and mixed (2–3 min). An amount of the solution, sufficient to fill the recess and overflow the annulus, is immediately transferred into the cell, which is held at 90 °C to allow release of trapped air bubbles. The cell is transferred to a cool surface and the mixture sets within a few seconds. After complete solidification of the sample, the excess is removed by running a clean metal edge, such as a single-edged razor blade, at an angle of 45° to the horizontal, across the annulus of the cell making sure that the thin film in the recess is not disturbed in the process. The cell is then inserted into the sample holder in the normal manner, *i.e.*, with the waxed oil facing downwards, carefully centred over the aperture in the mask and held in position with a foamed plastic pad. The sulphur count is measured using the following conditions: a 3-kW chromium anode tube, 50 kV with variable milliamps and the counting rate not exceeding 50000 s^{-1}; sulphur Kα; pentaerythritol crystal; coarse collimation; counting time, 20 s; and also under vacuum conditions. The cell is made from metal in order to ensure good heat dissipation.

Calibration

Calibration could be carried out using oils with analytically determined sulphur contents,[9] or using oil-soluble organic compounds of known composition, to prepare synthetic standards by solution in "sulphur-free" white oil.

Three organic sulphides dissolved in white oil (Liquid Paraffin BP, sulphur content approximately 80 p.p.m.) were used to prepare standards for assessment of their suitability for this

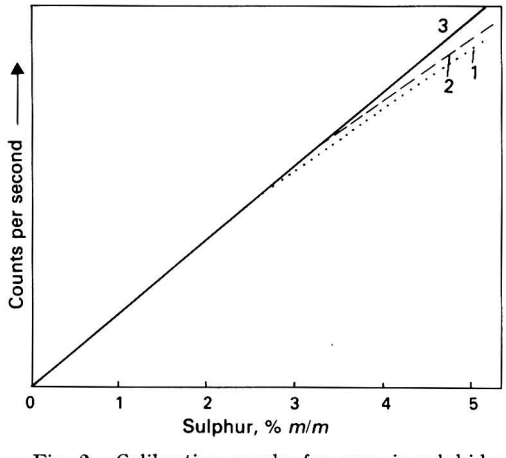

Fig. 2. Calibration graphs for organic sulphides in white oil: 1, dihexyl disulphide; 2, diphenyl sulphide; and 3, dibenzyl disulphide.

technique: 1, dihexyl disulphide (certified by NPL, purity greater than 99.97%); 2, diphenyl sulphide (BDH Chemicals, purity greater than 95%); 3, dibenzyl disulphide (organic analytical standard, BDH Chemicals, sulphur content 26.02% *m/m*).

A class A2 fuel oil (BS 2869 : 1970) was repeatedly analysed for sulphur using the bomb method and used as a secondary standard. The calibration covered the range 0–5% *m/m*. The lower limit of detection using this method was determined to be 0.005% (95% confidence limit).

Results

The graphs obtained from the three synthetic standards are shown in Fig. 2. The two liquid sulphides (Nos. 1 and 2 above) both showed a tendency to give low results, as compared with the solid disulphide (No. 3), at over 3–4% sulphur. It was thought that these losses, although reasonably reproducible, were due to a slight loss by evaporation of the added sulphides during the heating of the wax with the oil. Dibenzyl disulphide produced a reproducible straight-line calibration. Table I shows the calibration data obtained from the standards prepared with this compound.

TABLE I

CALIBRATION: DIBENZYL DISULPHIDE IN WHITE OIL

Sulphur, % *m/m*	Counts s^{-1}	Error of result using linear least-squares fit, %*
0	450	—
0.99	35700	0.0
2.00	72300	−1.4
4.00	140000	−0.4
4.40	156000	0.0
5.00	177000	+0.2

* The correlation coefficient from linear regression was found to be 0.9996.

Tests for repeatability were carried out using the Class A2 fuel oil, which contained 0.72% of sulphur (the mean of four determinations by the bomb method), a lubricating oil and a Class E heavy fuel oil both of which had an unknown sulphur content. The results are given in Table II.

TABLE II

DETERMINATION OF SULPHUR IN FUEL OILS AND LUBRICATING OIL

Oil type	Number of determinations	Mean sulphur content, % *m/m*	Relative standard deviation, % of the mean
Class A2 fuel oil ..	10	0.719	1.25
Lubricating oil (plain mineral oil, SAE 30)	8	0.58	0.52
Class E fuel oil ..	5	2.68	1.49

A number of analyses were carried out on four commercially available certified standards. The results are given in Table III.

Discussion

Most X-ray fluorescence methods are subject to interference effects from the matrix and/or other elements. Various methods have been suggested to compensate for these effects.[3,4,6,7] For sulphur analysis, the following are possible effects arising from the nature of the matrix: the carbon to hydrogen ratio of the matrix for hydrocarbon oils; absorption due to the presence of additives containing heavy metals; and the presence of oxygen in synthetic ester type oils or vegetable and animal oils.

TABLE III

DETERMINATION OF SULPHUR IN COMMERCIALLY CERTIFIED STANDARDS

Description of standard	Sulphur, % *m/m*	
	Certified analysis	Analysis by X-ray fluorescence method ± standard deviation
Kerosene 258 MAR 844	0.29 ± 0.01*	0.34 ± 0.005
Kerosene 258 MAR 845	0.50 ± 0.01*	0.54 ± 0.005
Lubricating oil 258 MAR 833	0.48 ± 0.02	0.47 ± 0.01
Lubricating oil 258 MAR 837	4.14 ± 0.08†	3.52 ± 0.03

* Because of the differences in the sulphur contents as between those obtained by the X-ray fluorescence method and the certified figures, the kerosene standard No. 844 was analysed six times by the IP bomb method[4] and found to contain 0.332% *m/m* of sulphur (standard deviation 0.020%). We felt that this cast some doubt on the reliability of the certified figures.

† Standard 837 contained a sulphur compound much more volatile than the sulphur compounds in normal lubricating oil (infrared and gas chromatographic - mass spectrometric examination indicated di-*tert*-butyl disulphide). Compounds of similar volatility in white oil tended to give low results when under consideration for calibration purposes. It was noted that standard 837 lost sulphur (compounds) when freely exposed at room temperature for several hours.

The carbon to hydrogen mass ratio in mineral oils can vary from approximately 6:1 to greater than 9:1, depending on the degree of aromaticity. Christensen and Agerbo[3] have shown the effect on the determination of sulphur is small for mineral oils. As a confirmation of these findings, two samples were prepared containing a known amount of sulphur (as dibenzyl disulphide) added to white oil, with a carbon to hydrogen ratio of approximately 6:1, and diphenyl with a carbon to hydrogen ratio of 14:1. These were analysed using the calibration in Table I with the results shown in Table IV.

TABLE IV

DETERMINATION OF SULPHUR IN MATRICES OF DIFFERING CARBON TO HYDROGEN RATIOS

Matrix	Sulphur expected, % *m/m*	Sulphur found (X-ray fluorescence method), % *m/m*
White oil (aliphatic)	1.86	1.87
Diphenyl (aromatic)	1.98	1.98

A number of samples of known sulphur content were prepared in white oil and various additives containing heavy metals were introduced into the oil. A high boiling-point ester (dibutyl phthalate) was also used as a matrix to investigate the effect of oxygen. Table V gives the uncorrected X-ray fluorescence results together with the corrected results using only mass absorption coefficients[10] to correct for attenuation.

TABLE V

DETERMINATION OF SULPHUR IN AN ESTER MATRIX AND IN A HYDROCARBON MATRIX CONTAINING HEAVY METALS

Matrix	Expected sulphur, % *m/m*	Sulphur found, % *m/m*	
		Uncorrected	Corrected
Dibutyl phthalate (oxygen = 23%)	1.00	0.79	0.95
	2.00	1.54	1.95
White oil with barium = 0.72%	1.39	1.34	1.38
	2.24	2.12	2.26
White oil with calcium = 1.2% and oxygen = 0.8%	1.19	1.16	1.18
	2.16	2.11	2.14
White oil with phosphorus = 1% and oxygen = 2%	1.00	0.89	0.98
	1.97	1.78	1.91

The method is also generally applicable to the determination of "heavy" elements in hydrocarbon oils where minimal or no correction would be necessary and especially to additives containing not only "heavy" metals but also chlorine and phosphorus.

Conclusions

The thin-layer cell method described above has been found to be rapid, accurate and reproducible for the analysis of sulphur in hydrocarbon oils. In most instances standards and samples can be reasonably matched and accurate results obtained without the need for any correction procedures. Where the oil to be analysed contains additives, not present in the calibration standards, the wax - oil mixture in the cell can be used both to identify and to determine most interfering elements. Simple corrections (usually small), based on published mass absorption coefficients, enable good results to be obtained. Very "light" oils, such as petroleum spirits, could give problems with volatility. Calibration using a recognised organic analytical standard of known composition is recommended.

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Application of Zirconium Molybdophosphate Gel for the Selective Separation of Thallium(I) Ions

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Zirconium molybdophosphate has been prepared in the form of hard yellow granules. The product possesses an ion-exchange capacity of 0.42 mequiv. g^{-1} and shows selectivity for Ag^+, Tl^+, UO_2^{2+} and Th^{4+} ions. It has been possible to separate Tl^+ ions selectively on columns of this ion-exchanger material, but only Tl^+ ions can be eluted completely.

Keywords: *Zirconium molybdophosphate gel*; *thallium(I) separation*

Ammonium heteropolyacid salts have been used for the separation of Cs^+ or Rb^+ ions.[1–4] Tungsto- and molybdoarsenates have not been tried for column procedures as these salts are highly colloidal. We have previously prepared and reported the ion-exchange properties of tungsto- and molybdoarsenate salts of organic bases,[5,6] which are stable and possess good sorption properties.

Recently, heteropolyacid salts of zirconium and titanium have been synthesised and utilised as ion exchangers.[7,8] Zirconium molybdophosphate[9,10] has also been prepared but had a low exchange capacity, low stability and very poor selectivity towards various cations. However, we have prepared this compound under different physical conditions and found the product to possess promising exchange characteristics, good stability and specific selectivity for thallium(I) ions. Separations of this ion from a variety of other cations are possible on a column of this ion-exchanger material. The results of these investigations are reported in this paper.

Experimental

Reagents

Zirconium oxychloride and dodecamolybdophosphoric acid (BDH Chemicals) were used for synthesis as received. For the determination of distribution coefficients, ^{58}Co, ^{54}Mn, ^{65}Zn, $^{85}+^{87}Sr$, ^{86}Rb, $^{110}Ag^m$, ^{133}Ba, ^{134}Cs, ^{203}Hg and ^{204}Tl isotopes were procured from the Bhabha Atomic Research Centre, Bombay, India.

Instrumentation

pH measurements were made with an ELICO, Model LI-10, pH meter. For the spectrophotometric determination of ions a Bausch and Lomb Spectronic 20 instrument was used, and infrared spectra were recorded in potassium bromide medium on a Beckman IR 20 spectrophotometer.

Thermogravimetric studies were performed on a Du Pont thermogravimetric analyser. X-ray diffraction patterns were obtained on a Philips X-ray diffraction unit using Mo Kα radiation.

For ^{58}Co, ^{54}Mn, ^{65}Zn, $^{85}+^{87}Sr$, ^{86}Rb, $^{110}Ag^m$, ^{133}Ba, ^{134}Cs and ^{203}Hg, gamma counting was carried out using a well-type NaI(Tl) scintillation detector coupled with a single-channel analyser. Beta counting of ^{204}Tl was performed on a Geiger - Müller counter.

Preparation of Exchanger

Other workers[9–10] prepared this compound at pH $\geqslant$ 8 in the presence of ammonium nitrate. The concentrations of the acid and zirconium salt were lower than those used here. The following procedure was adopted for the preparation of the ion-exchange material in this work.

12-Molybdophosphoric acid (36.12 g), dissolved in 400 ml of water, was acidified to pH 0.7 with dilute hydrochloric acid and then added with constant stirring to a solution of zirconium oxychloride (32.23 g in 500 ml of water, pH 0.6). The zirconium molybdophosphate thus precipitated was digested in a steam-bath for 30 min and then allowed to stand for 24 h. The product was filtered and thoroughly washed with distilled water and dried at 90 °C.

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The reproducibility of the samples prepared by this method is evident from the fact that the exchange capacities of different samples from the same batch had a standard deviation of 0.01 and the standard deviation of the exchange capacity of samples from different batches was 0.02.

Analysis

A 0.2-g amount of the sample was boiled with concentrated sodium hydroxide solution to precipitate zirconium as the hydroxide and the precipitate was filtered. The filtrate was diluted to 100 ml in a calibrated flask and molybdenum was determined spectrophotometrically[11] as the thiocyanate complex at 475 nm.

The precipitate on the filter-paper was dissolved in hydrochloric acid and zirconium was determined spectrophotometrically by the alizarin red S method at 510 nm.[12]

For determining phosphorus, 0.1 g of the powdered ion exchanger was treated with a known excess of sodium hydroxide solution. The excess of alkali was then back-titrated with hydrochloric acid. The phosphorus content was calculated from the volume of alkali consumed.

Determination of Ion-exchange Capacity (Batch Operation)

The exchange capacity was determined by a standard method.[13] The conditions such as the concentration of electrolyte used and the time of equilibration were fixed from the results of preliminary investigations.

Determination of distribution coefficients (K_d)

About 0.1 g of the ion exchanger was loaded with 10 ml of 0.002 M metal ion solution (pH 4–6) and at equilibrium the K_d values were calculated using the expression

$$K_d = \left(\frac{C_o}{C_e} - 1\right)\frac{A}{m}$$

where C_o = the original concentration of ion in solution, C_e = the concentration of ion in the solution at equilibrium, A = the total volume of solution (ml) and m = the mass of the exchanger (g).

Various cations were determined by titrimetric, spectrophotometric or radiometric methods (Table IV).

Column Operation

For separation studies, a glass column (30 × 0.50 cm i.d.) containing 1.0 g of ion exchanger (100–200-mesh particle size obtained after sieving the product) on a glass-wool support was used. The column was loaded with a mixture of ion pairs until they were completely adsorbed on the column material. Elution was started after 15 min at a flow-rate of about 0.2 ml min^{-1}. The cations eluted were determined in 2-ml fractions of the eluate.

Results and Discussion

Zirconium molybdophosphate, prepared as mentioned above, is yellow and shows no dispersion in either aqueous or salt solutions (3 M). It is also stable in alcohols, benzene and concentrated solutions (1 M) of hydrochloric, sulphuric, nitric and acetic acids. Dilute

TABLE I

CHEMICAL ANALYSIS DATA

Molecular formula	Component	Composition, % Observed	Calculated
$(ZrO)_3(Mo_{12}PO_{40})_2.26H_2O$	ZrO	5.90	6.17
	Mo	52.50	51.93
	P	1.38	1.40
	H_2O	10.42	10.55

alkaline solutions (up to 0.01 M) have no effect on the compound but it decomposes in strong alkali (>0.01 M). In general, molybdophosphates have been reported to be less stable than other heteropolyacid salts, but the zirconium compound is obtained in the form of hard granules and is stable in acidic, salt and dilute alkaline solutions.

The analytical results are given in Table I. The experimental values are in agreement with calculated values on the basis of the formula assigned to the compounds. The number of water molecules present was calculated from the decrease in mass in thermogravimetric analysis, and also by heating a known amount of exchanger at 400 °C to constant mass. The thermograms (not shown here) indicated a decrease in mass from 100 to 400 °C, and heating to 1000 °C gave no further decrease in mass. The absence of characteristic bands of water in the infrared spectra of samples heated to 400 °C also confirm the complete loss of water at this temperature.

TABLE II

VARIATION OF EXCHANGE CAPACITY (WITH RESPECT TO K^+) OF ZIRCONIUM MOLYBDOPHOSPHATE WITH DRYING TEMPERATURE

Drying temperature/°C	Exchange capacity of exchanger/mequiv. g^{-1}
90	0.42
150	0.22
200	0.08
300	0.03

The infrared spectrum of zirconium molybdophosphate has peaks at 3400, 1620, 1074, 968, 911, 869, 795 and 508 cm^{-1}. The broad band at 3400 cm^{-1} is characteristic of interstitial water molecules whereas the sharp bands at 1620 and 508 cm^{-1} may be assigned to the HOH bending mode[14] and to Zr–O stretching vibration,[15] respectively. According to the literature,[16] the characteristic bands corresponding to the molybdophosphate anion should appear at 1060, 945, 895 and 889 cm^{-1}. The same peaks in the present compound appear at 1074, 968, 911 and 869 cm^{-1}; the slight shift may be attributed to the presence of the quadrivalent zirconium ion instead of a monovalent cation in the heteropolyacid salt. Apart from this, the spectrum compares well with that reported by Dollimore *et al.*[17] for zirconium phosphates.

The X-ray diffraction spectrum shows only a few weak diffraction lines at 3.26, 2.39, 2.05, 1.46 and 1.23 Å, indicating the poorly crystalline nature of the product. Similar results have also been reported for the crystallinity of zirconium tungstoarsenate and titanium tungstophosphate.[7,8]

TABLE III

DISTRIBUTION COEFFICIENT VALUES FOR VARIOUS METAL CATIONS (0.002 M) ON THE ION EXCHANGER

Sample No.	Cation	Taken as	K_d/ml g^{-1}	Method
1	Rb^+	Chloride	61	Radiometry
2	Cs^+	Chloride	87	Radiometry
3	Tl^+	Nitrate	740	Radiometry
4	Ag^+	Nitrate	934	Radiometry
5	Mg^{2+}	Chloride	15	Titrimetry
6	Sr^{2+}	Chloride	13	Radiometry
7	Ba^{2+}	Chloride	2	Radiometry
8	Mn^{2+}	Chloride	5	Radiometry
9	Zn^{2+}	Sulphate	0	Radiometry
10	Hg^{2+}	Chloride	69	Radiometry
11	Co^{2+}	Chloride	32	Radiometry
12	Ni^{2+}	Chloride	22	Titrimetry
13	Cu^{2+}	Nitrate	40	Titrimetry
14	ZrO^{2+}	Chloride	30	Spectrophotometry
15	UO_2^{2+}	Nitrate	C.A.*	Spectrophotometry
16	Bi^{3+}	Nitrate	0	Titrimetry
17	Th^{4+}	Nitrate	934	Titrimetry

* C.A. = Completely adsorbed.

Normally in ammonium heteropolyacid salts[1–3] it is the ammonium ion that is exchanged with other cations. In this compound the zirconium ion is not released during the process of ion exchange. Probably hydrogen ions, which become incorporated in the structural network of the product during the course of its preparation in an acidic medium, account for the exchange capacity of the material. It is well established that ion exchange in zirconium phosphate also involves replaceable hydrogen in acid phosphate groupings.[18] Therefore, sorption and separation studies were performed after converting the exchanger into the H^+ form by treating it with 0.5 M hydrochloric acid for 50 h.

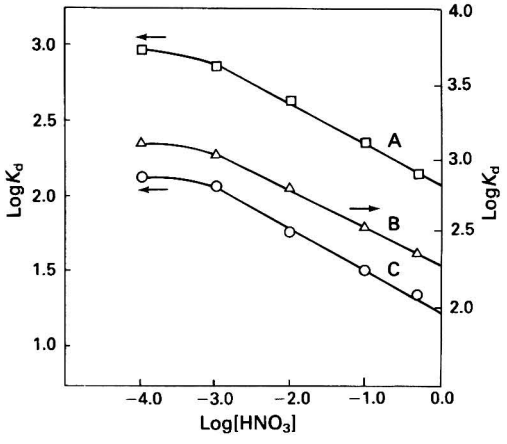

Fig. 1. Graphs showing variations of logK_d for 10^{-3} M Cs^+, Ag^+ and Tl^+ (A, B and C, respectively) on ZMP with nitric acid concentration.

The ion-exchange capacity was determined by equilibrating the original compound (as prepared) in 1 M potassium chloride solution for 50 h. The equilibration time and concentration of electrolyte were fixed after preliminary investigations. The ion-exchange capacity of the compound was found to be 0.42 mequiv. g^{-1}, which is much higher than that reported by other workers,[9,10] and it decreased sharply with increase in drying temperature (Table II). As with zirconium phosphate,[18] this decrease may be attributed to variable hydrolysis at different temperatures.

Distribution coefficients (K_d) for various cations are given in Table III. The uranyl ion is completely adsorbed, and the compound also exhibits significant affinity for Tl^+, Ag^+ and

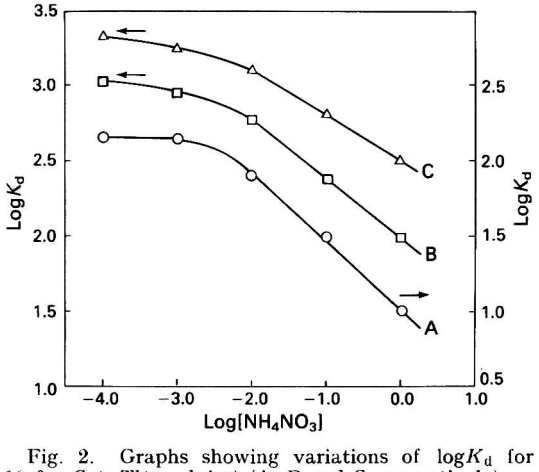

Fig. 2. Graphs showing variations of logK_d for 10^{-3} M Cs^+, Tl^+ and Ag^+ (A, B and C, respectively) on ZMP with ammonium nitrate concentration.

Th^{4+}. For the other cations the K_d values are low; the uptake of Mg^{2+}, Sr^{2+}, Ba^{2+} and Mn^{2+} is negligible and Zn^{2+} and Bi^{3+} are not adsorbed at all. Except for silver, the sequence for monovalent ions is parallel to the affinity series obtained for ammonium molybdophosphate.[19]

The variation of the distribution coefficient with nitric acid and ammonium nitrate concentration for Ag^+, Tl^+ and Cs^+ is shown in Figs. 1 and 2. The uptake of these ions decreases with increasing nitric acid or ammonium nitrate concentration. As the slope of linear portion of the graph is less than unity, the uptake of all three monovalent cations is non-stoicheiometric. As the ion-exchange material is stable in acids (but not in 0.01 M alkali) and the exchange capacity for Cs^+, Ag^+ and Tl^+ is reduced in the presence of H^+ and NH_4^+ ions, then the non-stoicheiometry is excellent evidence for a competitive ion exchange based upon a selectivity series of the type $H^+ > NH_4^+ > Cs^+$, Ag^+, Tl^+, *i.e.*, that H^+ is a counter ion.

Of the four cations Tl^+, Ag^+, Th^{4+} and UO_2^{2+}, silver is irreversibily adsorbed on the columns of the ion-exchange material and its elution is not possible. For Th^{4+} and UO_2^{2+}, only 60–70% recovery is possible with various eluents whereas Tl^+ can be completely recovered. A large number of separations of thallium from other cations have been performed on columns of this ion exchanger and some of these are shown in Fig. 3. The recovery of Tl^+ is 100% in all of the separations. Apart from the separations shown in Fig. 3, thallium has also been separated from nickel, cobalt, copper, magnesium, manganese and bismuth. In spite of large separation factors between Tl^+ and Cs^+ and between Tl^+ and Rb^+ ions, it has not been possible to separate these successfully. In these two separations, the recovery of Tl^+ is 100% whereas only 80% elution is possible for Rb^+ and Cs^+. Hence the ion exchanger can be used for the sorption and recovery of thallium ions from a mixture of other cations. In addition, some separations with low separation factors (Sr^{2+} from Cs^+ and Sr^{2+} from Rb^+) have also been performed (Fig. 3).

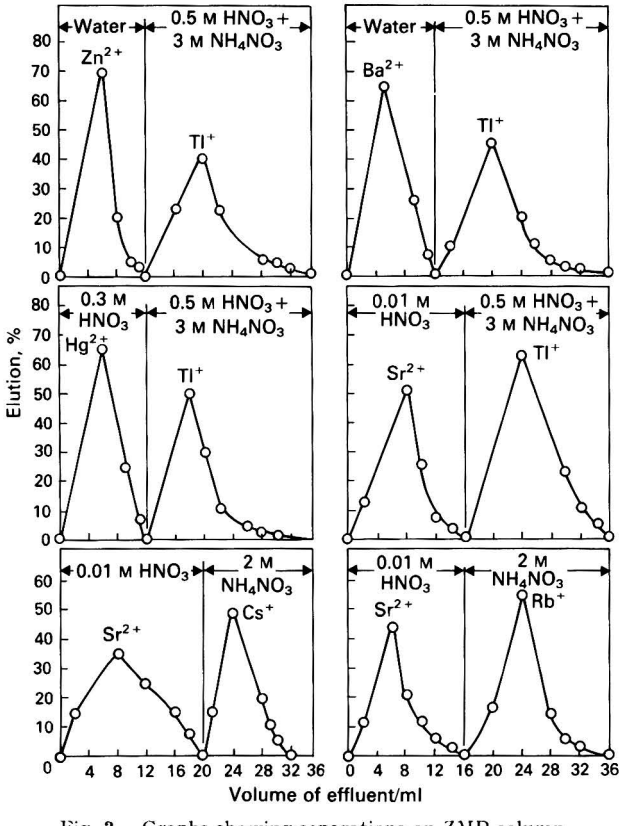

Fig. 3. Graphs showing separations on ZMP column.

It has been possible to use the material for eight to ten cycles without any decrease in its adsorption capacity or separation efficiency of Tl^+ with respect to other cations (Fig. 3).

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Liquid - Liquid Extractive Separation of Molybdenum(VI) in Malonate Solutions with High Relative Molecular Mass Amines

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Molybdenum can be quantitatively extracted with 0.08 M Amberlite LA-2 in xylene at pH 3.0 from 0.02 M malonic acid, stripped with 0.25 M ammonia solution and determined spectrophotometrically with Tiron at 390 nm. Four other liquid anion exchangers were examined as possible extractants and extraction of molybdenum was found to be quantitative over a limited pH range with two of these. Eight common solvents were tested as diluents; of these, hexane, cyclohexane, benzene and xylene were found to be satisfactory.

Molybdenum can be separated from elements that do not form complexes with malonic acid by prior stripping of the extract with water. The metal can be separated from elements forming weak complexes by selective stripping with acids. A novel feature of the method is the separation of molybdenum from multi-component mixtures.

The method has been applied to the determination of molybdenum in steel and soil.

Keywords: *Molybdenum separation*; *molybdenum determination*; *liquid - liquid extraction*; *steel analysis*; *soil analysis*

The separation of molybdenum by solvent extraction has been carried out either with solvating solvents such as diethyl ether or with alkyl esters.[1] Chelating extractants of the quinolin-8-ol and azonaphthol type[2] have also been used. However, few attempts have been made to use liquid anion exchangers for the extractive separation of molybdenum.

Molybdenum has been extracted from sulphuric acid with trioctylamine[3] and tributylamine,[4] and from chloride media it has been extracted with Amberlite LA-2 in kerosene,[5] benzylamine[6] and triisooctylamine.[7] Such extractions are not quantitative, showing random variations in extractability with change in acidity. Molybdenum(V) has been extracted from mixtures of hydrochloric acid and alkali metal thiocyanates with tribenzylamine[8] and Amberlite LA-1 in chloroform,[9,10] but large numbers of elements were found to interfere.

There appears to be a lack of systematic work on the solvent extraction of molybdenum with high relative molecular mass amines. This paper presents a method for the solvent extractive separation of molybdenum(VI) from aqueous solutions containing malonic acid with Amberlite LA-2 in xylene. Other liquid exchangers are unsatisfactory because their systems do not extract quantitatively or because the pH for the quantitative extraction is low. The method has been extended to the determination of molybdenum in samples of soil and alloys.

Experimental

Apparatus

A digital pH meter, Type 822 (ECIL, India), with glass and calomel electrodes, an ECIL GS 866 C spectrophotometer with matched 10-mm Corex glass cells and a wrist-action flask shaker (Toshniwal and Co., India) were used.

Reagents

Molybdenum(VI) stock standard solution, 5 mg ml^{-1}. Prepared by dissolving 2.30 g of ammonium molybdate tetrahydrate in 250 ml of distilled water. The solution was standardised gravimetrically as the quinolin-8-olate.[11]

Molybdenum(VI) working standard solution, 100 μg ml^{-1}. Prepared by appropriate dilution of the stock standard solution.

Ion-exchange resins. Amberlite LA-1 [*N*-dodecyl(trialkylmethyl)amine], Amberlite LA-2 [*N*-lauryl(trialkylmethyl)amine], Primene JM-T (a mixture of primary amines in the C_{18}–C_{22} range) (Rohm and Haas, Philadelphia, PA, USA), Aliquat 336S (tricaprylmonomethylammonium chloride) (General Mills Ltd., UK), trioctylamine and triisooctylamine (Riedel-de Haen, Hannover, Germany) were used without further purification. The exchangers were converted into the malonate form by shaking for 10 min with an equal volume of 0.1 M malonic acid. A second equilibration with a further 10 ml of 0.1 M malonic acid gave no further exchange. The optimum concentration of malonic acid was 0.1 M, as at higher concentrations emulsification or turbidity was encountered.[12]

Buffer solution (pH 7.0). Prepared by mixing 50 ml of 0.2 M potassium dihydrogen orthophosphate with 35 ml of 0.2 M sodium hydroxide solution and diluting to 200 ml with water.

Tiron (disodium 1,2-*dihydroxybenzene*-3,5-*disulphonate) solution*, 2%.

General Procedure

To an aliquot of sample solution containing up to 100 μg of molybdenum(VI), add 2 ml of 0.1 M malonic acid followed by dilute sodium hydroxide solution or malonic acid to adjust the pH to 3.0. Dilute the solution with water to 10 ml. Transfer the solution into a separating funnel and equilibrate with 10 ml of 0.08 M Amberlite LA-2 in xylene for 5 min using the flask shaker. Allow the layers to separate and discard the aqueous phase. Strip molybdenum from the organic phase with 10 ml of 0.25 M ammonia solution. Add to the separated strippant 5 ml of buffer solution and 2 ml of Tiron solution. Dilute the resulting solution to 25 ml. Measure the absorbance of the pale yellow complex at 390 nm against a reagent blank. Calculate the mass of molybdenum in the portion of sample taken by means of a calibration graph,[13] constructed by taking 5–250 μg of molybdenum per 25 ml of solution, developing the coloured complex with Tiron and measuring the absorbance at 390 nm.

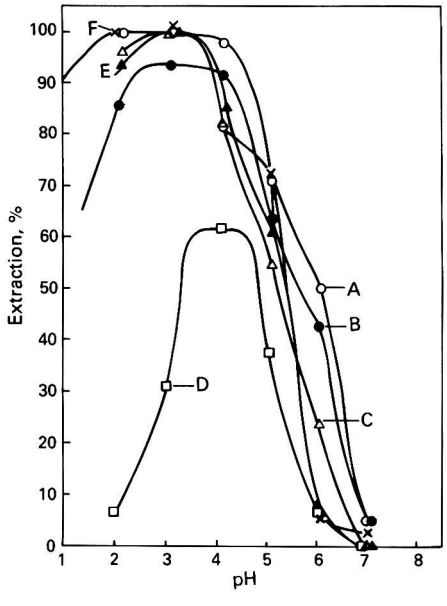

Fig. 1. Extraction of molybdenum(VI) from malonic acid solution with various 4% amine solutions in xylene: A, Amberlite LA-2; B, Amberlite LA-1; C, Primene JM-T; D, Aliquat 336S; E, TOA; and F, TIOA.

Results and Discussion

Extraction as a Function of pH

The optimum pH for the extraction of molybdenum was ascertained by extracting with 4% solutions of liquid exchangers in xylene in the pH range 1–8. The pH was adjusted with 0.01 M malonic acid or sodium hydroxide solution. The phase-volume ratio was maintained at 1:1. The optimum pH for the quantitative extraction was 2–4 with Amberlite LA-2, 2–3 with Primene JM-T or triisooctylamine (TIOA) and 2.5–3.0 with trioctylamine (TOA). The extraction was incomplete with Aliquat 336S (maximum 62%) and Amberlite LA-1 (93%). The results in Fig. 1 indicate that the exchanger that extracts molybdenum quantitatively over the widest pH range is Amberlite LA-2.

Effect of Various Diluents

Benzene, toluene, chloroform, carbon tetrachloride, xylene, hexane, cyclohexane and isobutyl methyl ketone were examined for use as diluents in the extraction of molybdenum(VI) with Amberlite LA-2 (Table I). The phase-volume ratio was kept at unity during all the work to mitigate the problem of turbidity. The most efficient diluents were found to be benzene, xylene, hexane and cyclohexane. When toluene, carbon tetrachloride, chloroform or isobutyl methyl ketone was used as a diluent, quantitative extraction of molybdenum was not obtained. The results showed a regular trend of a decrease in extraction with increasing dielectric constant of the diluent, with the exception of carbon tetrachloride. The optimum periods for extraction and stripping were found to be 5 and 2 min, respectively. On safety grounds xylene was preferred to benzene as the diluent.

TABLE I

EFFECT OF VARIOUS DILUENTS

Mo(VI) = 100 μg; pH = 3.0; Amberlite LA-2 concentration = 8×10^{-2} M.

Diluent	Extraction, %	Diluent	Extraction, %
Hexane	100.0	Xylene	100.0
Cyclohexane	100.0	Toluene	98.8
Carbon tetrachloride	86.5	Chloroform	70.3
Benzene	100.0	Isobutyl methyl ketone	64.8

Effect of Variation of Malonic Acid Concentration

The optimum concentration of malonic acid for the purpose of complexation was ascertained by extracting molybdenum with 4% Amberlite LA-2 in xylene at various concentrations of malonic acid (Table II). Some extraction was observed when 1×10^{-3} M malonic acid was used, but extraction was not quantitative unless the concentration exceeded 1.2×10^{-2} M. To ensure complete complexation, 0.02 M malonic acid is recommended.

TABLE II

EFFECT OF MALONIC ACID CONCENTRATION

Mo(VI) = 100 μg; pH = 3.0; Amberlite LA-2 concentration in xylene = 8×10^{-2} M.

Malonic acid concentration/M $\times 10^{-3}$	Extraction, %	Distribution ratio, D
1	45.6	0.84
2	72.0	2.58
3	85.7	5.99
4	91.4	10.63
5	94.3	16.54
6	95.7	22.25
7	97.1	33.48
9	98.4	61.50
11	99.6	249.00
12–30	100.0	∞

Effect of Variation of Amberlite LA-2 Concentration

The concentration of Amberlite LA-2 required for quantitative extraction of molybdenum was studied by keeping all other factors constant. The extractions were carried out with 0.01–0.08 M Amberlite LA-2 in xylene (Table III). Some extraction was observed at concentrations less than 0.01 M, but extraction was not quantitative unless the concentration exceeded 0.07 M. Hence, an Amberlite LA-2 concentration of 8×10^{-2} M was selected as optimum for the quantitative extraction of molybdenum.

TABLE III

EFFECT OF AMBERLITE LA-2 CONCENTRATION

Mo(VI) = 100 μg; pH = 3.0; malonic acid concentration = 0.02 M.

Amberlite LA-2 concentration/M	Extraction, %	Distribution ratio, *D*
0.01	42.4	0.74
0.02	68.0	2.13
0.03	82.9	4.85
0.04	90.0	9.00
0.05	92.8	12.89
0.06	94.8	18.23
0.07	96.0	24.00
0.076	100.0	∞
0.08	100.0	∞

Choice of Stripping Agents

After extraction into the amine phase, the molybdenum was stripped with 10 ml of various stripping agents at different concentrations (Table IV). The stripping was found to be incomplete with any concentration of mineral acid in the range 0.05–1.0 M. Under alkaline conditions, stripping was quantitative when the concentration of strippant exceeded 0.05 M sodium hydroxide, 0.1 M sodium carbonate or 0.2 M ammonia. Alkali metal hydroxide concentrations greater than 0.5 M were not used owing to emulsion formation. Ammonia solution (0.25 M) was preferred to sodium hydroxide or sodium carbonate because it was easier to adjust the pH prior to determining molybdenum spectrophotometrically with Tiron.

TABLE IV

EFFECT OF STRIPPING AGENTS

Mo(VI) = 100 μg; Amberlite LA-2 concentration in xylene = 8×10^{-2} M.

	Back-extraction, %								
Stripping agent	0.05 M	0.1 M	0.2 M	0.5 M	1.0 M	2.0 M	4.0 M	6.0 M	8.0 M
HCl	0.0	0.0	31.1	58.9	60.0	64.4	35.5	36.0	35.5
HNO_3	0.0	0.0	—	51.1	83.3	100.0	80.0	84.4	100.0
H_2SO_4	0.0	0.0	<2.0	16.7	56.0	86.6	100.0	92.0	—
NaOH	100.0	100.0	100.0	100.0	100.0	—	—	—	—
NH_3	92.4	92.0	100.0	100.0	100.0	—	—	—	—
Na_2CO_3	96.0	100.0	100.0	100.0	100.0	—	—	—	—

Nature of Extracted Species

A possible mechanism for the extraction is

$$2R_2NH_2^+ + [MoO_2(malonate)_2]^{2-} = (R_2NH_2)_2[MoO_2(malonate)_2]$$

This was confirmed by plotting graphs of log *D versus* log Amberlite LA-2 concentration at fixed malonate concentration and log *D versus* log malonic acid concentration at fixed Amberlite LA-2 concentration. The slopes were found to be 1.95 and 2.05, respectively, confirming the composition of the extracted species as $(R_2NH_2)_2[MoO_2(malonate)_2]$.

Separation of Molybdenum from Binary Mixtures

Molybdenum was extracted in the presence of various ions (Table V). The tolerance limit was set as the amount of foreign ion required to cause a $\pm 2\%$ error in the recovery of molybdenum.[12] Alkali and alkaline earth metals, thallium(I), iron(II), silver, arsenic(III), yttrium and lanthanides were not extracted with molybdenum because malonato complexes are not formed. Zinc, cadmium, nickel(II), cobalt(II), manganese(II), lanthanoids, aluminium and chromium(III) form weaker malonato complexes; these metals were, therefore, stripped with water before stripping molybdenum with 0.25 M ammonia solution. Bismuth, iron(III), vanadium(V), titanium(IV) and gallium form stronger malonato complexes, which were extracted with molybdenum; these metals were stripped from the extract with 0.25 M sulphuric acid. Under these conditions molybdenum remains behind as an anionic sulphato complex and can be stripped with 0.25 M ammonia solution.

Molybdenum was separated from platinum(IV), gold(III), thallium(III), mercury(II), indium(III), cerium(III), thorium(IV) and uranium(VI) in nitrate media. After extraction of these metals as malonato complexes, molybdenum was first stripped with 8 M nitric acid. Under these conditions, the other metals remain in the organic phase as anionic nitrato complexes and, if necessary, can be stripped with 0.1 M sodium hydroxide solution.

TABLE V

SEPARATION OF MOLYBDENUM FROM BINARY MIXTURES

Mo(VI) = 100 μg; pH = 3.0; Amberlite LA-2 concentration = 8×10^{-2} M.

Foreign ion	Added as	Tolerance limit/μg	Foreign ion	Added as	Tolerance limit/μg
Li^+	$Li_2SO_4.H_2O$	4500	Co^{2+}	$Co(NO_3)_2.6H_2O$	280
Na^+	NaCl	6000	Mn^{2+}	$MnSO_4.7H_2O$	400
K^+	KCl	6000	Al^{3+}	$Al(NO_3)_3.9H_2O$	820
Rb^+	RbCl	1200	Cr^{3+}	$Cr(NO_3)_3.9H_2O$	1000
Cs^+	CsCl	1000	Bi^{3+}	$Bi(NO_3)_3.5H_2O$	500
Mg^{2+}	$MgSO_4.7H_2O$	1200	Cu^{2+}	$CuSO_4.5H_2O$	600
Ca^{2+}	$CaCl_2.6H_2O$	2000	Fe^{3+}	$FeCl_3$	4200
Sr^{2+}	$Sr(NO_3)_2.2H_2O$	2500	V^{5+}	NH_4VO_3	600
Ba^{2+}	$Ba(NO_3)_2.4H_2O$	1400	Ti^{4+}	$Ti(SO_4)_2$	400
Tl^+	Tl_2SO_4	5000	Ga^{3+}	$GaCl_3$	1600
Fe^{2+}	$FeSO_4.7H_2O$	4500	Pt^{4+}	$H_2PtCl_6.xH_2O$	800
Ag^+	$AgNO_3$	350	Au^{3+}	$AuCl_3$	700
As^{3+}	$AsCl_3$	400	Tl^{3+}	$TlCl_3$	820
Y^{3+}	$Y(NO_3)_3$	1400	Hg^{2+}	$HgCl_2$	900
La^{3+}	$La(NO_3)_3$	350	In^{3+}	$In_2(SO_4)_3.5H_2O$	600
Zn^{2+}	$ZnSO_4.7H_2O$	600	Ce^{3+}	$Ce(NO_3)_3$	480
Cd^{2+}	$3Cd(NO_3)_2.8H_2O$	320	Th^{4+}	$Th(NO_3)_4.4H_2O$	1300
Ni^{2+}	$Ni(NO_3)_2.6H_2O$	500	U^{6+}	$UO_2(NO_3)_2.6H_2O$	760

Separation from Tertiary Mixtures

It was found to be possible to separate molybdenum from elements such as cerium, titanium, vanadium, chromium, uranium and iron in multi-component mixtures (Table VI).

Vanadium(V), chromium(III) and molybdenum were separated by extracting them together, followed by stripping of chromium with water, vanadium(V) with 0.25 M sulphuric acid and molybdenum with 0.25 M ammonia solution.

After extraction of chromium(III), iron(III) and molybdenum from malonate solution, they were separated by stripping chromium with water, iron with 0.25 M sulphuric acid and finally molybdenum with 0.25 M ammonia solution.

Titanium, molybdenum and chromium(III) were separated, after their extraction, by stripping chromium with water, titanium with 0.25 M sulphuric acid and molybdenum with 0.25 M ammonia solution.

TABLE VI

SEPARATION FROM TERTIARY MIXTURES

pH = 3.0; Amberlite LA-2 concentration in xylene = 8×10^{-2} M.

No.	Mixture	Taken/μg	Found/μg	Recovery, %	Eluent
1	Cr(III)	406.5	406.0	99.9	Water
	V(V)	300.0	296.0	99.7	0.25 M H_2SO_4
	Mo(VI)	100.0	100.0	100.0	0.25 M NH_3
2	Cr(III)	406.5	402.5	99.0	Water
	Fe(III)	500.0	491.2	98.2	0.25 M H_2SO_4
	Mo(VI)	50.0	49.7	99.4	0.25 M NH_3
3	Cr(III)	813.0	811.2	99.8	Water
	Ti(IV)	300.0	298.4	99.5	0.25 M H_2SO_4
	Mo(VI)	50.0	50.3	100.6	0.25 M NH_3
4	Cr(III)	813.0	807.0	99.3	Water
	Mo(VI)	100.0	100.0	100.0	8 M HNO_3
	Ce(III)	400.4	398.2	99.5	1 M HCl
5	Cr(III)	406.5	405.6	99.8	Water
	Mo(VI)	50.0	49.8	99.6	8 M HNO_3
	U(VI)	310.8	308.0	99.1	0.1 M NaOH

The separation of chromium(III), molybdenum and cerium(III) or chromium(III), molybdenum and uranium(VI) was accomplished after their extraction from malonate media by stripping chromium with water, followed by molybdenum with 8 M nitric acid and finally cerium or uranium with 1 M hydrochloric acid or 0.1 M sodium hydroxide solution, respectively.

Application to Analysis of Steel

Molybdenum was determined in a sample of stainless steel (BCS No. 466) by the following procedure. A 0.5-g amount of sample was dissolved in a mixture of hydrochloric, nitric and hydrofluoric acids (2 + 1 + 0.5). The solution was evaporated to dryness, the residue dissolved in 2 ml of concentrated hydrochloric acid and the resulting solution diluted to 100.0 ml with water. An aliquot (1.0 ml) of the diluted solution was treated and extracted as described under General Procedure. The organic phase (containing chromium, molybdenum and iron) was washed with water to remove chromium, then with two 10-ml volumes of 0.25 M sulphuric acid to remove iron and finally with 0.25 M ammonia solution to strip the molybdenum. In triplicate determinations, the molybdenum concentration was found to be 2.20, 2.22 and 2.20%; the reported value was 2.21%.

Application to Analysis of Soil Samples

A 5.0-g amount of finely powdered soil sample was digested and brought into solution by the standard procedure of Smythe and co-workers.[14,15] Molybdenum, together with other micronutrients such as iron, copper and zinc, was converted into its malonate complex and extracted with 0.08 M Amberlite LA-2 in xylene at pH 3.0 as described under General Procedure. The organic phase was washed successively with water and 0.25 M sulphuric acid to remove all interfering ions. Finally, molybdenum was stripped with 0.25 M ammonia solution. In triplicate analyses, the molybdenum concentration was found to be 2.82, 2.85 and 2.78 μg g^{-1}; the reported value was 2.82 μg g^{-1}.

The important feature of this proposed method is that it permits the separation of molybdenum from vanadium, titanium, chromium, manganese, iron, nickel and zinc, which are generally associated with any steel. Also, the method permits the separation of molybdenum from copper, a metal with which it is associated in soils. The method is simple and rapid (30 min per sample separation); it is reproducible and compares favourably with other methods for the extractive separation of molybdenum.

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Determination of the Active-ingredient Content of Technical and Formulated DNOC and Dinoseb and Technical Dinobuton by Spectrophotometry

A Collaborative International Pesticides Analytical Council Study

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A spectrophotometric method is described for the determination of the active-ingredient content of tecnical and formulated DNOC (2-methyl-4,6-dinitrophenol) and dinoseb (2-*sec*-butyl-4,6-dinitrophenol), and technical dinobuton (2-*sec*-butyl-4,6-dinitrophenyl isopropyl carbonate). The method involves a preliminary clean-up on Woelm neutral alumina (grade I). Impurities are eluted from the column while the active ingredient is retained at the top of the column. The active ingredient is then eluted as its butylammonium salt and determined spectrophotometrically. Dinobuton samples require a preliminary separation from free dinoseb, de-esterification on the column and determination using the dinoseb procedure. Results obtained from international collaborative studies are presented and discussed.

Keywords: *DNOC*; *dinoseb*; *dinobuton*; *spectrophotometry*; *collaborative studies*

In 1958 the Herbicides Panel of the Pesticides Analysis Advisory Committee (PAAC) of the Ministry of Agriculture, Fisheries and Food set up a dinoseb sub-committee. The terms of reference of this sub-committee were to consider and agree methods for the analysis of technical and formulated dinoseb (2-*sec*-butyl-4,6-dinitrophenol). The most promising method available when work began was based on the procedure described by Bouwman and Westenberg.[1] This employed liquid - liquid chromatography on Kieselguhr, with hexane as the eluting solvent. After separation from interfering substances, the dinoseb content was determined spectrophotometrically. This method was tedious and the sub-committee suspended work in 1962, pending the provision of improved methods. Later, in 1967 with the setting up of the Dinitro Pesticides Panel, work was restarted and expanded to encompass the analysis of dinobuton (2-*sec*-butyl-4,6-dinitrophenyl isopropyl carbonate) and DNOC (2-methyl-4,6-dinitrophenol). The spectrophotometric determination was replaced by a method involving reduction, with titanium(III) chloride, of the nitro groups but this had to be abandoned as the semi-micro techniques involved were insufficiently precise. A brief trial involving formation of the nitron complex of the dinitro compounds also failed as it was found to be applicable only to dinoseb in an excess of 95% purity. This left only the method based on Bouwman's procedure which, although it had shortcomings, was subsequently published in the Collaborative International Pesticides Analytical Council (CIPAC) handbook.[2]

A suitable substitute for the first CIPAC dinoseb method was found with the publication of a procedure for the analysis of dinobuton by Lynch.[3] This method involves preliminary de-esterification of dinobuton to dinoseb on neutral alumina; the dinoseb is then eluted from the column as its butylammonium salt and determined spectrophotometrically. This method, in a modified form, proved suitable for the analysis of dinoseb and DNOC in addition to dino-

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buton, and through the efforts of the Dinitro Pesticides Panel suitable adaptations were made for formulations and the method was subjected to international collaborative trial. The finalised methods for technical pesticides and oil-based formulations of DNOC and dinoseb were published in the 1980 Proceedings of the Collaborative International Pesticides Analytical Council (CIPAC)[4] and methods for the salt formulations of DNOC and dinoseb followed in the 1981 Proceedings.[5] The basic method is also applicable to analysis of binapacryl (2-*sec*-butyl-4,6-dinitrophenyl-3-methyl crotonate) and dinoterb (2-*tert*-butyl-4,6-dinitrophenol) but no collaborative work has been carried out on these materials.

An independent check on the accuracy of the results has been provided by high-performance liquid chromatographic (HPLC) analysis of DNOC, dinoseb and dinobuton,[6] in which no statistical difference was found between the spectrophotometric and HPLC results (at the 95% confidence limit).

Results and Discussion

The whole of the work reported here was not completed in a single exercise but over a period of a number of months, though as far as possible efforts were made to get collaborators to analyse individual samples at roughly the same time. The method first submitted by Lynch[3] was basically applicable to technical and oil-based formulated material. Initial work in a small number of laboratories was undertaken to reveal any specific difficulties, and once an apparently satisfactory procedure was agreed upon, potential collaborators were notified on an

TABLE I

RESULTS FROM THE COLLABORATIVE TRIALS OF RECOMMENDED METHODS FOR THE ANALYSIS OF DNOC

	Sample, % *m/m*			
	DNOC technical		DNOC ammonium salt formulation	DNOC - petroleum oil formulation
Laboratory	1	2		
I	95.4	95.3	43.8	2.0
	94.0	95.8	45.2	2.0
II	98.9	95.7	46.8	2.1
	98.3	96.6	47.4	2.1
III	—	99.0	43.7	2.0
	—	97.9	44.3	1.9
IV	—	—	46.7	—
	—	—	46.2	—
V	—	—	47.6	—
	—	—	48.2	—
VI	97.1	98.1	47.2	1.9
	97.7	98.7	47.4	2.0
VIII	98.9	99.2	46.0	2.1
	99.0	98.8	46.9	2.1
IX	—	—	44.3	—
	—	—	45.0	—
X	96.7	95.4	44.9	2.3
	94.2	95.8	44.4	2.3
$\bar{x}$	97.1	97.2	45.9	2.0
n	10	12	18	12
r	2.39	1.40	1.47	0.12
R	5.20	4.60	4.22	0.39

international basis by a CIPAC trawl notice. Eleven laboratories agreed to participate in the experimental work, though for a variety of reasons not every laboratory has submitted results for all the samples. Difficulties were encountered at the collaborative stage with the analysis of the DNOC and dinoseb ammonium salts, and it was found that an extraction procedure was required prior to chromatography. The methods were duly modified and sent out again for collaborative study. Sample collection was undertaken by the Panel Secretary at the Ministry of Agriculture, Fisheries and Food, Harpenden Laboratory, and each was mixed thoroughly before sub-sampling for despatch to individual laboratories. Collaborators were asked to record any departures from the methods that they received and each collaborator was instructed to analyse the samples in duplicate. The results obtained from all collaborators were examined using Dixon's test and outliers were rejected, prior to any further statistical consideration. Where methods were strictly adhered to few difficulties were encountered; most of the problems that did occur could be traced to the use of alumina of a different type to that which was recommended. Woelm alumina was found by the originator of the method to give repeatable results and, over a period of 15 years, has been of consistent quality. Difficulties can also be

TABLE II

RESULTS FROM COLLABORATIVE TRIALS OF RECOMMENDED METHODS FOR THE ANALYSIS OF DINOSEB

	Sample, % *m/m*					
	Dinoseb technical		Dinoseb ammonium salt formulation		Dinoseb - petroleum oil formulation	
Laboratory	1	2	1	2	1	2
I	99.4	99.0	16.2	16.5	9.9	9.9
	99.6	97.6	17.8	16.3	10.4	9.7
II	98.4	98.3	18.8	16.9	10.5	10.5
	99.1	99.0	18.9	17.2	10.7	10.6
III	—	100.3	17.1	16.0	10.0	9.3
	—	99.3	16.5	—	10.3	9.9
IV	—	—	—	—	10.7	10.3
	—	—	—	—	10.6	10.1
V	—	—	—	—	10.2	10.2
	—	—	—	—	10.5	10.0
VI	98.5	97.9	18.1	17.2	10.3	10.2
	98.3	97.7	18.2	17.6	10.3	10.3
VII	—	—	16.7	16.0	10.6	10.3
	—	—	16.8	15.9	10.6	10.4
VIII	98.2	98.5	—	—	10.0	10.2
	97.8	98.9	—	—	10.3	10.0
IX	—	—	17.9	16.8	10.4	10.1
	—	—	17.6	16.9	10.3	10.2
X	98.2	98.9	—	—	—	—
	97.5	99.1	—	—	—	—
XI	97.6	96.9	17.3	17.1	—	—
	97.5	95.6	17.6	17.3	—	—
$\bar{x}$	98.3	98.3	17.6	16.7	10.4	10.4
n	12	14	17	16	18	18
r	0.9	1.43	1.34	0.48	0.51	0.50
R	2.10	3.37	2.39	1.47	0.68	0.87

encountered if sintered-glass supports are used in the chromatographic columns as these can cause de-esterification of dinobuton; as a general precaution against adsorption cotton-wool supports were used throughout.

The repeatability (r) and reproducibility (R) figures were determined using calculations based on International Standard ISO 5725.[7] The values were determined at the 95% probability level; hence, a difference between two results from one operator within a single laboratory should not exceed more than one time in twenty. Similarly, the difference between two single independent results from different laboratories should not exceed R more than one time in twenty. The results were considered satisfactory by the Panel and the methods have subsequently been accepted by CIPAC, and are being recommended as the methods of analysis in the appropriate Food and Agriculture Organisation draft specifications currently in preparation.

The results of the studies are given in Tables I–III.

TABLE III

RESULTS FROM COLLABORATIVE TRIALS OF RECOMMENDED METHODS FOR THE ANALYSIS OF TECHNICAL DINOBUTON

	Sample, % *m/m*			
	Dinobuton content		Dinoseb content	
Laboratory	1	2	1	2
I	97.9	99.0	0.02	0.05
	98.3	99.0	0.02	0.05
II	99.1	98.5	0.01	0.04
	100.0	98.2	0.01	0.04
IV	99.1	97.8	—	—
	98.7	98.4	—	—
VI	97.5	98.7	0.01	0.05
	99.0	98.8	0.01	0.05
VIII	99.0	98.5	—	0.07
	99.2	98.6	—	0.06
X	97.2	98.1	0.02	0.04
	98.6	98.3	0.01	0.05
$\bar{x}$	98.6	98.5	0.014	0.05
n	12	12	8	10
r	1.89	0.58	—	—
R	2.27	1.10	—	—

APPENDIX I

Recommended Methods for the Determination of the Active-ingredient Content of DNOC, Dinoseb and Dinobuton Samples

DNOC

Outline of Method

The sample dissolved in xylene - light petroleum (or an extract in light petroleum with ammonium salts) is added to a column of neutral alumina. Impurities are eluted with chloroform and acetonitrile - propan-2-ol. The DNOC is eluted from the column as its butylammonium salt and determined spectrophotometrically at 414 nm.

Apparatus

Chromatographic columns with glass tubes 100 × 14 ± 1 mm i.d., fitted with PTFE taps (do not use columns with fritted glass discs), a spectrophotometer, wavelength range 340–480 nm, and separating funnels, 250-ml capacity, were used.

Reagents

All reagents should be of analytical-reagent grade except where otherwise specified.

Acetone.

Acetonitrile.

Alumina Woelm, neutral, grade I.

Butylamine solution. A 1% *V/V* solution in acetone - water (1 + 1) was freshly prepared.

Chloroform. This was free from hydrochloric acid and phosgene.

DNOC pure. Obtainable from the National Physical Laboratory. Standard solutions were prepared in solvent mixture (I) containing 0, 12.5, 25, 37.5, 50 and 75 μg ml^{-1} of DNOC.

Hydrochloric acid, 0.5 M.

Methanol. This was dry and free from aldehydes.

Light petroleum. Boiling-range 60–80 °C.

Propan-2-ol.

Solvent mixtures. These were as follows: (I) xylene - light petroleum (2 + 3); (II) acetonitrile - propan-2-ol (3 + 2) and (III) light petroleum - acetone (10 + 1).

Xylene.

Procedure

Insert a small plug of cotton-wool into the chromatographic column. Transfer 8 g of alumina to the column and tap down to a depth of 48–52 mm. Place a plug of cotton-wool on top of the alumina. Add solvent mixture (I) to the column and allow it to pass through until the solvent level is just above the cotton-wool plug. Use a separate column for each sample. The same column preparation is used for dinoseb and dinobuton analysis.

Preparation of calibration graph

Carry out the following procedure on each of the standard DNOC solutions. Pipette a 2-ml aliquot of the standard solution on to the chromatographic column. Allow the liquid to pass through the column until the level is just above the cotton-wool. Rinse the walls of the column three times using 1-ml aliquots of solvent mixture (I) and elute until the liquid is just above the cotton-wool each time. Elute the column with 20 ml of chloroform followed by 20 ml of solvent mixture (II) and discard the eluate. Elute the column with butylamine solution, collecting the eluate when the coloured DNOC band reaches a point about 10 mm above the cotton-wool support and collect a total volume of 10.0 ml. Measure the absorbance at 414 nm against the reagent blank. Plot a graph of absorbance *versus* DNOC in the eluate.

Analysis of samples

DNOC technical. Melt the sample by immersing the container in hot water (> 85 °C), mix thoroughly and take duplicate samples each containing the equivalent of 0.5 g of active ingredient. Treat each sample as follows: transfer into a 100-ml calibrated flask, dissolve in 50 ml of methanol and dilute to 100 ml with solvent mixture (I). Carry out sequential dilutions with solvent mixture (I) to obtain a solution containing approximately 50 μg ml^{-1} of DNOC. Pipette a 2-ml aliquot on to an alumina column and proceed as described under *Preparation of calibration graph.* Calculate the DNOC content by reference to the calibration graph.

DNOC - petroleum oil formulations. Mix thoroughly and take duplicate samples, each containing the equivalent of approximately 0.02 g of DNOC. Treat each sample as follows: transfer into a 100-ml calibrated flask, dissolve in 50 ml of methanol and dilute to 100 ml with solvent mixture (I). Carry out further sequential dilutions with solvent mixture (I) to obtain a solution containing approximately 20 μg ml^{-1} of DNOC. Transfer by pipette a 5-ml aliquot of this solution on to an alumina column and proceed as described under *Preparation of calibration graph.* Calculate the DNOC content by reference to the calibration graph.

DNOC ammonium salt formulations. Mix thoroughly and take duplicate samples, each containing the equivalent of approximately 0.4 g of DNOC. Treat each sample as follows: transfer into a 100 ml calibrated flask and dissolve in 100 ml of solvent mixture (I). Dilute an aliquot of this solution to 0.2 mg ml^{-1} with 0.5 M hydrochloric acid. Transfer by pipette 5 ml of this solution into a separating funnel, add 95 ml of 0.5 M hydrochloric acid and extract

by shaking with 25 ml of solvent mixture (III). Repeat the extraction using two further 25-ml aliquots of solvent mixture (III). Combine the extracts and dilute to 100 ml with light petroleum. Pipette 5 ml of this solution on to an alumina column and proceed as described under *Preparation of calibration graph*. Calculate the DNOC content by reference to the calibration graph.

Dinoseb

Outline of Method

The methods for dinoseb active-ingredient analysis are essentially the same as those described for DNOC.

Apparatus

As for DNOC analysis; in addition filter-paper, Whatman No. 52, is required.

Reagents

As for DNOC analysis.

Dinoseb pure. Obtainable from the National Physical Laboratory. Standard solutions were prepared in solvent mixture (I) containing 0, 12.5, 25, 37.5, 50 and 75 μg ml^{-1} of dinoseb.

Sodium chloride.

Procedure

The procedure previously described was again followed.

Preparation of calibration graph

Pipette 2-ml aliquots of each of the dinoseb standard solutions on to separate alumina columns and treat each column as described under *Preparation of calibration graph*. Measure the absorbance at 420 nm against the reagent blank. Plot a graph of absorbance *versus* micrograms of dinoseb in the eluate.

Analysis of samples

Dinoseb technical. Melt the sample by immersing the container in warm water (>40 °C), mix thoroughly and take duplicate samples, each containing the equivalent of 0.5 g of dinoseb. Treat each sample as follows. Transfer into a 100-ml calibrated flask and dissolve in 100 ml of solvent mixture (I). Carry out sequential dilutions with solvent mixture (I) to obtain a solution containing approximately 50 μg ml^{-1} of dinoseb. Pipette a 2-ml aliquot on to an alumina column and proceed as described under *Preparation of calibration graph*, measuring the absorbance at 420 nm. Calculate the dinoseb content by reference to the calibration graph.

Dinoseb - petroleum oil formulations. Mix thoroughly and take duplicate samples, each containing the equivalent of approximately 0.1 g of dinoseb. Treat each sample as follows: transfer into a 100-ml calibrated flask and dissolve in 100 ml of solvent mixture (I). Carry out further sequential dilutions with solvent mixture (I) to obtain a solution containing approximately 10 μg ml^{-1} of dinoseb. Transfer by pipette a 5-ml aliquot of this solution into an alumina column and proceed as described under *Preparation of calibration graph*, measuring the absorbance at 420 nm. Calculate the dinoseb content by reference to the calibration graph.

Dinoseb ammonium salt formulations. Mix thoroughly and take duplicate samples, each containing the equivalent of approximately 0.3 g of dinoseb. Treat each sample as follows: transfer into a 250-ml separating funnel containing 100 ml of 0.5 M hydrochloric acid that has been saturated with sodium chloride. Extract by shaking with 25 ml of solvent mixture (I) and filter. Repeat the extraction using two further 25-ml aliquots of solvent mixture (I). Combine the filtered extracts and dilute to 100 ml with solvent mixture (I), then carry out sequential dilutions with solvent mixture (I) to obtain a dinoseb content of approximately 30 μg ml^{-1}. Pipette 2 ml of this solution on to an alumina column and proceed as described under *Preparation of calibration graph*, measuring the absorbance at 420 nm. Calculate the dinoseb content by reference to the calibration graph.

Dinobuton Technical

Outline of Methods

Free dinoseb

Selectively adsorb the free dinoseb, from a solution of dinobuton technical in xylene, light petroleum and acetone, on a column of neutral alumina, grade V, which allows unchanged dinobuton to elute. Elute the dinoseb from the column as its butylammonium salt and determine spectrophotometrically at 420 nm.

Dinobuton content

Add the sample retained from free dinoseb determination to a column of neutral alumina, grade I, which de-esterifies the dinobuton to dinoseb. Elute the impurities with chloroform and acetonitrile - propan-2-ol, elute the dinoseb from the column as its butylammonium salt and determine spectrophotometrically at 420 nm.

Apparatus

As for DNOC analysis.

Reagents

As for DNOC analysis.

Alumina Woelm, neutral, grade V.

Solvent mixture (IV). Solvent mixture I - acetone (9 + 1).

Dinobuton pure. Obtainable from the National Physical Laboratory. Standard solutions were prepared in solvent mixture (I) containing 0, 25, 50, 75 and 100 μg ml^{-1} of dinobuton.

Dinoseb pure. Standard solutions were prepared in solvent mixture (I) containing 0, 5, 10, 15 and 20 μg ml^{-1} of dinoseb.

Procedure

An alumina column (grade V) is prepared as described for DNOC, but using grade V alumina in place of grade I and solvent mixture (IV) in place of solvent mixture (I).

Preparation of calibration graphs

Free dinoseb. Pipette 5-ml aliquots of the standard dinoseb solutions on to separate alumina columns (grade V) and treat each column as described under *Preparation of calibration graph* but using solvent mixture (IV) in place of solvent mixture (I). Measure the absorbance at 420 nm against the reagent blank. Plot a graph of absorbance *versus* micrograms of dinoseb in the eluate.

Dinobuton. Pipette 2-ml aliquots of the standard dinobuton solutions on to separate alumina columns (grade I) and treat each column as described under *Preparation of calibration graph.* Measure the absorbance at 420 nm against the reagent blank. Plot a graph of absorbance *versus* micrograms of dinobuton in the eluate.

Analysis of samples.

Free dinoseb content. Melt the sample by immersing the container in hot water (> 60 °C), mix thoroughly and take duplicate samples, each containing the equivalent of 0.5 g of dinobuton. Treat each sample as follows. Transfer into a 100-ml calibrated flask and dissolve in 100 ml of solvent mixture (IV). Transfer by pipette a 10-ml aliquot to an alumina column containing grade V alumina prepared as described above. Elute until the liquid level is just above the cotton-wool. Rinse the walls of the column three times using 1-ml aliquots of solvent mixture (IV), allowing the level of the liquid to fall to just above the cotton-wool each time. Immediately elute with solvent mixture (IV), collecting a total of 100 ml of eluate in a calibrated flask. Retain the solution for subsequent dinobuton analysis. Elute the column with 20 ml of chloroform, then elute with butylamine solution, collecting the eluate when the coloured dinoseb band reaches a point about 10 mm above the cotton-wool support, and collect a total volume of 10.0 ml. Measure the absorbance at 420 nm against the reagent blank. Calculate the dinoseb content by reference to the calibration graph.

Dinobuton content. Dilute a 10-ml aliquot of the solution retained from *Free dinoseb content* to 100 ml with solvent mixture (I). Pipette a 2-ml aliquot on to an alumina column (grade I) and proceed as described under *Preparation of calibration graph*, measuring the absorbance at 420 nm. Calculate the dinobuton content by reference to the calibration graph.

APPENDIX II

Membership of the Panel

The following laboratories, represented by the workers named, contributed to the work of the Panel: Arbitral Central de Ensayos y Analisis Agricolas, Madrid, Spain (Miss P. Hitos); Estacion Experimental Del Zaidin, Granada, Spain (Dr. F. Sanchez-Rasero); FBC Ltd. (M. Brown); Harpenden Laboratory (J. F. Lovett); Hoechst AG, Frankfurt, Federal Republic of Germany (Dr. J. Asshauer); Laboratorio Agrario Cordova, Spain (M. Magallanes); Laboratorio Agrario Regional del Centro, Madrid, Spain (E. Celma-Calamita); Laboratory of the Government Chemist (D. S. Farrington); A. H. Marks Co. Ltd. (S. Greaves); Murphy Chemical Ltd. (V. P. Lynch, Chairman); Pennwalt, The Netherlands (P. Bank).

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Spectrophotometric Determination of Phosphate in River Waters with Molybdate and Malachite Green

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On the basis of the coloration formed with molybdate and malachite green in aqueous solution, trace amounts of phosphate were determined. The molar absorptivity was 7.8×10^4 l mol^{-1} cm^{-1} at 650 nm. The absorbance of the reagent blank was about 0.02, and its relative standard deviation was less than 10%. The recommended concentration range of phosphorus was 0.1–5 μg and the limit of detection was 0.01 μg of phosphorus. The sample solution was acidified with sulphuric acid and heated in a water-bath above 90 °C for 40 min, and subsequently it was coloured with molybdate and malachite green. The colour was stabilised by adding poly(vinyl alcohol). The method was applied to the determination of parts per billion (10^9) amounts of phosphorus in river and tap waters; the relative standard deviation was less than 4% and the recovery was 95–101%.

Keywords: Spectrophotometry; phosphate determination; molybdate; malachite green; river waters

Most of the procedures for the spectrophotometric determination of inorganic phosphate are based on the formation of heteropolyacids such as molybdophosphate and vanadomolybdophosphate in an acidic medium. The heteropolyacid of phosphate formed is used as a light-absorbing species either as it is, after reduction to a heteropoly blue species or after extraction into an organic solvent as a protonated species or as an ion pair with a bulky cation. Recently, we reported the extraction of molydophosphate with ethyl violet and a sensitive spectrophotometric method for the determination of phosphate (the molar absorptivity was 2.7×10^5 l mol^{-1} cm^{-1}).[1] Although the method is very sensitive, it is troublesome in routine work because of the necessity for a solvent extraction. Itaya and Ui[2] reported a simple and sensitive spectrophotometric method for the determination of phosphate in serum in acidic medium with malachite green; the method was subsequently modified and applied to the determination of phosphate in serum, plasma and urine.[3–10] Altmann *et al.*[11] reported the mechanism of the colorimetric reaction between molybdophosphate and malachite green, and the possibility of the determination of low levels of phosphate in natural waters. Fogg *et al.*[12] used crystal violet instead of malachite green; this method seems to be less satisfactory because it requires heating of the solution and is time consuming.

In this work, we modified the method with malachite green reported by Itaya and Ui[2] and Altmann *et al.*[11] and applied it to the determination of trace amounts of phosphorus in water.

Experimental

Apparatus

Absorption measurements were made on a Hitachi Perkin-Elmer, Model 139, spectrophotometer in a glass cell of 20-mm path length.

Reagents

Malachite green (MG) solution, 2×10^{-3} M. Commercially available malachite green (oxalate), 0.91 g, was dissolved in distilled water to give 1 l of solution.

Molybdate solution, 0.68 M. Ammonium molybdate, $(NH_4)_6(Mo_7O_{24}).4H_2O$ (Nakarai Chemicals) (120 g) was dissolved in distilled water to give 1 l of solution.

Standard phosphate solution. Potassium dihydrogen orthophosphate was dried at reduced pressure (about 5 mmHg) at 60 °C to constant mass. The dried compound (0.2722 g) was dissolved in distilled water to give 1 l of solution (2×10^{-3} M). For calibration purposes, the solution was diluted to 4×10^{-5} M with distilled water before use; the diluted solution contains 1.24 μg ml^{-1} of phosphorus and 0–4 ml of this solution were used.

Poly(vinyl alcohol) (PVA) solution. Commercially available PVA (number-average degree of polymerisation 500) (1 g) was dissolved in 100 ml of hot water and was used after filtration through filter-paper.

Sulphuric acid. Commercially available concentrated sulphuric acid (97%, 18.2 M) was used.

Preparation of Reagent Solution

The reagent solution was prepared by mixing 300 ml of 0.68 M molybdate solution, 47 ml of a concentrated sulphuric acid and 250 ml of 2×10^{-3} M malachite green solution. About 30 min after mixing, the solution was filtered through a membrane filter (pore size 0.45 μm). The filtrate was as the reagent solution. The optimum composition of the reagent solution was established from preliminary results obtained with the proposed method.

Results and Discussion

Selection of Dye

Belle[13] recommended methyl green instead of malachite green owing to its higher solubility, lower concentration and lesser attachment to the vessel. In this work, we examined several cationic dyes: ethyl violet, crystal violet, malachite green, methyl violet, Victoria blue 4R, methyl green, methylene blue and brilliant green with no addition of PVA. With ethyl violet, methyl violet, crystal violet, Victoria blue 4R and methylene blue, coloured precipitates were easily formed. With brilliant green, the colour faded gradually. With methyl green, no precipitation occurred, but the coloration was less than that with malachite green, and faded gradually. With malachite green, a precipitate was formed with difficulty and the coloration was the best of all dyes examined. Malachite green was therefore selected as the colour reagent.

Effect of Acidity

When the dye solution was acidified, the monovalent malachite green cation, which shows an absorption maximum at 620 nm, changes slowly to a dark yellow species, which is probably protonated. If the acidified malachite green solution is converted into a neutral solution or molybdophosphate is present, a maximum absorption occurs at 620 or 650 nm. Altmann *et al.*[11] and Fogg *et al.*[12] used solutions of malachite green dissolved in aqueous PVA solution and of crystal violet in water, respectively. In those procedures, the absorbance measurements must be made 30–60 min after the addition of the dye solutions, because a longer time is necessary for monovalent dye cations to change to protonated species. In this work, to reduce the time required, malachite green solution previously acidified with sulphuric acid was used.

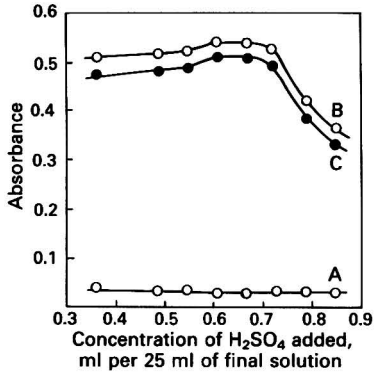

Fig. 1. Effect of sulphuric acid concentration on absorbance. A, Reagent blank (reference water); B and C, with 2.48 μg of phosphorus (B, reference water and C, reference reagent blank).

The acidity of the solution also affects the formation of molybdophosphate and is therefore very important in such a colour reaction. The effect of sulphuric acid was examined by varying the content of acid in the reagent solution. The results are shown in Fig. 1. The largest, constant absorbance was obtained in the range 0.6–0.67 ml of concentrated sulphuric acid per 25 ml of the final solution. In this work, the total amount of the concentrated sulphuric acid contained in 25 ml of the final solution was fixed at 0.65 ml; 0.24 ml were supplied by 3 ml of the reagent solution and 0.41 ml by 1 ml of 7.5 M sulphuric acid, which was added to hydrolyse the condensed phosphates.

Effect of Amount of Molybdate

Fig. 2 shows the effect of molybdate. In the range 0.03–0.045 M in the final solution, the absorbance of the reagent blank is relatively small and the coloration is at a maximum. In this work, the final concentration of molybdate was adjusted to about 0.04 M, that is, the concentration in the reagent solution was fixed at 0.34 M.

Effect of Amount of Malachite Green

Fig. 3 shows the effect of malachite green. At levels above 8×10^{-5} M in the final solution, the highest, constant absorbance was obtained. In this work, the final concentration of malachite green was adjusted to 10^{-4} M, that is, the concentration in the reagent solution was fixed at 8.4×10^{-4} M.

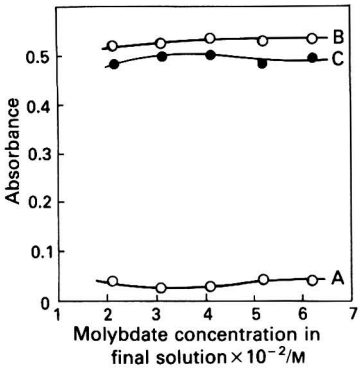

Fig. 2. Effect of molybdate concentration on absorbance. A, Reagent blank (reference water); B and C, with 2.48 μg of phosphorus (B, reference water and C, reference reagent blank).

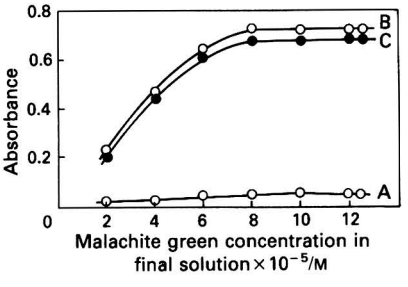

Fig. 3. Effect of malachite green concentration on absorbance. A, Reagent blank (reference water); B and C, 3.72 μg of phosphorus (B, reference water and C, reference reagent blank).

Effect of Poly(vinyl Alcohol) on the Stabilisation of the Coloration

The effect of PVA was examined and the results are shown in Fig. 4. Without PVA the colour fades gradually, whereas on addition of PVA before adding the reagent solution the colour is developed gradually. When the PVA was added after adding the reagent solution, the absorbances of the reagent blank and the phosphate were almost constant for at least 1 d, although the absorbances of the phosphate were lower than those obtained by the addition of PVA before adding the reagent solution. In this work, to reduce the time required and to improve the reproducibility of the absorbance, PVA was transferred into the calibrated flask after adding the reagent solution and mixing.

Comparison of Sulphuric acid with Hydrochloric acid

Sulphuric and hydrochloric acids were compared, the molar concentration of hydrochloric acid being adjusted to twice that of sulphuric acid. On addition of PVA, the constancy of the absorbance of 2.48 μg of phosphorus against the reagent blank in sulphuric acid medium is almost the same as that in hydrochloric acid medium, and the differences in the absorbances of 2.48 μg of phosphorus against the reagent blank during 2 h were less than 0.01 absorbance

unit in each medium. Without PVA, however, the absorbance in hydrochloric acid medium decreases more rapidly than that in sulphuric acid medium, and the decreases in the absorbance of 2.48 μg of phosphorus against the reagent blank during 2 h were 0.17 and 0.21 absorbance unit in the sulphuric acid and hydrochloric acid media, respectively.

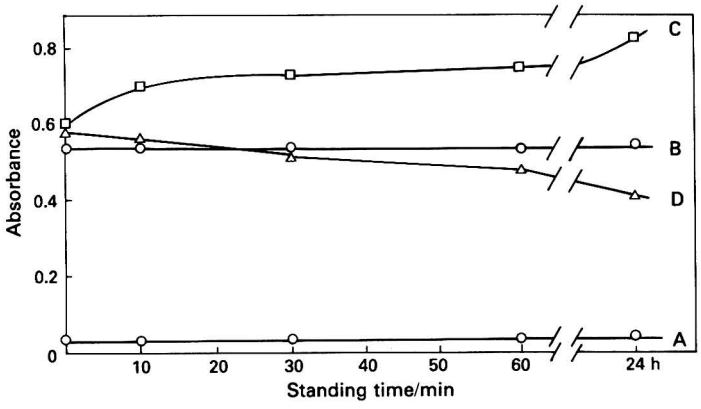

Fig. 4. Effects of poly(vinyl alcohol) and standing time on absorbance. A and B, poly(vinyl alcohol) (PVA) was added after the addition of the reagent solutions: A, reagent blank; and B, with 2.48 μg of phosphorus. C, PVA was added before the addition of the reagent solution, with 2.48 μg of phosphorus; and D, without PVA, but with 2.48 μg of phosphorus. Reference, reagent blank.

Hydrolysis of Condensed Phosphate to Orthophosphate

Only orthophosphates react with molybdate to form molybdophosphate, but condensed phosphate probably exists in river waters. The hydrolysis of several condensed phosphates to orthophosphates was examined and the results obtained are shown in Fig. 5. The four kinds of condensed phosphates examined were completely hydrolysed to orthophosphate in sulphuric acid medium (0.35 M) by heating at about 90 °C in a water-bath for about 30 min. Therefore, in the determination of total inorganic phosphate, the sample solution must be acidified and heated.

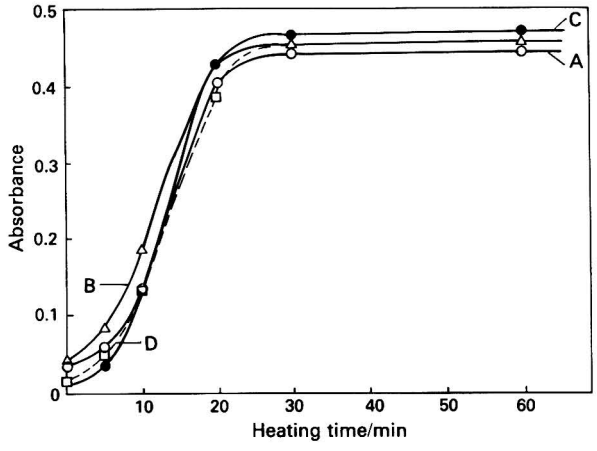

Fig. 5. Effect of heating time on hydrolysis of condensed phosphate. A, Pyrophosphate ($Na_4P_2O_7.10H_2O$), 17.8 μg; B, tripolyphosphate ($Na_5P_3O_{10}$), 9.76 μg; C, metaphosphate [$(NaPO_3)_n$, $n > 3$], 8.16 μg; D, polyphosphate (sodium salt), 7.68 μg. Sulphuric acid, 0.35 M at 90 °C.

Recommended Procedure for the Determination of Total Inorganic Phosphate in Water

After sampling a test water in a glass bottle, acidify it with sulphuric acid to pH 2–3 and, if necessary, filter it through a membrane filter (pore size 0.45 μm). Transfer up to 20 ml of the acidified sample solution, containing up to 5 μg of phosphorus, into a 25-ml calibrated flask and dilute to 20 ml with distilled water. Add 1 ml of 7.5 M sulphuric acid and heat above 90 °C for 40 min. Cool the flask to the room temperature and add 3 ml of the reagent solution. Within 2 min of mixing the sample and reagent solution, add 0.5 ml of PVA solution, dilute to the mark with water and mix. Measure the absorbance at 650 nm within 2 h. When only orthophosphate is to be determined, the procedure without heating is required.

Absorption Spectra and Calibration Graph

Fig. 6 shows the absorption spectra. The maximum absorption wavelength is 650 nm, and at this wavelength the calibration graph was linear for amounts of phosphorus from 0 to 5 μg. The molar absorptivity was 7.8×10^4 l mol^{-1} cm^{-1}.

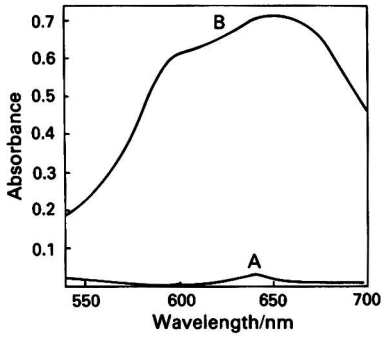

Fig. 6. Absorption spectra. A, Reagent blank (reference water); B with 3.72 μg of phosphorus (reference water); glass cells of 20-mm path length.

Effect of Diverse Ions

The interference of diverse ions was examined and the results obtained are shown in Table I. The amounts of ions, except silicate, generally present in river water are much smaller than those listed in Table I. Silicate ion at concentrations above 5×10^{-5} M reacts gradually with molybdate to form heteropolyacid and this results in positive errors, but large amounts of silicate ion were eliminated by acidifying the sample solution and filtering it through a membrane filter (pore size 0.45 μm).

TABLE I

EFFECT OF DIVERSE IONS

Phosphorus taken: 3.72 μg.

Ion	Tolerance limit*
HCO_3^-	6 mg
Al^{3+}	3 mg
Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Ca^{2+}, K^+, NO_3^-	1 mg
Mg^{2+}, Na^+, NH_4^+	0.5 mg
Si(IV)	15 μg
ClO_4^-	5 μg
W(VI)	4 μg
As(V)	2 μg
V(V)	1 μg

* The tolerance limit is defined as the concentration level at which the interference causes an error of not more than $\pm 2\%$.

TABLE II

STANDARD DEVIATIONS (SD) AND RELATIVE STANDARD DEVIATIONS (RSD) FOR THE REAGENT BLANK AND FOR THE DETERMINATION OF PHOSPHORUS

Sample	Parameter	Time after preparation of reagent solution: 1 h	1 d	2 d	4 d
Reagent blank	$\bar{x}$*	0.022 ± 0.004	0.023 ± 0.002	0.022 ± 0.004	0.021 ± 0.001
	SD	0.0023	0.0012	0.0017	0.0008
	RSD, %	10.4	5.1	7.6	3.7
Phosphorus (1.55 μg) ..	$\bar{x}$†	0.330 ± 0.009	0.327 ± 0.006	0.327 ± 0.006	0.320 ± 0.005
	SD	0.0036	0.0036	0.0027	0.0026
	RSD, %	1.1	1.1	0.8	0.8

* Average of the absorbances of the reagent blanks in 10 experiments. Reference: water.
† Average of the absorbances of the phosphorus (1.55 μg) in 10 experiments. Reference: reagent blank.

Determination of Phosphorus in Waters

The precision of the method was evaluated by the recommended procedure without pre-treatment, by determining the same amounts (1.55 μg) of phosphorus in ten experiments. The results obtained are shown in Table II. The standard deviation of the absorbance of the reagent blank is less than 0.002 absorbance unit, which corresponds to 0.009 μg of phosphorus. The standard deviation and relative standard deviation for 1.55 μg phosphorus are less than 0.005 absorbance unit and 1.1%, respectively.

Table III gives the results for the standard deviation and relative standard deviation in ten experiments on real water samples. The relative standard deviations are less than 4%. The results of recovery tests in Table IV show that the recoveries ranged between 95.0 and 101.3%.

TABLE III

STANDARD DEVIATIONS (SD) AND RELATIVE STANDARD DEVIATIONS (RSD) FOR THE DETERMINATION OF PHOSPHORUS IN WATERS

Phosphorus was determined with pre-treatment.

	Water sample: Tap water*	Asahi River†	Zasu River†
Volume taken/ml	20	20	10
Average absorbance‡	0.039 ± 0.002	0.052 ± 0.002	0.389 ± 0.006
Average phosphorus content, p.p.b.§	9.5 ± 0.4	12.6 ± 0.5	188.3 ± 2.3
SD	0.35	0.42	4.6
RSD, %	3.7	3.3	2.4

* Sampled on August 24th, 1982.
† Sampled on August 21st, 1982.
‡ Reference: reagent blank.
§ From 10 experiments.

TABLE IV

RECOVERY TEST

Sample waters as in Table III. Phosphorus was determined with pre-treatment.

	Water sample: Tap water	Asahi River	Zasu River
Volume taken/ml	17	17	10
Phosphorus added/μg*	0.078	0.078	0.78
Absorbance†	0.192 ± 0.002	0.205 ± 0.002	0.539 ± 0.003
Recovery, %‡	100.3	101.2	95.0

* Potassium dihydrogen orthophosphate was added.
† Reference: reagent blank.
‡ Average of five experiments.

By using the recommended procedure with pre-treatment or without heating, phosphorus was determined in river waters. The results obtained are shown in Table V. There are some differences between the phosphorus results obtained with and without heating. Some part of these differences can be attributed to the condensed phosphate and the remainder to organophosphate compounds, especially esters.

TABLE V

DETERMINATION OF PHOSPHORUS IN RIVER WATERS

Sample taken: 20 ml.

Water sample*		Phosphorus, p.p.b.
Asahi River	A	35.1 ± 0.3†
		37.5
	B	14.8 ± 1.4
		24.6
	C	10.6 ± 0.2†
		17.1
Yoshii River	A	27.2 ± 0.5†
		33.0
	B	21.9 ± 0.5†
	C	32.2 ± 0.8†
	D	29.2 ± 0.3†
		32.8
Takahashi River	A	28.6 ± 0.5†
		30.8
	B	14.9 ± 1.4†
	C	22.4 ± 0.5†
	D	13.2 ± 0.1†

* Asahi, Yoshii and Takahashi Rivers are the three largest rivers in Okayama Prefecture. The waters were sampled on September 10th, 1981. Symbols A–C and A–D denote order of sampling downstream.

† Values obtained without heating; average of three experiments.

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Spectrophotometric and Fluorimetric Determination of Boron in Soils, Plants and Water by Extraction with 2-Methylpentane-2,4-diol in Isobutyl Methyl Ketone

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Two methods for the determination of boron, by molecular absorption spectrophotometry with curcumin and by molecular fluorescence with dibenzoylmethane, after extraction of boron into isobutyl methyl ketone (IBMK) with 2-methylpentane-2,4-diol, are proposed. The development of the colour or the fluorescence is carried out in the organic phase used for extraction by addition of curcumin in glacial acetic acid or dibenzoylmethane in IBMK and phosphoric acid as dehydrating agent. The different conditions for both spectrophotometric and fluorimetric methods have been established. A study has been made of the influence in aqueous solution of several ions as potential interferents. The spectrophotometric method has been applied to the determination of boron in soils and plants and the fluorimetric method to plants and natural waters.

Keywords: *Boron determination*; *2-methylpentane-2,4-diol extraction*; *spectrophotometry*; *fluorimetry*

There is increasing interest in the determination of boron in several fields, such as in geochemistry[1] and as an oligoelement in the physiology of plants,[2] in the nuclear industry owing to the large cross-section neutron capture of boron and in metallurgy owing to the increase in the hardenability of steels on addition of boron. The determination of boric acid, *e.g.*, as a contaminant in foodstuffs,[3] is also common.

Suitable methods for the determination of boron at the microgram level are atomic-absorption spectrophotometry,[4,5] inductively coupled plasma emission spectroscopy,[6] nuclear activation,[7] flame photometry,[8–10] molecular-absorption spectrophotometry with curcumin,[11] carminic acid[12] and azomethine-H[13] and molecular fluorescence with Thoron,[14] 2-hydroxyphenone,[15] quinalizarin,[16] morin[17] and dibenzoylmethane.[18] The fluorescence methods show the greatest sensitivity, allowing the determination of boron at the nanogram level. However, the above methods are not highly selective, it being necessary to eliminate numerous interferents by separation of boric acid by distillation of methyl borate ester,[19] by use of ion-exchange resins[20] or by extraction.[4,8,9] In addition, it is necessary to prevent the contamination of boron from laboratory glassware, especially in operations of long duration or at temperatures higher than ambient. A further difficulty in some methods is the need to work in corrosive media such as concentrated sulphuric acid or glacial acetic acid.

The selectivity of the extraction of boric acid with 2-methylpentane-2,4-diol (MPD) into isobutyl methyl ketone (IBMK) previously[4,8] provides a pre-concentration method and the simultaneous elimination of numerous interferents. In this work the determination of boron was carried out in the organic phase used for extraction by absorption spectrophotometry with curcumin or by fluorimetry with dibenzoylmethane (DBM). Chromotropic acid, quinalizarin and 1,1′-dianthrimide have also been studied as boron spectrophotometric reagents, but they show low solubility in IBMK or their boron compounds have low molar absorptivities.

Experimental

Apparatus

The apparatus used was a Pye-Unicam SP8 100 spectrophotometer, with special equipment for fluorescence measurements, an Orion Research Microprocessor Ionanalizer/901 for pH measurements, a Haake thermostatic bath and a Kötterman mechanical shaker.

Glass materials must be avoided in order to eliminate boron contamination, so PTFE, polyethylene or platinum materials were used.

Reagents

All solutions were prepared with analytical-reagent grade chemicals (Merck), using doubly distilled water.

Boron stock standard solution, 1000 μg ml^{-1}. Prepared by dissolving 2.8600 g of dried boric acid in 500 ml of water.

Boron working standard solutions. Prepared by diluting the stock standard solution just before use.

Extraction solution, 20% V/V MPD in IBMK. Stored in a polyethylene bottle.

Curcumin solution, 0.1% m/V in glacial acetic acid. Prepared just before use.

Dibenzoylmethane solution, 0.1% m/V in IBMK.

Quinine sulphate solution, 0.05% m/V in 0.1 M *sulphuric acid.*

Procedure

Soil sample solution

Weigh exactly 0.2–1 g of finely ground soil sample, depending on boron content, and digest it with 5 ml of concentrated nitric acid - perchloric acid (3 + 1) in a PTFE-lined pressure pump at 150 °C for 2 h (FAO recommendation). Cool, dilute and filter off any residue through Albet 242 filter-paper. Neutralise with 6 M sodium hydroxide solution and dilute to 100 ml with hydrochloric acid (1 + 1) in a calibrated flask.

Plant sample solution

Weigh exactly 0.2–0.3 g of plant sample, after pulverising and drying it at 60 °C, in a platinum crucible. Ash the sample in a muffle furnace at 500 °C for 4 h and leave it to cool inside the furnace. Add 10 ml of hydrochloric acid (1 + 1) and heat the crucible carefully at 80 °C on a hot-plate for 1 h. Cool and transfer with hydrochloric acid (1 + 1) into a 100-ml polyethylene separating funnel.

Natural water samples

Dilute 50 ml of natural water with hydrochloric acid (1 + 1) to 100 ml in a calibrated flask.

Extraction Procedure

Place a measured volume of sample solution (less than 50 ml) containing 10–100 μg of boron (spectrophotometric method) or 0.5–5 μg of boron (fluorimetric method) in a separating funnel. Extract three times with 10-ml volumes of IBMK in order to eliminate iron interference. Add 10 ml of extraction solution and shake for about 5 min with a mechanical shaker. Dry the organic phase with about 1 g of anhydrous sodium sulphate.

Spectrophotometric Procedure

Pipette 3 ml of the organic phase into a polyethylene test-tube with a hermetic cap and add 2 ml of curcumin solution and 2 ml of concentrated phosphoric acid. Shake the sealed test-tube for 2 min and heat at 70 $\pm$ 3 °C for 1 h in a thermostated bath. After rapid external cooling to room temperature, measure the absorbance of the solution at 510 nm against a reagent blank solution within 45 min.

Prepare a calibration graph as follows. To different volumes of standard solution containing 10–100 μg of boron add an equal volume of hydrochloric acid (1 + 1) and extract with 10 ml of extraction solution according to the above extraction procedure. Pipette 3 ml of the organic phase and carry out the same spectrophotometric procedure.

Fluorimetric Procedure

Pipette 3 ml of the organic extraction phase into a polyethylene test-tube with a hermetic cap and add 2 ml of DBM solution and 2 ml of concentrated phosphoric acid. Shake the sealed test-tube for 2 min and heat at 80 $\pm$ 3 °C for 30 min in a thermostated bath. After rapid external cooling to room temperature, measure the relative fluorescence intensity of the solution at 400 nm or by using a Kodak 2B cut-off filter, with excitation at 390 nm and quinine sulphate solution as reference, within 45 min.

Prepare a calibration graph as follows. To different volumes of standard solution containing 0.5–5 μg of boron, add an equal volume of hydrochloric acid (1 + 1) and extract with 10 ml of extraction solution according to the above extraction procedure. Pipette 3 ml of the organic phase and carry out the same fluorimetric procedure.

Results and Discussion

Boron Extraction Characteristics

The extraction of boron by MPD into IBMK was studied in our previous work.[4,8] It has been found that the extraction is quantitative at the microgram level (boron extraction yields higher than 95%) using 1.8–2.5 M sulphuric acid. However, in the presence of alkaline earth metal cations, low results for boron have been obtained owing to adsorption by the precipitate formed. Boron extraction can also be carried out quantitatively using 2.4–7.2 M hydrochloric acid. This extraction procedure has the inconvenience of the simultaneous extraction of oxonium chloro complexes of Fe(III), Sb(III), Sb(V) and Au(III), which interfere in the boron determinations. This difficulty has been overcome by washing the aqueous phase before boron extraction with IBMK, without any appreciable loss of boric acid.

Spectrophotometric Characteristics

The absorption spectrum of the boron - curcumin compound in IBMK obtained following the above procedure exhibits maximum absorbance at 510 nm when measured against a reagent blank solution, as shown in Fig. 1. The absorbance at 510 nm remained constant for at least 45 min after rapid external cooling to room temperature.

The effects of warming time, temperature, reagent concentration and phosphoric acid to organic phase ratio were also studied and optimised.

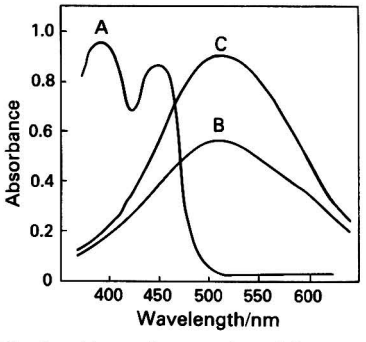

Fig. 1. Absorption spectra of A, reagent blank solution measured against IBMK as reference; B, boron - curcumin compound against reagent blank, 3 μg ml^{-1} of boron; and C, as B, 5 μg ml^{-1} of boron.

Calibration graph, sensitivity and precision

The calibration graph at 510 nm is a straight line and Beer's law is obeyed from 0.5 to 5 μg ml^{-1} of boron in the final measured solution (corresponding to 10–110 μg of boron in the aqueous phase). The molar absorptivity, calculated from the slope of the statistical working calibration graph at 510 nm, was 2905 l mol^{-1} cm^{-1}. The Sandell sensitivity was 0.011 μg cm^{2} of boron. The precision of the method for ten replicate determinations was 0.6%. The absorbance of the reagent blank solution at 510 nm was 0.010 ± 0.003 for ten replicate determinations. Therefore, the detection limit was 0.04 μg ml^{-1} of boron in the final measured solution.

Interference studies

The effect of those ions most frequently present in soils and plant ashes on the boron determinations is shown in Table I. The interference of iron at concentrations higher than

TABLE I

EFFECT OF FOREIGN IONS ON BORON DETERMINATION

Determination of 58.3 μg of boron by the spectrophotometric method and 2.38 μg of boron by the fluorimetric method.

Foreign ion	Maximum concentration tested without giving interference/M	
	Spectrophotometric method	Fluorimetric method
Cl^-	7.2	7.2
SO_4^{2-}	2.5	2.5
NH_4^+	1.0	0.8
Na^+	0.2	0.2
K^+, Ca^{2+}, Al^{3+}	0.5	0.4
NO_3^-, NO_2^-, Cr^{3+}	0.2	0.2
Mg^{2+}, HCO_3^-, CO_3^{2-}	0.05	0.05
Mn^{2+}	0.05	0.01
F^-	0.02	0.01
Cu^{2+}, Zn^{2+}, Ni^{2+}	4×10^{-3}	4×10^{-3}
Sr^{2+}, PO_4^{3-}, SiO_3^{2-}, Br^-	3×10^{-3}	3×10^{-3}
Fe^{3+}	7×10^{-5}*	7×10^{-5}*
Fe^{3+} (by elimination with three 10-ml IBMK washes)	0.1	0.1

* Tolerance limit (M) as the concentration level at which the interferent causes an error of not more than ±2% (spectrophotometric method) or ±3% (fluorimetric method).

7×10^{-5} M can be eliminated as the chloro complex by extraction with IBMK. The total elimination of Fe(III) was not necessary as the phosphoric acid masked the residual Fe(III) in the boric acid - curcumin reaction.

Fluorimetric Characteristics

The fluorescent excitation spectrum of the boric acid - DBM compound in IBMK against quinine sulphate solution is shown in Fig. 2. The wavelength of the maximum excitation radiation was 390 nm. The maximum relative fluorescence intensity was measured at 400 nm or by using a Kodak 2B cut-off filter (400 nm cut-off).

The effects of heating time, temperature, reagent concentration, phosphoric acid to organic phase ratio and stability were studied and optimised.

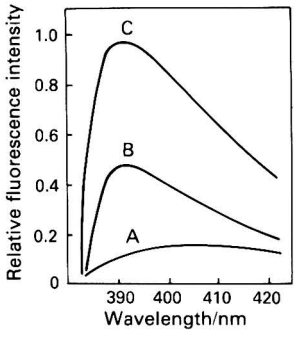

Fig. 2. Fluorescence excitation spectra against quinine sulphate solution as reference: A, reagent blank solution; B, boron - DBM, 50 μg l^{-1} of boron; and C, boron - DBM, 100 μg l^{-1} of boron.

Calibration graph, detection limit and precision

The calibration graph was linear in the range 0.5–5 μg of boron in aqueous solution (20–200 μg l^{-1} of boron in the final solution to be measured). The detection limit and precision were 1 μg l^{-1} of boron and 3% for ten replicate determinations of 1.2 μg of boron, respectively.

Interference studies

The selectivity of the proposed fluorimetric method was studied on the determination of 2.38 μg of boron, as shown in Table I.

TABLE II

DETERMINATION OF BORON IN SOILS BY THE SPECTROPHOTOMETRIC METHOD

Soil No.	Source	Mean boron content ± standard deviation*/μg g^{-1}	Boron added to spiked samples/μg	Mean recovery,† %
1	El Bayo (H_1)	249 ± 3.4	250	97.2
2	El Bayo (H_2)	181 ± 1.3	200	104.0
3	El Bayo (H_3)	260 ± 2.4	250	101.6
4	El Bayo (H_4)	288 ± 1.0	300	102.7
5	El Espinal (H_1)	78 ± 0.6	80	98.8
6	Aula Dei (H_1)	165 ± 0.5	150	100.7
7	Villamayor (H_1)	79 ± 0.3	80	98.8
8	Villamayor (H_2)	94 ± 0.4	100	99.0

* Eight determinations.
† Three determinations.

Determination of Boron in Natural Samples

The results of boron determinations in soils by the spectrophotometric method are shown in Table II. Soil samples were provided by Aula Dei, Experimental Station of the Consejo Superior de Investigaciones Cientificas (CSIC), and correspond to alluvial ground or soils with a high limestone content (35–45% of calcium carbonate).

The results of boron determinations in plant samples by both the spectrophotometric and fluorimetric methods are shown in Table III. The plant samples were analysed by the Foliar Analysis Inter-Institutes Committee and were kindly supplied by M. Pinta (Office de la Recherche Scientifique et Technique Outre-Mer).

TABLE III

DETERMINATION OF BORON IN PLANT SAMPLES

Plant	Spectrophotometric method: Mean boron content ± standard deviation*/μg g^{-1}	Spectrophotometric method: Mean recovery,† %	Fluorimetric method: Mean boron content ± standard deviation*/μg g^{-1}	Fluorimetric method: Mean recovery,‡ %
Hevea	60.1 ± 0.4	99.9	58.53 ± 1.11	97.6
Phoenix dactylibera	15.6 ± 0.2	100.0	15.65 ± 0.26	98.4
Eucaliptus globulus	35.5 ± 0.2	98.3	35.73 ± 0.43	100.3
Vitis vinifera	46.5 ± 0.4	99.2	50.45 ± 0.48	99.7
Citrus sinensis	36.2 ± 0.3	100.0	38.15 ± 0.41	100.4
Olea europea	19.6 ± 0.4	100.5	18.81 ± 0.23	98.7
Prunnus perzica	37.1 ± 0.2	98.5	38.02 ± 0.63	98.1
Codia	26.0 ± 0.3	99.3	26.02 ± 0.44	103.0
Zea mays	21.2 ± 0.2	99.5	23.31 ± 0.47	99.2
Malus communis (Cox)	30.6 ± 0.2	100.7	31.28 ± 0.33	100.1
Malus communis (Golden)	28.0 ± 0.3	99.7	26.25 ± 0.23	99.8
Gossypium herbaceum	23.2 ± 0.4	100.0	25.26 ± 0.31	98.7

* Ten determinations.
† Boron added to spiked samples, 20 μg. Three determinations.
‡ Boron added to spiked samples, 2.5 μg. Three determinations.

TABLE IV

DETERMINATION OF BORON IN NATURAL WATERS BY THE FLUORIMETRIC METHOD

Origin	Mean boron content ± standard deviation*/$\mu g\ l^{-1}$	Mean recovery,† %
Guadalope–1	200 ± 3	98.2
Guadalope–2	182 ± 3	97.5
Guadalope–3	132 ± 2	99.3
Acequia–1	143 ± 2	99.7
Acequia–2	124 ± 2	100.2
Acequia–3	151 ± 2	100.8
Guadalopillo–1	253 ± 5	101.0
Guadalopillo–2	237 ± 3	99.9
Guadalopillo–3	186 ± 4	99.8
Guadalopillo–4	157 ± 2	100.6
Martin–1	255 ± 3	98.6
Martin–2	152 ± 2	97.7
Martin–3	208 ± 3	97.8
Martin–4	249 ± 2	99.9
Pozo	201 ± 3	99.6

* Ten determinations.
† Boron added to spiked samples, 2.0 μg. Three determinations.

The results for boron determinations in natural waters from the province of Teruel (Spain) are shown in Table IV.

In order to detect any losses of boron, the standard additions method was also used. The boron recovery obtained for determinations on three replicate spiked solutions are shown in Tables I, II, III and IV for both the spectrophotometric and fluorimetric methods.

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Monoethanolamine as an Absorbing Reagent for the Spectrophotometric Determination of Atmospheric Sulphur Dioxide

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The possible use of monoethanolamine as an absorbing reagent for sulphur dioxide has been investigated. The proposed reagent has an absorption efficiency of about 100% and is superior to the sodium tetrachloromercurate method with respect to ease of manipulation, speed, stability of absorbed sulphur dioxide and the use of stable and readily available pure and non-toxic reagents. The effects of reagent concentration, flow-rate and temperature on the absorption of sulphur dioxide were studied. The absorbed sulphur dioxide was subsequently determined spectrophotometrically using *p*-aminoazobenzene - formaldehyde reagent in hydrochloric acid medium. The interference of nitrogen dioxide is eliminated by the use of sulphamic acid. The procedure is suitable for air pollution studies.

Keywords: *Atmospheric sulphur dioxide absorption; monoethanolamine; spectrophotometry*; p-*aminoazobenzene - formaldehyde reagent*

Sulphur dioxide, one of the most harmful air pollutants, is widely used as a fungicide, preservative and bleaching agent and in pharmaceutical preparations because of its toxic action upon vegetative organisms.[1] Burning of sulphur-bearing coal and oil and smelting of ores, etc., are the main sources of its presence in the environment. The threshold limit value as fixed by American Conference of Governmental Industrial Hygienists is 5 p.p.m., but lower values of 2–3 p.p.m. have been reported to be toxic to plants and corrosive to metallic construction materials.[2] Several methods have been reported for its determination in air.[3] The method based on the Schiff reaction[4] proposed by West and Gaeke,[2] using sodium tetrachloromercurate (TCM) as absorbing reagent for sulphur dioxide and acid-bleached pararosaniline and formaldehyde for colour development, for the measurement of ambient sulphur dioxide is the method of choice for environmental chemists. Several modifications to this method have been suggested, which include improvement in the purity of the dye,[5] temperature dependence and stability of the coloured product[6] and studies on interferents.[7,8] The method is still retained because of its sensitivity, simplicity, specificity and easy adaptability.

Most of the methods for determination of sulphur dioxide have two stages: absorption of sulphur dioxide from air in a suitable medium and subsequent determination by using a suitable reagent to liberate the trapped sulphur dioxide.

Aqueous iodine solution,[9] hydrogen peroxide,[10] glycerol - alkali[11] and EDTA solution[12] have been used by earlier workers. West and Gaeke recommended TCM as an efficient absorber for atmospheric sulphur dioxide.[2] The major disadvantages with this method are the instability of sulphite solution on storage (1% per day decay at room temperature[7]) and the use of toxic and expensive mercury(II) chloride. To remove these defects, a modified West and Gaeke method has been proposed in which formaldehyde[13] and triethanolamine[14] are recommended as the absorbing medium for sulphur dioxide. In a search for a fast and reliable method for absorption of sulphur dioxide, we found that monoethanolamine, which is readily available and similar to triethanolamine, can be employed for the efficient absorption of atmospheric sulphur dioxide. The procedure has been optimised with regard to absorption efficiency. The method is comparable to the TCM method in absorption and recovery efficiency. After absorption, the amount of sulphur dioxide present is determined by using *p*-aminoazobenzene and formaldehyde, a reagent pair recommended by Kniseley and Throop[15] owing to large variation in purity and selectivity of pararosaniline dye. *p*-Aminoazobenzene gives more reliable and reproducible results as it is available in a pure form and can be synthesised in the laboratory.[16]

Experimental

Apparatus

Carl Zeiss ultraviolet - visible Specord and Spekol instruments were used for spectral measurements.

Midget impingers of 35 ml capacity with a jet diameter of 1 mm, the distance above the base of the tube being 5 mm, were used for absorption. Calibrated rotameters were used for air flow measurements.

Reagents

De-ionised, de-aerated water was used for preparing the reagent solutions. The chemicals used were of analytical-reagent grade or the purest grade available.

Monoethanolamine (MEA) solution, 0.05 M.

p-*Aminoazobenzene solution*, 0.02%. *p*-Aminoazobenzene (0.02 g) was dissolved in 20 ml of ethanol; 20 ml of 4 N hydrochloric acid were added and the solution was diluted to 100 ml with water. The reagent was recrystallised from alcohol before use.

Formaldehyde, 0.2%. Prepared from approximately 40% reagent-grade formaldehyde.

Sodium sulphite solution. Sodium sulphite (0.08 g) was dissolved in 100 ml of water. This solution, which contains approximately 320 μg ml^{-1} of sulphur dioxide, was standardised iodimetrically.[9] Working solutions were prepared by appropriate dilution of the stock solution with 0.05 M monoethanolamine solution.

Sulphamic acid, 0.6%. The solution was freshly prepared.

Procedure

Collection of sample

Two impingers, each containing 10 ml of 0.05 M MEA solution, were connected in series to a source of suction. Sulphur dioxide-containing air was passed through the impinger at a rate of 500 ml min^{-1}. Air samples of 38.2 l* were collected and analysed later for sulphur dioxide concentration in each impingers. No antifoaming reagent was used for collection of the sample.

Analysis

After sampling, 10-ml aliquots (the volume depending on the concentration of sulphur dioxide) were transferred into 25-ml calibrated flasks. To each flask were added 1 ml of 0.02% *p*-aminoazobenzene solution, 1 ml of 0.2% formaldehyde and 1 ml of concentrated hydrochloric acid. The contents were mixed and the volume was made up to the mark with water. Each flask was allowed to stand for 20 min and the absorbance was measured at 505 nm using a 1-cm cell against water as reference. The absorbance of the reagent blank was subtracted from that of the sample.

A calibration graph was prepared by placing aliquots of sodium sulphite solution in 0.05 M MEA solution containing 2.5–25 μg of sulphur dioxide in a 25-ml calibrated flask and developing the colour as described above.

Results and Discussion

The absorbing efficiency of MEA, the stability of sulphur dioxide in MEA and the effects of time, temperature and aeration on absorbed sulphur dioxide were studied. The effect of interferences on adherence to Beer's law was also studied.

Generation of synthetic sulphur dioxide

As standard samples of sulphur dioxide were not available, the gas was initially generated synthetically by bubbling air through a solution of sodium metabisulphite[17] and absorbing the liberated sulphur dioxide in 0.05 M MEA solution for subsequent analysis. This is a tedious process as it requires the determination of sulphur dioxide concentration before and after aeration in the generating chamber.

* The reason for taking 38.2 l of air in the determination of atmospheric sulphur dioxide is that with this sample size each microgram of sulphur dioxide conveniently represents 0.01 p.p.m. of sulphur dioxide in air.

However, it was found that a controlled amount of sulphur dioxide can be generated by placing a 15 μg ml^{-1} solution, prepared in 0.05 M MEA solution, in a microburette and adding it dropwise to a vessel containing 50 ml of 0.5 M hydrochloric acid and passing air through this solution at a rate of 0.5 l min^{-1}. The sulphur dioxide evolved was absorbed in 0.05 M MEA solution and was analysed for sulphur dioxide by the recommended procedure. It was found that the generation of sulphur dioxide is quantitative (Table I). The standard deviation and relative standard deviation for 15 μg of sulphur dioxide per 25 ml were 0.061 μg and 0.4%, respectively, showing that synthetic generation is reproducible and accurate.

This method was followed in our experiments as sodium metabisulphite solution is unstable and the sulphur dioxide concentration decreases if aeration is carried out for a long time.[17] However, sulphur dioxide liberated by the metabisulphite method also gave reproducible results when dilute solutions were used and aeration time was small.

TABLE I

REPRODUCIBILITY OF THE METHOD

Amount of sulphur dioxide = 15 μg. Volume of air passed = 38.2 l.

Sample No.	SO_2 found/μg	Mean	Standard deviation/μg	Relative standard deviation, %
1	15.08			
2	15.05			
3	14.98			
4	15.07	15.00	0.061	0.4
5	14.90			
6	14.96			
7	15.00			

Absorption Efficiency

Two midget impingers (capacity 35 ml) containing 10 ml of MEA solution were connected in series. A 38.2-l volume of air containing 2.5–1000 μg of sulphur dioxide was passed through them for various lengths of time at different flow-rates. After sampling, the sulphur dioxide was determined by the recommended procedure. Samples containing more than 300 μg of sulphur dioxide were analysed after dilution with distilled water. In such instances the reagent blank was also diluted accordingly. It was found that 99–100% of sulphur dioxide was absorbed by the first impinger. The second impinger gave a negative test for sulphur dioxide.

TABLE II

EFFECT OF CONCENTRATION OF MEA ON ABSORPTION EFFICIENCY

Flow-rate = 0.5 l min^{-1}.

Sample No.	Concentration of MEA/ M	SO_2 passed/ μg	SO_2 found in 1st impinger/ μg	Absorption, %
1	0.005	2.5	2.49	99.60
		12.5	12.43	99.44
		125.0	124.06	99.25
		1000.0	990.00	99.00
2	0.01	2.5	2.49	99.60
		12.5	12.44	99.50
		125.0	124.38	99.50
		1000.0	992.00	99.20
3	0.05	2.5	2.50	100.00
		12.5	12.50	100.00
		125.0	124.50	99.60
		1000.0	995.40	99.54
4	0.1	2.5	2.49	99.60
		12.5	12.50	100.00
		125.0	125.00	100.00
		1000.0	996.50	99.65

The concentration of the MEA solution, from 0.005 to 0.1 M, had no effect on the absorption efficiency. Even variation of the flow-rate from 0.25 to 2 l min^{-1} and of temperature from 15 to 40 °C during collection of the sample had no effect on absorption efficiency. In our subsequent experiments 0.05 M MEA solution was used. The data on absorption efficiency are presented in Tables II and III.

TABLE III

EFFECT OF FLOW-RATE ON ABSORPTION EFFICIENCY

Concentration of MEA solution = 0.05 M.

Sample No.	Flow-rate/ l min^{-1}	SO_2 passed/ μg	SO_2 found in 1st impinger/ μg	Absorption, %
1	0.25	2.50	2.49	99.60
		12.50	12.45	99.60
		125.00	124.75	99.80
		1000.00	994.50	99.45
2	0.5	2.50	2.50	100.00
		12.50	12.50	100.00
		125.00	125.00	100.00
		1000.00	996.00	99.60
3	1.0	2.50	2.50	100.00
		12.50	12.46	99.70
		125.00	125.00	100.00
		1000.00	995.00	99.50
4	2.0	2.50	2.50	100.00
		12.50	12.50	100.00
		125.00	124.88	99.90
		1000.00	999.00	99.90

Stability of Collected Sulphur Dioxide Samples

Sulphur dioxide was absorbed to the extent of 100 μg ml^{-1} in 0.05 M MEA solution. The solution was made up to a known volume and aliquots were taken and analysed on subsequent days. It is reported that sulphur dioxide absorbed in 0.1 M TCM solution is oxidised at the rate of 1 %per day at room temperature. When MEA is used as the absorber, the loss of sulphur dioxide is negligible for up to 25 days. Even aeration of the absorbed sample for 8 h had no effect on the stability of sulphur dioxide. Although the MEA solution used was very dilute (0.05 M), the decrease in absorbance after 25 days was found to be only 1–2%.

Colour Development

The order of addition of reagents to the absorbed sulphur dioxide solution in MEA was investigated. The order of addition for maximum colour development was *p*-aminoazobenzene solution, formaldehyde solution and hydrochloric acid. After addition of *p*-aminoazobenzene solution, hydrochloric acid concentration between 0.02 and 0.15 M was found to be the most suitable. After addition of formaldehyde solution, the maximum colour development was found to take place at hydrochloric acid concentrations between 0.2 and 1.5 M. This is in agreement with the earlier reported method (0.6 M hydrochloric acid using *p*-aminoazobenzene).[15] In the proposed method, the hydrochloric acid concentration was maintained at 0.08 and 0.5 M after addition of *p*-aminoazobenzene and formaldehyde solutions, respectively, to the MEA solution containing absorbed sulphur dioxide solution. In the earlier method 10 min were required for full colour development and the product was stable for 60 min. In this method, full colour development takes 20 min but the product is stable for more than 12 h, which is an advantage.

Beer's Law and Reproducibility

Beer's law was found to be valid between 2.5 and 25 μg per 25 ml of sulphur dioxide in 0.05 M MEA solution. The method was found to be reproducible.

Effect of Interferents

Nitrogen dioxide interferes if present at concentrations above 2 p.p.m. by decreasing the colour intensity. This interference can be removed by addition of 1 ml of 0.6% sulphamic acid solution to a sulphur dioxide sample containing 15 μg per 25 ml. This, however, gave about a 15% lower absorbance. Better results (8–10% lower absorbance) were obtained when air samples containing sulphur dioxide and nitrogen dioxide were passed through a tube containing 20 ml of 0.6% sulphamic acid solution before passing through the absorbing solution. Owing to non-availability of ozone, its interference could not be studied. Hydrogen sulphide was removed from the air by passage through a tube containing lead acetate solution. Other gases such as ammonia, chlorine and halogen acids do not interfere.

The effects of Fe^{3+}, Cu^{2+}, V^{5+} and Mn^{2+}, which are known to catalyse the oxidation of sulphur dioxide, were studied by adding 200 μg of each ion to a sulphur dioxide solution containing 15 μg per 25 ml. No significant interference was observed, except with Mn^{2+}. However, up to 50 μg of Mn^{2+} can be tolerated.

Application of the Method

The method has been applied successfully to the determination of sulphur dioxide in various samples of air. The sampled air was divided into two parts, each having a flow-rate of 0.5 l min^{-1} and was passed through two impingers, one containing 0.04 M TCM solution and the other 0.05 M MEA solution. The absorbed sulphur dioxide was subsequently determined by Kniseley and Throop's method.[15] The results obtained were in good agreement with each other, as shown in Table IV. The correlation coefficient for the two methods was 0.999, which shows that the method is accurate for absorption of sulphur dioxide.

TABLE IV

APPLICATION OF THE METHOD TO AIR SAMPLES

	SO_2 found, p.p.m.	
Sample No.	TCM	MEA
1	0.0300	0.0301
2	0.0803	0.0800
3	0.1052	0.1050
4	0.1710	0.1708
5	0.2030	0.2030
6	0.2430	0.2431

Conclusion

The proposed method, using monoethanolamine as absorbing reagent in combination with *p*-aminoazobenzene, provides a simple and selective spectrophotometric procedure for the measurement of ambient sulphur dioxide. The stability and absorption efficiency of the method compare favourably with those obtained using buffered formaldehyde solution and the reaction conditions are less stringent. Interference from nitrogen dioxide has been eliminated. The *p*-aminoazobenzene method has advantages over the West and Gaeke method[2] in terms of simplicity, wide acidity range, stability of the coloured product and reproducibility.

We are grateful to Prof. S. G. Tandon, Head, Department of Chemistry, Ravishankar University, Raipur, for providing facilities. Thanks are also due to the University Grants Commission for the award of a Senior Research Fellowship to one of us (A.B.).

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Spectrophotometric Method for the Determination of Dobutamine Hydrochloride

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A rapid, accurate and simple method is proposed for the determination of dobutamine hydrochloride in the bulk drug and in vials. This method is based on measuring the intensity of the pink colour that develops when dobutamine hydrochloride is allowed to react with thiosemicarbazide in an alkaline - acetone medium. The colour is stable for at least 3 h and can be quantified spectrophotometrically at 510 nm ($\epsilon_{max.} = 1.7 \times 10^4$ l mol^{-1} cm^{-1}). Beer's law is obeyed in a concentration range 0.5–20 μg ml^{-1} in the final assay solution. The effects of reagent concentration, alkali concentration and solvent on colour formation were investigated. A Job's plot of absorbance *versus* the molar ratio of dobutamine to thiosemicarbazide indicates a 1 : 1 ratio. As the catecholic group with free adjacent positions is required for colour development, the method is highly specific.

Keywords: *Spectrophotometry*; *dobutamine hydrochloride determination*; *thiosemicarbazide*

Dobutamine hydrochloride {(±)-4-[2-(3-*p*-hydroxyphenyl-1-methylpropylamino)ethyl]-benzene-1,2-diol hydrochloride} is a synthetic catecholamine that has been developed recently in an effort to find an ideal inotropic drug,[1] increasing ventricular contractility and cardiac output without substantially increasing heart rate or systemic blood pressure.[2–4] Although the drug has not yet been introduced in the British Pharmacopoeia or in the United States Pharmacopeia, it is widely used in the form of Dobutrex vials. Two chemical methods are now available for the determination of dobutamine hydrochloride: gas chromatography of the trimethylsilyl derivative[5] and high-performance liquid chromatography.[6] For routine quality control, development of a simple, rapid, sensitive and specific spectrophotometric method is highly desirable.

Experimental

Apparatus

Spectra were recorded on a Zeiss PM2 DL spectrophotometer, using 1-cm cells.

Samples

Dobutamine hydrochloride. Pharmaceutical grade, from Eli Lilly Co. Ltd., utilised as a working standard.

Dobutamine hydrochloride injection. Dobutrex vials, Lilly, containing dobutamine hydrochloride equivalent to 250 mg of dobutamine.

Preparation of sample solutions

An accurately weighed amount of dobutamine hydrochloride, or the contents of Dobutrex vials, were dissolved in water and diluted stepwise to obtain a concentration of 100 μg ml^{-1} of dobutamine hydrochloride.

Reagents

Solvents. Solvents used were of spectroscopic grade or rendered so by appropriate treatment.[7]

Thiosemicarbazide solution. Dissolve 0.3 g of thiosemicarbazide (BDH Chemicals Ltd.) in 75 ml of water with the aid of gentle heat, cool to room temperature and dilute to 100 ml with water.

Sodium hydroxide solution, 0.2 N.

Assay Procedure

A 1-ml aliquot of the prepared solution was transferred into a 10-ml calibrated flask; 1 ml of thiosemicarbazide solution and 1 ml of 0.2 N sodium hydroxide solution were then added, in order. The contents were mixed thoroughly and allowed to stand for 30 min at constant temperature (20 ± 5 °C). The solution was then diluted to volume with acetone and the absorbance was measured at 510 nm in a 1-cm cell against a blank prepared under the same conditions, but using 1 ml of distilled water instead of the thiosemicarbazide solution.

Construction of Calibration Graph

An amount of dobutamine hydrochloride (about 40 mg) was accurately weighed, dissolved in water and diluted to volume in a 100-ml calibrated flask. The solution was diluted stepwise to give a series of concentrations suitable for construction of the calibration graph in the range 5–200 μg ml^{-1}; 1 ml of each solution was utilised for colour formation with thiosemicarbazide and sodium hydroxide as described under the Assay Procedure.

Stoicheiometric Relationship

Job's method of continuous variation was employed.[8] Standard aqueous solutions of dobutamine hydrochloride (2×10^{-4} M) and thiosemicarbazide (2×10^{-4} M) were used. A series of standard solutions of dobutamine hydrochloride and thiosemicarbazide in different complementary proportions totalling 5 ml (from 0 + 5 to 5 + 0 inclusive) were prepared in 10-ml calibrated flasks and each treated with 1 ml of 0.2 N sodium hydroxide solution. After 30 min, the solutions were made up to volume with acetone and the absorbances were measured at 510 nm against blanks prepared under the same conditions, but by replacing the thiosemicarbazide solution with an equal volume of water.

Results and Discussion

A characteristic pink colour, with an absorption maximum at 510 nm, develops when dobutamine hydrochloride reacts with thiosemicarbazide in an alkaline - acetone medium. Fig. 1 shows the spectra of the reactants and the products of the reaction.

It must be noted that dobutamine hydrochloride will form a pink colour when it is mixed with sodium hydroxide solution. However, this colour disappears completely after 5 min and at the time of measurement blank solutions are colourless. This colour is not formed at all in the assay solutions because of the prior reaction of dobutamine hydrochloride with thiosemicarbazide, as the thiosemicarbazide is introduced into the assay solution first.

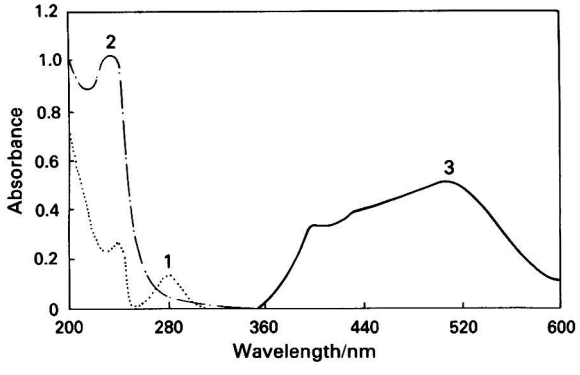


Fig. 1. Absorption spectra of (1) aqueous solution of dobutamine hydrochloride, (2) aqueous solution of thiosemicarbazide and (3) solution of the chromogen in aqueous acetone medium. Concentration, 10 μg ml^{-1}.

Optimisation of Variables

Effect of thiosemicarbazide concentration

The optimum concentration of thiosemicarbazide leading to a maximum intensity of colour was found to be 0.03% in the final solution, which corresponds to 1 ml of 0.3% thiosemicarbazide reagent per 10 ml of reaction mixture (Table I).

TABLE I

EFFECT OF THIOSEMICARBAZIDE CONCENTRATION ON COLOUR INTENSITY

Results are absorbances at 510 nm.

Final thiosemicarbazide concentration, %	Concentration of dobutamine hydrochloride in final assay solution/μg ml^{-1}		
	8	12	16
0.01	0.372	0.541	0.715
0.02	0.400	0.582	0.768
0.03	0.414	0.595	0.792
0.04	0.415	0.595	0.790
0.05	0.412	0.595	0.790

Effect of alkali concentration

The optimum concentration of sodium hydroxide leading to a maximum intensity of colour was found to be 0.02 N in the final solution (corresponding to 1 ml of 0.2 N sodium hydroxide per 10 ml of reaction mixture). Alkali concentrations higher than 0.02 N may lead to partial decomposition of the coloured chromogen (Fig. 2).

Effect of solvent

The solvent affects both the wavelength and intensity of maximum absorption. The solvents studied were water, methanol, ethanol, propan-1-ol, propan-2-ol and acetone. Fig. 3 shows that acetone gives the highest absorption intensity and the longest λ_{max}. These solvent effects can be explained on the basis of a tautomeric equilibrium of the coloured product.[9]

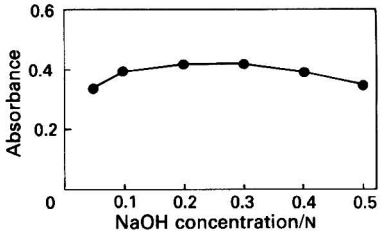


Fig. 2. Absorption intensity of the chromogen as a function of concentration of sodium hydroxide solution. Concentration of dobutamine hydrochloride, 8 μg ml^{-1}.

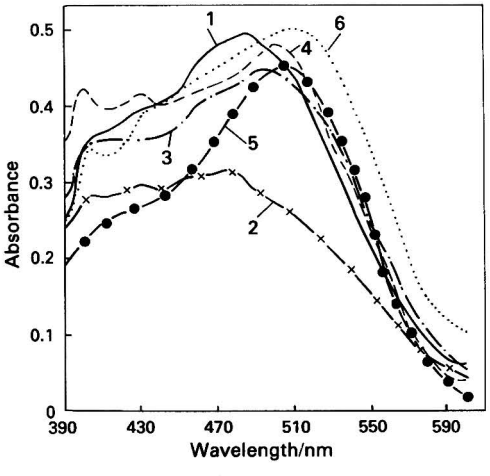


Fig. 3. Absorption spectra of chromogen in (1) water, (2) methanol, (3) ethanol, (4) propan-1-ol, (5) propan-2-ol and (6) acetone. Concentration of dobutamine hydrochloride, 10 μg ml^{-1}.

Effect of reaction time

Maximum colour intensity was obtained after 30 min at 20 $\pm$ 5 °C. The colour was stable for a further 3 h (Table II).

Quantification, Linearity of Beer's Law Plot, Accuracy and Precision

A linear correlation ($r = 0.9997$) was found between the absorbance at 510 nm and the concentration of dobutamine hydrochloride in the range 0.5–20 μg ml^{-1} in the final assay solution. The apparent molar absorptivity was found to be 1.7×10^4 l mol^{-1} cm^{-1}. The

TABLE II

EFFECT OF REACTION TIME ON COLOUR INTENSITY

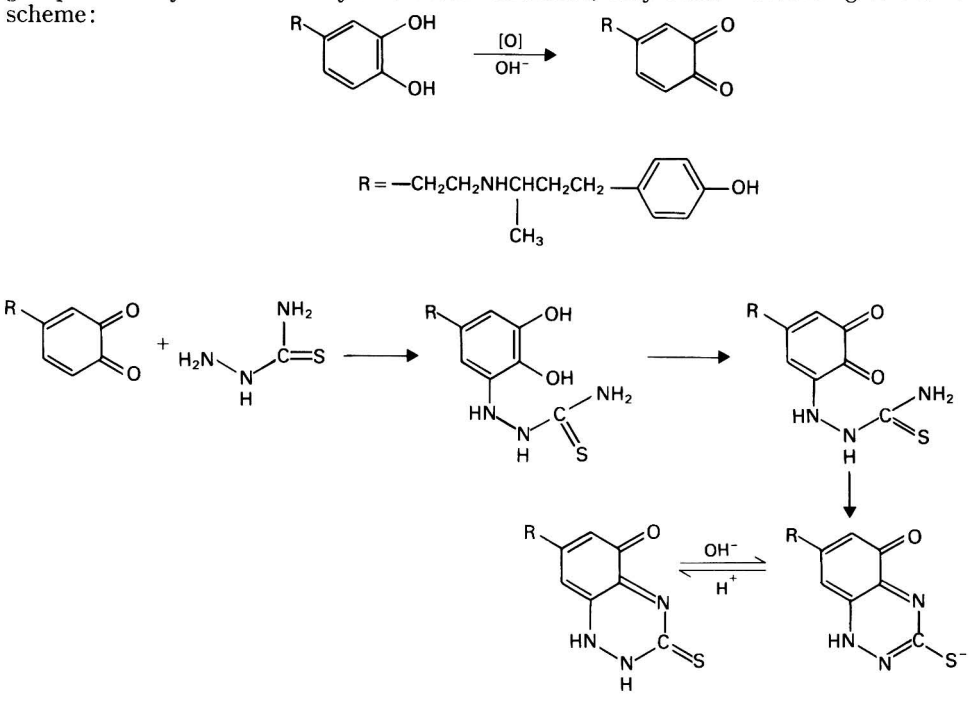
Concentration of dobutamine hydrochloride in the final assay solution is 10 μg ml^{-1}.

Time/ min	Absorbance at 510 nm	Time/ min	Absorbance at 510 nm
5	0.356	40	0.499
10	0.395	45	0.496
15	0.434	50	0.498
20	0.460	55	0.499
25	0.478	60	0.498
30	0.499	120	0.498
35	0.498	180	0.496

reproducibility of the procedure was determined by running replicate samples, each containing 10 μg ml^{-1} of dobutamine hydrochloride in the final test solution. At this concentration level, the standard deviation did not exceed 1.5%.

Dobutamine to Thiosemicarbazide Ratio in the Chromogen

The continuous molar variation of dobutamine and thiosemicarbazide (Fig. 4) showed that the interaction between these two compounds occurs on an equimolar basis. Trials made for the separation of the chromogen formed were unsuccessful owing to the formation of a resinous coloured mass. The possibility of formation of the thiosemicarbazone of the *o*-quinone, formed *in situ*, is unlikely as thiosemicarbazone formation is generally an acid-catalysed reaction.[10] In addition, no colour was produced when dobutamine hydrochloride was allowed to react with semicarbazide under the same conditions. Addition of thiosemicarbazide through the sulphur to the *o*-quinone (newly formed) in analogy to the addition of thiourea to catechol[11,12] is also unlikely, as dobutamine hydrochloride was found to react with thiourea under the same conditions giving only a faint yellow colour with an absorption maximum at 405 nm. A nucleophilic attack of the *o*-quinone, formed *in situ* by the amino group of the hydrazide moiety of thiosemicarbazide, may occur[13] according to the following scheme:



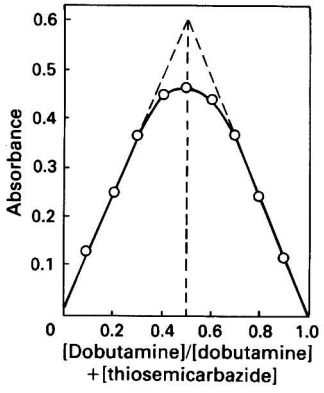


Fig. 4. Continuous variation plot obtained from solutions of dobutamine hydrochloride and thiosemicarbazide (2×10^{-4} M).

Sensitivity and Specificity

The lower limit of detection of dobutamine hydrochloride using thiosemicarbazide was found to be 1 μg ml^{-1}, which indicates the sensitivity of the reaction and it thus can be recommended for detection of minute amounts of the drug. The catecholic function with free adjacent positions is responsible for development of the colour. Phenol, resorcinol, phloroglucinol, guaiacol and thymol are known to give negative responses to the reaction, while pyrocatechol, epinephrine, norepinephrine, isoprenaline and methyldopa give coloured products with thiosemicarbazide in alkaline medium.[14–17] This reveals that the method is highly specific.

Application to the Bulk Drug and Dosage Form

The suggested method was applied to the quantitative determination of dobutamine hydrochloride in bulk and in Dobutrex vials (Table III). The data in Table III indicate the suitability of the method for routine quality control analysis.

TABLE III

ASSAY OF DOBUTAMINE HYDROCHLORIDE IN BULK DRUG AND DOSAGE FORM BY THE THIOSEMICARBAZIDE METHOD

	Dobutamine hydrochloride sample or vials			Standard dobutamine hydrochloride	
Sample or vials	Amount taken/mg	Recovery ± standard deviation, %*		Added/ mg	Recovery ± standard deviation, %*
Bulk drug	20	100.9	0.92		
Bulk drug	40	100.3	1.12		
Bulk drug	60	99.5	1.04		
Bulk drug	80	99.8	1.32		
Dobutrex vials . .	100	99.1	0.88	100	99.6 1.22
Dobutrex vials . .	200	99.4	1.48	200	100.2 1.36

* Three determinations.

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Spectrophotometric Study of the Ruthenium(III) - 2-Thiobarbituric Acid System

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Ruthenium(III) forms a 1:2 complex with 2-thiobarbituric acid, with an absorption maximum at 338 nm; the molar absorptivity is 1.1×10^4 l mol^{-1} cm^{-1} and the Sandell's sensitivity of the reaction is 0.0091 μg cm^{-2} per 0.001 absorbance unit. Beer's law is obeyed for up to 28 μg ml^{-1} of ruthenium. The molar composition of the complex was determined by the molar ratio method and confirmed by means of elemental analysis. Co-ordinate bonding involving the sulphur atom of 2-thiobarbituric acid was proved by infrared spectroscopy. The tolerance of the system to platinum metals and other common cations is reported.

A statistical evaluation of the proposed method has been undertaken and a comparison with important spectrophotometric reagents for ruthenium(III) used in recent years is presented.

Keywords: *Ruthenium determination*; *2-thiobarbituric acid*; *spectrophotometry*

Ruthenium is one of the most effective hardeners in high-density alloys; it is alloyed with other platinum metals to make electrical contacts for severe wear resistances and it is also a versatile catalyst. Because of its commercial importance, numerous reagents have been proposed for its spectrophotometric determination. The various methods differ considerably in sensitivity, tolerance to other ions, rate of reaction, useful concentration range, etc., and there appears to be scope for the development of further procedures giving good sensitivity and accuracy.

During the investigation on the chromogenic properties of 4,6-dihydroxy-2-mercaptopyrimidine (2-thiobarbituric acid, TBA), it was observed that it yields a stable 1:2 ruthenium(III) - TBA complex, with an absorption maximum at 338 nm.

TBA and its derivatives are mostly employed for their pharmacological and anaesthetic properties, and in the recent years derivatives of TBA have been synthesised that exhibit hypotensive,[1] psychopharmacological[2] and anticonvulsant[3] activity.

TBA has also found some applications as an analytical reagent. Recently, it has been used in the determination of lipid peroxidation in tissues[4] and for the determination of gold[5] and bismuth - copper mixtures (by derivative spectrophotometry).[6] In an investigation on the reaction of six sulphur-containing compounds with the platinum metals, Knight *et al.*[7] observed that TBA forms a coloured compound with ruthenium with an absorbance maximum at 570 nm, but little information was given about its use as a spectrophotometric reagent for ruthenium.

In this paper, a detailed study of the ruthenium(III) - TBA system is described. The method is sensitive and accurate for the determination of ruthenium. Beer's law is obeyed for up to 28 μg ml^{-1} of ruthenium and the molar absorptivity is 1.1×10^4 l mol^{-1} cm^{-1}. The 1:2 molar ratio of ruthenium to TBA has been demonstrated by the molar ratio method and confirmed by elemental analysis; co-ordinate bonding involving the sulphur atom of the 2-thiobarbituric acid has been demonstrated by infrared spectroscopy. The effects of acidity, temperature and heating time and the tolerance to platinum metals and other foreign ions have been investigated. A statistical analysis of the results and a comparison with spectrophotometric reagents for ruthenium developed in the last 10 years are also presented.

Experimental and Results

Reagents

All chemicals were of analytical-reagent grade unless specified otherwise.

Ruthenium(III) standard solution. Stock solutions of ruthenium(III) were prepared by

dissolving approximately 0.095 g of ruthenium(III) chloride trihydrate in 100 ml of concentrated hydrochloric acid - 95% ethanol (1 + 1) (preliminary tests showed that a high concentration of ethanol is desirable to prevent precipitation when ruthenium solutions are mixed with TBA solutions); the concentration of the resulting solution was about 3.6×10^{-3} M.

The ruthenium content was determined by precipitating it as the hydrated oxide, followed by ignition in air and then reduction in hydrogen and cooling in a carbon dioxide atmosphere.

2-Thiobarbituric acid solution. Volumes of 100 ml of 3.0×10^{-2} M TBA solution were prepared by dissolving with stirring 0.4324 g of the solid in distilled water. As TBA is difficult to dissolve in water, about 0.9 ml of 4 M sodium hydroxide solution was added to speed up the process; hydrochloric acid solutions were then used for pH adjustment.

Foreign ion solutions. Reagent-grade salts of various elements were used. The solutions contained 2–10 mg ml^{-1} of the ions.

Apparatus

Ultraviolet - visible absorbance measurements were made with a Perkin-Elmer 555 spectrophotometer using 1-cm quartz cells and a 1-nm slit width.

Infrared measurements were made with a Perkin-Elmer 577 infrared spectrophotometer. The spectra of the solid substances were obtained in Nujol between potassium bromide discs.

pH measurements were made with an Orion Research Digital Ionalyzer/501.

Development of Colour and Analytical Procedure

In a preliminary study to determine the conditions for maximum colour development of the complex, heating of the samples for at least 30 min on a water-bath at 75 °C was found

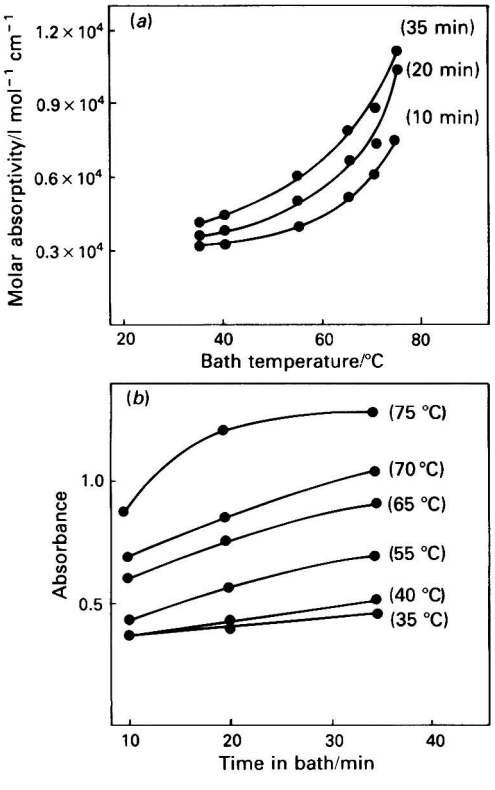


Fig. 1. Effect of temperature and heating time on (*a*) molar absorptivity and (*b*) absorbance of Ru(III) - TBA complex. Reference, reagent blank; pH, 2.2; 338 nm; in (*b*), 59 μg of Ru per 5 ml.

necessary. Therefore, a development time of 35 min at 75 °C was adopted in subsequent studies to ensure complete complexation. At higher temperatures, considerable amounts of ethanol could be lost. At lower temperatures, the rate of colour development was slow; in particular, at room temperature no stable colour intensity was reached, even after several hours. Colour-developed solutions, measured after different heating times and temperatures, showed no change in the wavelength of maximum absorbance.

Some results of this preliminary study are shown graphically in Fig. 1. Fig. 1(*a*) shows a large increase in the molar absorptivity of the ruthenium - TBA complex with increasing bath temperature with different times of heating; Fig. 1(*b*) shows that a constant absorbance of the complex is attainable after standing for about 30 min in a water-bath at 75 °C [the lines in Fig. 1(*b*) were obtained with samples of 59 μg of ruthenium per 5 ml, by following the procedure described below].

The procedure for the determination of ruthenium(III) using TBA was as follows: to a 2-ml aliquot of the 3.0×10^{-2} M TBA solution in a 5-ml calibrated flask were added a few microlitres of 3.6×10^{-3} M ruthenium(III) standard solution and the resulting solution was made up to volume with ethanol. The mixture was heated for 35 min in a water-bath at 75 °C, then the solution was rapidly cooled to room temperature and the absorption was measured at 338 nm against a reagent blank. The blanks were solutions 1.2×10^{-2} M in TBA prepared by transferring 2 ml of the 3.0×10^{-2} M TBA solution into a 5-ml calibrated flask and diluting to volume with ethanol.

Absorption Spectra

Absorption spectra were scanned in the double-beam mode with a slit width of 1 nm. The scan rate was 120 nm min^{-1}. The automatic base-line corrector was employed, the base line being determined with both sample and reference cuvettes filled with reagent blank solution.

Fig. 2 shows the absorption spectrum of the ruthenium(III) - TBA complex against a reagent blank, obtained by following the above procedure. The complex shows an absorption maximum at 338 nm. The ligand, however, exhibits appreciable absorption around 338 nm, which sharply decreases and becomes insignificant from 370 nm onwards; all absorbance measurements were therefore made against reagent blanks (by operating with a large excess of TBA, *i.e.*, under conditions of a pseudo-first-order reaction, the results are not affected by the fact that ruthenium consumes some of the reagent).

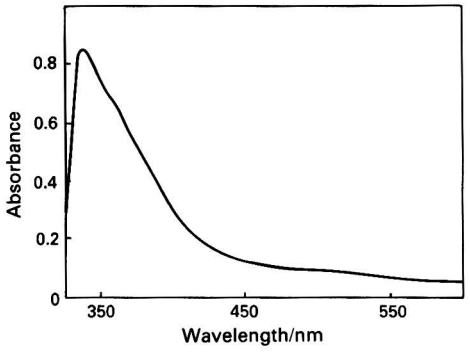


Fig. 2. Absorption spectrum of Ru(III) - TBA complex. Ru(III), 39 μg per 5 ml; reference, reagent blank; pH, 2.3.

Effect of Acidity

The effect of pH on the absorbance of the complex was determined by preparing a series of solutions varying in apparent pH from 0.6 to 12.5 by addition of hydrochloric acid or sodium hydroxide solution. The absorbance was found to be maximal and constant up to pH 3, then decreased sharply to very low values in strongly alkaline medium, as shown in Fig. 3. For this reason, all further measurements were made in the optimum pH range, *i.e.*, at pH $\leqslant 3$.

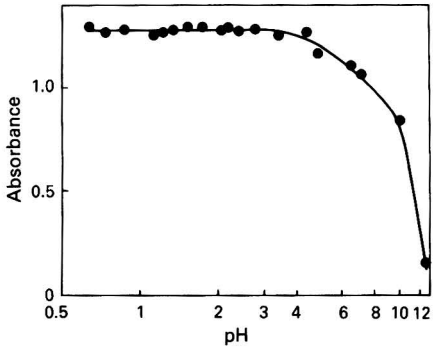


Fig. 3. Effect of acidity on absorbance of Ru(III) - TBA complex. Ru(III), 59 μg per 5 ml; reference, reagent blank; 338 nm.

Stability of the Reagent

Calibration graphs recorded with fresh reagent agreed very well with graphs obtained using TBA stored at room temperature for 24–48 h.

Calibration Graphs

The calibration graphs for the determination of ruthenium were obtained under optimum conditions by following the described procedure. The ruthenium(III) - TBA system has been found to obey Beer's law for up to 28 μg ml^{-1} of metal ion. The molar absorptivity of the complex at 338 nm, calculated from the linear regression coefficient, is 1.1×10^4 l mol^{-1} cm^{-1}. The sensitivity of the reaction for ruthenium was calculated to be 0.0091 μg cm^{-2} per 0.001 absorbance unit (in accordance with Sandell's notation).

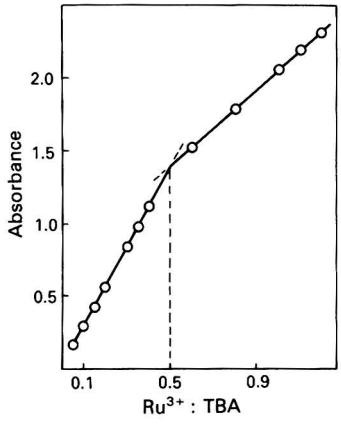


Fig. 4. Molar ratio of Ru(III) - TBA complex determined by the molar ratio method. TBA, 5.4×10^{-4} M; reference, water - ethanol; pH, 0.8; 338 nm.

Composition of the Complex

To determine the molar composition of the complex, the molar ratio method was employed in the optimum pH range. From the spectrophotometric data, the molar ratio of ruthenium to TBA was found to be 1:2. Fig. 4 shows a typical experimental plot at pH 0.8, obtained with samples containing 5.4×10^{-4} M TBA and increasing amounts of ruthenium as required; absorbance measurements were made against water - ethanol at 338 nm.

The composition was also determined by elemental analysis of the complex isolated from solution. The dry solid was black and soluble in ethanol to give solutions with an absorption spectrum identical with that obtained with samples prepared by the above procedure. The results of the elemental analysis of the compound postulated to be $Ru(C_4H_4N_2O_2S)_2Cl_4H$ were as follows: calculated, Ru 18.99, C 18.05, H 1.70, N 10.53, Cl 26.65, S 12.05%; found, Ru 18.80, C 16.80, H 2.40, N 9.15, Cl 27.60, S 11.99% (ruthenium was determined by the proposed method). The high values found for H and Cl (and, therefore, low values for C, N and S) could be due to contamination of the complex during its isolation; however, the results seem to be in better agreement with the proposed structure than with any other reasonable stoicheiometry, confirming the 1:2 molar ratio of ruthenium to TBA.

Infrared Spectroscopic Investigation

In order to establish the manner in which TBA molecules co-ordinate with ruthenium atoms, infrared spectroscopy was used on the complex isolated and dried *in vacuo*. The infrared spectra of solid TBA (broken line) and the ruthenium - TBA complex (continuous line) in the 2.5–14-μm region, obtained in Nujol between potassium bromide discs, are illustrated in Fig. 5.

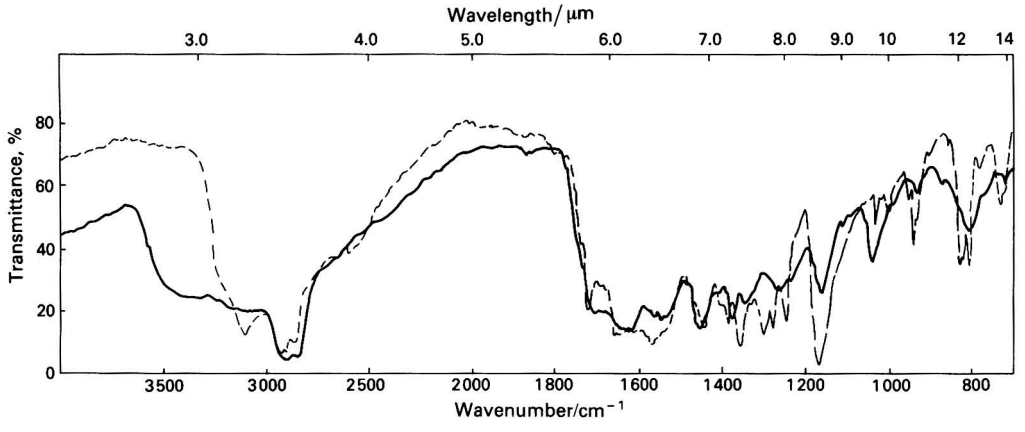
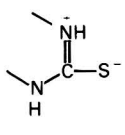


Fig. 5. Infrared spectra of TBA (broken line) and Ru(III) - TBA complex (solid line).

The most important difference between the spectra of the ligand and of the complex is displayed in the region 1160–1170 cm^{-1}. At 1170 cm^{-1} a strong absorption band is observable in the TBA spectrum, which can be assigned to C=S stretching vibrations. This absorption is considerably weakened and shifted to 1160 cm^{-1} on formation of the ruthenium - TBA complex. These effects may be interpreted by the change in the C=S bond on co-ordination of TBA through the sulphur atom; the formation of an S→Ru bond is expected to increase the single-bond character of the carbon–sulphur bond; in consequence, the C=S stretching frequency in the complex is lowered, the contribution of the C=S vibration to the 1170 cm^{-1} band is decreased and the intensity of this band is reduced.

Moreover, the band observed at 1445 cm^{-1} in the spectrum of TBA, which was assigned to the N–C–N stretching vibration, corresponds to the 1460 cm^{-1} band in the complex. This frequency increase can be interpreted by the greater double bond character of the carbon–nitrogen bond on complex formation as consequence of the formation of an S→Ru bond (which is expected to increase the contribution of the structure to the TBA molecule).



The above considerations, all of which indicate the presence of a sulphur–metal bond in the ruthenium - TBA complex and the assignment of the absorption bands, were made by analogy with infrared studies on similar compounds, *viz.*, thiourea and metal - thiourea complexes.[8] Sulphur–metal bonds have also been observed in diphenylthiourea - platinum metal ion complexes by means of X-ray photoelectron spectroscopy.[9] Finally, the presence in the ruthenium - TBA complex of co-ordinate bonding involving the sulphur atom is also strictly in accordance with the results of analogous infrared studies on the 1:2 copper(II) - TBA complex.[10]

Effect of Foreign Ions

The extent of interference by the other platinum metals and by common cations was determined by measuring the absorbance of solutions containing a constant amount of ruthenium and various amounts of diverse ions. The tolerance of the ruthenium - TBA system to an interfering substance was taken as the largest amount of that substance that would give an absorbance not more than 1% absolute different from that of ruthenium alone. Table I lists the substances tested and the tolerances as defined above.

TABLE I

TOLERANCE OF RUTHENIUM(III) - 2-THIOBARBITURIC ACID SYSTEM TO DIVERSE IONS

All solutions contained 48 μg of ruthenium per 5 ml. The tolerance to a foreign ion was taken as the largest amount that gives an absorbance not more than 1% absolute different from that of ruthenium alone.

Foreign ion	Amount tolerated relative to Ru, %	Foreign ion	Amount tolerated relative to Ru, %
Li(I)	141.0	Ag(I)	10.4
K(I)	135.4	Zn(II)	335.4
Rb(I)	53.3	Cd(II)	39.0
Cs(I)	143.7	Hg(II)	6.3
Mg(II)	21.2	Al(III)	104.2
Ca(II)	59.6	Sn(II)	82.9
Sr(II)	41.7	Pb(II)	1.6
Ba(II)	500.0	As(III)	8.1
La(I)	41.7	Sb(III)	83.3
Cr(III)	24.8	Bi(III)	15.0
Mn(II)	6.0	Rh(III)	2.1
Fe(III)	12.5	Pd(II)	4.1
Co(II)	59.4	Os(VIII)	17.7
Ni(II)	127.1	Ir(III)	23.3
Cu(II)	1.4	Pt(II)	3.6

For the analysis of samples containing significant amounts of interfering substances, ruthenium can be conveniently separated by the conventional distillation method as ruthenium tetraoxide. Table II shows the results of the analysis of samples containing ruthenium alone and in mixtures with other platinum metals, after distillation and recovery of ruthenium. Distillation was carried out for 15–20 min; suitable aliquots of the collected ruthenium solutions were analysed by the proposed method under the optimum conditions.

TABLE II

DETERMINATION OF RUTHENIUM(III) USING 2-THIOBARBITURIC ACID AFTER SEPARATION BY DISTILLATION

Ru taken/mg	Ru found/mg
2.0	1.95
2.5	2.45
4.5*	4.39
5.5†	5.38

* 9 mg each of Pt, Pd, Ir and Os.
† 16.5 mg each of Pt, Pd, Ir and Os.

Statistical Evaluation of the Method

By following the proposed procedure, linear graphs of absorbance *versus* ruthenium(III) concentration, with an intercept of zero, were obtained.

The excellent linearity of the calibration graphs is clear from the correlation coefficient of $r = 0.9999$, calculated from the linear regression coefficient; the intercept is 0.003, *i.e.*, close to zero.

Statistical analysis of the absorbance *versus* concentration graph allows the detection limit to be calculated.[11] At the $p = 0.01$ level of significance the variance was calculated to be $s_0^2 = 3.69 \times 10^{-5}$ and the detection limit was 0.165 μg ml^{-1}. These values indicate that the method allows the detection of ruthenium at very low levels with good precision.

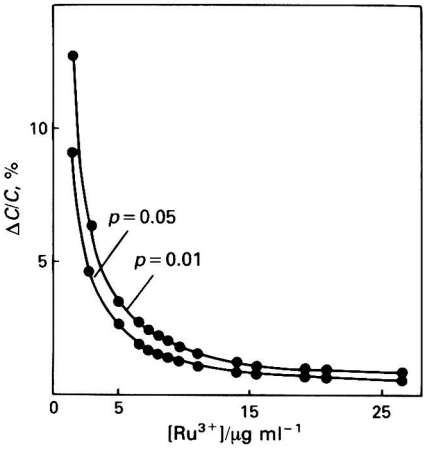


Fig. 6. Variation of confidence limits at levels of significance of 0.01 and 0.05, in form of uncertainty (%) on concentration.

The confidence limits[12] were also determined. Fig. 6 shows the confidence limits in the form of uncertainty (per cent.) on concentration, *i.e.*, as $\Delta C/C\%$, for both $p = 0.01$ (99% probability) and $p = 0.05$ (95% probability) levels of significance. This is a useful way of representing confidence limits because it allows a direct calculation of the relative uncertainty on concentration over the full range of concentrations tested.

Conclusions

The results indicate that TBA is a useful reagent for the determination of ruthenium. The method is fast and accurate. A comparison with spectrophotometric reagents for ruthenium(III) developed in recent years (Table III) shows that TBA ranks among the most sensitive reagents. Other sensitive methods are utilisable in narrower concentration ranges and require prior extraction of the complex with organic solvents and/or very long times of standing of the samples at high temperatures, making the procedure more tedious and time consuming.

The tolerance of the ruthenium(III) - TBA system to a large number of cations is fairly good. An advantage of the proposed method is that 2-thiobarbituric acid can be used for the determination of ruthenium in the presence of the reported amounts of other platinum metals without the use of masking agents; concentration levels higher than those listed in Table I are not a particular disadvantage, because a sharp separation of ruthenium can be made using an appropriate distillation procedure, as previously reported.

TABLE III

COMPARISON OF SPECTROPHOTOMETRIC REAGENTS FOR RUTHENIUM(III)

Reagent	Range of concentration/ $\mu g\ ml^{-1}$	Sandell's sensitivity/ $\mu g\ cm^{-2}$	Molar absorptivity/ $l\ mol^{-1}\ cm^{-1}$	Reference
2-Thiobarbituric acid	0.16–28	0.0091 (338 nm)	1.1×10^4	This work
2-Mercaptobenzimidazole	6–80	0.026 (680 nm)	3.9×10^3	13
3,4-Diaminobenzoic acid	10–40	0.024 (550 nm)	4.16×10^3	14
Diphenylcarbazone	0.8–5	0.062 (530 nm)	1.62×10^3	15
Acenaphthenequinone monoxime	Up to 6.57	0.0065 (550 nm)	1.5×10^4	16
Diphenylthiovioluric acid	5–60	0.0044 (520 nm)	2.23×10^4	17
Thiovioluric acid	Up to 5.6	0.0046 (540 nm)	2.2×10^4	18
o-Hydroxythiobenzhydrazide	0.44–8.8	0.0074 (540 nm)	1.37×10^4	19
3-Nitroso-4-hydroxy-5,6-benzocoumarin	Up to 7.4	0.0097 (520 nm)	1.04×10^4	20
4,5-Diamino-6-hydroxypyrimidine sulphate	Up to 10	0.015 (530 nm)	6.5×10^3	21
2-Amino-3-hydroxypyridine	up to 16.5	0.022 (520 nm)	4.46×10^3	22
β-Dithionaphtholic acid	0.05–2.4	0.0043 (490 nm)	2.36×10^4	23
3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine	0.5–3.4	0.0048 (485 nm)	2.1×10^4	24
Thiobenzhydrazide	0.73–7.33	0.008 (520 nm)	1.24×10^4	25
Diethazine hydrochloride	1.0–10.4	0.019 (515 nm)	5.35×10^3	26
4-Amino-2-mercapto-5-nitroso-6-pyrimidinol	Up to 2.7	0.006 (530 nm)	1.53×10^4	27
Pyridine-2-aldoxime	3.0–3.5	0.043 (420 nm)	2.34×10^3	28
2,2′,2″-Terpyridine	2.0–10	0.012 (690 nm)	8.3×10^3	29
Promethazine hydrochloride	0.4–4.4	0.016 (514 nm)	6.3×10^3	30
	0.5–11.6	0.024 (518 nm)	4.2×10^3	30
Trifluoroperazine dihydrochloride	1.0–8.8	0.023 (500 nm)	4.28×10^3	31
Chlorpromazine hydrochloride	0.4–12	0.021 (530 nm)	4.6×10^3	32
1-Pentyl-4,6-dihydroxy-5-nitroso-pyridine 3-carboxylate	0.7–2.8	0.024 (535 nm)	4.2×10^3	33
Promazine hydrochloride	0.2–11.94	0.014 (515 nm)	7.33×10^3	34
Isonitrosoacetylacetone	1.0–12	0.022 (530 nm)	4.6×10^3	35
Monothiourea-3-nitrophthalic acid	2.0–24	0.035 (598 nm)	2.9×10^3	36
Isonitrosodibenzoylmethane	1.0–12	0.024 (530 nm)	4.14×10^3	37
3-Hydroxy-3-(*p*-dimethylaminophenyl)-1-phenyltriazine	0.2–20	0.004 (490 nm)	2.5×10^4	38
Ammonium thiocyanate	Up to 20	0.018 (570 nm)	5.5×10^3	39
Isonitrosobenzoylacetone	2.0–12	0.018 (470 nm)	5.4×10^3	40
2-Methyl-1,4-naphthoquinone monoxime	0.19–3.4	0.0036 (470 nm)	2.76×10^4	41
Thiophene-2-aldoxime	0.5–10	0.015 (360 nm)	6.38×10^3	42
Dibenzylidenethiocarbohydrazide	0.5–3.2	0.0031 (370 nm)	3.31×10^4	43
	1.0–7	0.0077 (530 nm)	1.32×10^4	43

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Complexometric Back-titrations: a Theoretical Restatement

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The requirements for complexometric titrations to be theoretically feasible are examined, employing a simple criterion based on the evaluation of the titration error in a given range of the measured variable around the equivalence point. It is shown that the feasibility of a back-titration is essentially conditioned by the complex formed by the chelating agent either with the analyte or the titrant metal ion, having the lower stability constant. In contrast, the ratio between the stability constants of such complexes is not so important as is commonly believed; however, it determines the choice of a suitable indicator.

The same conclusions are reached by using newly developed logarithmic diagrams of equilibrium concentrations for back-titration systems.

Keywords: *Complexometric titrations*; *back-titrations*; *titration error*; *logarithmic diagrams*; *potentiometric titrations*

Back-titrations are a well established practice in complexometric analysis, when slow rates or some other reason do not allow direct titrations. An excess of chelating agent Y is allowed to react with the analyte metal ion M^{m+}; the excess is then titrated with a standard solution of a different metal ion, N^{n+}, in the presence of a system able to act as an end-point indicator, such as a metallochromic indicator or an electrode responsive to the titrant cation.

In spite of the wide use of this titration method, the theoretical prerequisites for the feasibility of back-titrations, as regards the ratio between the conditional stability constants of the chelates of N, K'_{NY}, and of M, K'_{MY}, appear to be incorrectly stated in the current literature. It is usually claimed that "the conditional stability constant of NY must not be greater than that of MY, for otherwise N would displace M from MY,"[1] according to the reaction

$$N + MY \rightarrow M + NY \qquad (1)$$

and "all of the complexing agent added to the solution would be titrated"[2]; in other terms, the equilibrium constant of reaction (1)

$$K'_{N,M} = \frac{[M'][NY']}{[N'][MY']} = \frac{K'_{NY}}{K'_{MY}} \qquad (2)$$

"must" not be higher than one.

In practice, however, one can find in the literature[3] several back-titration methods for which an inspection of the relevant constants shows that $K'_{MY} < K'_{NY}$, *i.e.*, $K'_{N,M} > 1$. Moreover, mathematical expressions for potentiometric back-titration curves have recently been obtained and it has been shown, by calculating and representing titration curves at diverse $K'_{N,M}$ values, that also titrations with $K'_{N,M} \gg 1$ can give sharp end-points.[4,5] However, these conclusions have not reached a wide audience, probably owing to the indirect and mathematically elaborate way in which they have been obtained.

The aim of this work is to show how the feasibility of complexometric back-titrations can be directly evaluated. A simple relationship between the titration error and the titration parameters (equilibrium constants and analytical, *i.e.*, total concentrations), which allows an easy appreciation of the "sharpness," *i.e.*, of the intrinsic precision of back-titrations, will be obtained. As a consequence, the feasibility criterion quoted above will be shown to be definitively incorrect.

The sharpness of a titration can also be judged by means of a logarithmic diagram of equilibrium concentrations[6]; for this purpose, diagrams for back-titrations will be developed.

In order to illustrate the approach chosen, a direct titration will be considered first. Symbols for true equilibrium quantities will be used; conditional (primed) quantities can be substituted if necessary.

Titration Error and Theoretical Accuracy and Precision in Direct Complexometric Titrations

The theoretical accuracy of a titration can be determined by calculating the titration error, ϵ, at the pre-selected value of a relevant variable that defines the end-point. For instance, in the titration of N by Y, the end-point is generally defined by a given value of the equilibrium concentration of the metal ion, $[N]_e$, and the relative error is expressed by the equation

$$\epsilon = \frac{n_Y - n_N}{n_N} = \frac{C_Y - C_N}{C_N} = \frac{[Y] - [N]}{C_N} \quad .. \quad .. \quad .. \quad (3)$$

where n is the amount of a reagent in moles, $C_Y = [Y] + [NY]$, and $C_N = [N] + [NY]$ are analytical concentrations. When [Y] is expressed as a function of [N], equation (4) is obtained[7]:

$$\epsilon = \frac{C_N - [N]}{C_N\,[N]\,K_{NY}} - \frac{[N]}{C_N} \quad .. \quad .. \quad .. \quad .. \quad (4)$$

If NY is a stable complex, [N] is negligible with respect to C_N in the proximity of the equivalence point (and, in every instance, when an excess of Y is present); then equation (4) reduces to

$$\epsilon = \frac{1}{[N]\,K_{NY}} - \frac{[N]}{C_N} \quad .. \quad .. \quad .. \quad .. \quad (5)$$

A more significant evaluation of the titration method can be obtained by taking into consideration the uncertainty in the identification of the end-point. For example, when the end-point is obtained by means of a dichromatic indicator for N, having the stability constant K_{NIn}, the relative titration error can be calculated by means of equation (4) at both extremes of the transition range of the indicator or, conventionally, at $pN \equiv -\log[N] = \log K_{NIn} + 1$ and at $pN = \log K_{NIn} - 1$. In this way, two limiting values of ϵ are obtained, which can be taken as a measure of the theoretical precision range of the method.

On the other hand, the intrinsic precision of the titration, independent of the method chosen for the end-point and depending only on the titration parameters, can be determined by calculating ϵ at the extremes of a pN interval, symmetric with respect to the equivalence or stoicheiometric point, pN_s, and having a conventionally fixed amplitude[8]; the interval $pN_s \pm 1$, corresponding to the transition range of an optimum indicator, can be conveniently taken. It can be noted that the intrinsic precision is inversely related to the mean slope of the titration curve, pN *versus* ϕ (where $\phi = C_N/C_Y = 1 + \epsilon$ is the titration ratio), around the equivalence point. Therefore, its calculation corresponds to the determination of the "steepness" of the potentiometric end-point,[8] an allowance being made for the effect of the charge of the ion towards which the electrode is responsive.

In a titration of N with Y,

$$[N]_s = (C_N/K_{NY})^{\frac{1}{2}} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (6)$$

as derived from equation (5) for $\epsilon = 0$. Therefore, ϵ must be calculated at $pN = \frac{1}{2}(\log K_{NY} - \log C_N) \pm 1$. In order to have an intrinsic precision better than $\pm 1\%$, *i.e.*, $|\epsilon| < 0.01$, $[N]_s$ must be lower than $0.001\ C_N$. Therefore, K_{NY} must be higher than $10^6/C_N$. For example, when $C_N = 10^{-2.0}$ and $K_{NY} = 10^{8.0}$, equation (4) gives $-0.01 < \epsilon < 0.01$ in the range $4.0 < pN < 6.0$. At this value of C_Y, lower values of K_{NY} are not consistent with the specified theoretical precision.

Back-titrations

In the titration by N of the amount of Y exceeding the amount of analyte M, the stoicheiometric condition at the equivalence point is $n_N = n_Y - n_M$, *i.e.*, $C_N = C_Y - C_M \equiv \Delta C_Y$. Consequently, the relative titration error is defined by the equation

$$\epsilon = \frac{C_Y - C_M - C_N}{C_M} \quad .. \quad .. \quad .. \quad .. \quad (7)$$

On introduction of the mass balance equations for Y, M and N,

$$C_M = [M] + [MY] \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (8)$$

$$C_N = [N] + [NY] \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (9)$$

and

$$C_Y = [Y] + [MY] + [NY] \quad .. \quad .. \quad .. \quad .. \quad (10)$$

the following expression is obtained:

$$\epsilon = \frac{[Y] - [M] - [N]}{C_M} \quad .. \quad .. \quad .. \quad .. \quad (11)$$

Also in this instance, the titration can be appraised by calculating ϵ either for $pN = pN_S \pm 1$ when the intrinsic precision of the titration is wanted, or for $pN = \log K_{NIn} \pm 1$ when one is concerned with a given indicator. For this purpose, [Y] and [M] in equation (11) must be expressed as functions of [N]. If a precision of better than $\pm 5\%$ on calculated values of [Y], [M] and ϵ is not required,[9] a simple relationship between ϵ and [N] can be obtained as follows.

If NY is a stable complex, the [N] term in equation (9) can be omitted provided that $C_N < 1.05\Delta C_Y$ (*i.e.*, for $1 > \epsilon C_M/\Delta C_Y > -0.05$); therefore, when C_N differs by less than 5% from ΔC_Y (for $|\epsilon| < 0.05\Delta C_Y/C_M$), one can write

$$[NY] \approx C_N \approx \Delta C_Y \quad .. \quad .. \quad .. \quad .. \quad .. \quad (12)$$

and consequently the following expression for [Y] is obtained:

$$[Y] = \frac{[NY]}{K_{NY}[N]} \approx \frac{C_N}{K_{NY}[N]} \approx \frac{\Delta C_Y}{K_{NY}[N]} \quad .. \quad .. \quad .. \quad (13)$$

If MY is also a stable complex, the amount of free M will be negligible in the mass balance of M until 5% of it is displaced by N, that is, at least as long as $C_N < C_Y - 0.95C_M$ ($1 > \epsilon > -0.05$); thus

$$[MY] \approx C_M \quad .. \quad .. \quad .. \quad .. \quad .. \quad (14)$$

From equations (2), (12) and (14), the following expression for [M] is derived:

$$[M] = K_{N,M}\left(\frac{C_M[N]}{\Delta C_Y}\right) \quad .. \quad .. \quad .. \quad .. \quad (15)$$

Finally, taking into account equations (13) and (15), equation (11) becomes

$$\epsilon = \frac{\Delta C_Y}{K_{NY}C_M[N]} - \frac{K_{N,M}[N]}{\Delta C_Y} - \frac{[N]}{C_M} \quad .. \quad .. \quad .. \quad (16)$$

In order to deal more conveniently with equation (16), one can assume $\Delta C_Y = C_M$ (which is in agreement with the common practice of using an excess of chelating agent comparable to the amount of analyte), so obtaining the equation

$$\epsilon = \frac{1}{K_{NY}[N]} - \frac{[N]\,(K_{N,M} + 1)}{C_M} \quad .. \quad .. \quad .. \quad .. \quad (17)$$

which, according to previous assumptions, is valid for $|\epsilon| < 0.05$.

When $K_{N,M} \ll 1$, equation (17) reduces to

$$\epsilon = \frac{1}{K_{NY}[N]} - \frac{[N]}{C_M} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (18)$$

By comparing this equation with equation (5), it is seen that for the back-titration of M by N, ϵ is represented by the same function of [N] as that found for the direct titration of an equivalent amount of N by Y. Therefore, even the intrinsic precision must be the same in both instances.

When $K_{N,M} \gg 1$, equation (17) reduces to

$$\epsilon = \frac{1}{K_{NY}[N]} - \frac{[N]K_{N,M}}{C_M} \quad .. \quad .. \quad .. \quad .. \quad (19)$$

According to equation (15), the ratio between the equilibrium concentrations of both metal ions is

$$\frac{[M]}{[N]} \approx K_{N,M}\left(\frac{C_M}{\Delta C_Y}\right) \quad .. \quad .. \quad .. \quad .. \quad (20)$$

and, for $\Delta C_Y = C_M$,

$$\frac{[M]}{[N]} \approx K_{N,M} = \frac{K_{NY}}{K_{MY}} \quad .. \quad .. \quad .. \quad .. \quad (21)$$

Taking into account equation (21), equation (19) can be written as

$$\epsilon = \frac{1}{K_{MY}[M]} - \frac{[M]}{C_M} \quad .. \quad .. \quad .. \quad .. \quad (22)$$

As equation (22) is evidently identical with the equation for the relative error of the direct titration of M with Y, the intrinsic precision must also be the same. Moreover, owing to equation (20), a given pM interval corresponds to a pN interval of equal amplitude; therefore, both $pM = pM_s \pm 1$ and $pN = pN_s \pm 1$ correspond to the same error range. As a consequence, the intrinsic precision does not change whether indicators and electrodes for M or for N are employed.

Although the above deductions are strictly valid only when the excess of chelating agent is equal, or at least comparable, to the amount of M, it can be concluded that the intrinsic precision of a back-titration, and thus its theoretical feasibility, depends essentially on which is the lowest stability constant, either K_{MY} or K_{MY}. Neither the higher constant nor the ratio between K_{NY} and K_{MY} is a truly determining factor. A sufficiently precise back-titration is theoretically feasible when, and only when, *both* constants are high enough [$|\epsilon| < 1\%$ for K_{MY} and K_{NY} both higher than about $10^6/C_M$; see discussion of equation (5)].

The value of $[N]_S$, which is required for the evaluation of the intrinsic precision of a specific titration and also for the choice of the proper indicator, can be calculated by means of the equation

$$[N]_S = \Delta C_Y K_{N,M}^{-\frac{1}{2}}(K_{MY}\Delta C_Y + K_{NY}C_M)^{-\frac{1}{2}} \quad .. \quad .. \quad .. \quad (23)$$

or

$$[N]_S = \left[\frac{C_M}{K_{N,M}(K_{NY} + K_{MY})}\right]^{\frac{1}{2}} \quad .. \quad .. \quad .. \quad .. \quad (24)$$

which are obtained by imposing $\epsilon = 0$ in equation (16) or (17), respectively. As an example, when $C_M = \Delta C_Y = 10^{-2.0}$ mol dm^{-3}, $K_{MY} = 10^{8.0}$ and $K_{NY} = 10^{12.0}$, we have $K_{N,M} = 10^{4.0}$ and $[N]_S = 10^{-9.0}$, and equation (22) gives ± 0.01, *i.e.*, $\pm 1\%$, as the intrinsic precision. On the other hand, when $K_{MY} = 10^{12.0}$, $K_{NY} = 10^{8.0}$ and the concentrations are the same as above, we have $K_{M,N} = 10^{-4.0}$ and $[N]_S = 10^{-5.0}$, and the intrinsic precision again is $\pm 1\%$.

A comparison of equation (23) with equation (6) shows that when $K_{NY} \gg K_{MY}$, the $[N]_S$ value for the back-titration of M with N is very different from its value in the direct titration

of an identical concentration of N with Y, or of Y alone with N. Therefore, the back-titration can be affected by a large systematic error when the indicator most suitable for the direct titration of N is chosen. In this instance, according to equation (6) the equilibrium concentration of N at the end-point is

$$[\mathrm{N}]_e = \left(\frac{\Delta C_Y}{K_{NY}}\right)^{\frac{1}{2}} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (25)$$

and the theoretical accuracy of the back-titration, according to equation (16), is

$$\epsilon = -\frac{K_{NY}^{\frac{1}{2}}}{K_{MY}\Delta C_Y^{\frac{1}{2}}} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (26)$$

On the basis of equation (26), it is possible to predict, for a given set of C_M, C_Y and K_{MY} values, the maximum value of the stability constant of the titrant metal ion, $(K_{NY})_{max.}$, which allows an absolute value of the systematic error lower (that is, a theoretical accuracy better) than a required limit. In Fig. 1 the value of $\log(K_{NY})_{max}$ for $|\epsilon| < 0.001$ at $C_M = \Delta C_Y = 1.0 \times 10^{-2}$ mol dm^{-3} is plotted against log K_{MY}, according to the equation

$$\log(K_{NY})_{max.} = 2\log|\epsilon| + \log\Delta C_Y + 2\log K_{MY} \quad .. \quad .. \quad (27)$$

which is obtained by rearranging equation (26) into a logarithmic form. K_{MY} and K_{NY} values lower than 10^8, for which the intrinsic precision of the titration would be worse than $\pm 1\%$ (see above), were not taken into account. Fig. 1 shows that in no instance does K_{NY} need to be lower than K_{MY}; with increasing values of K_{MY}, the ratio between both constants, $K_{N,M}$, can increase from one to several powers of ten.

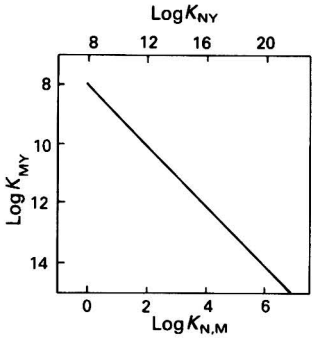

Fig. 1. Maximum values of K_{NY} and of $K_{N,M}$ at which $|\epsilon| < 0.1\%$ for [N] = [Y]. $C_M = \Delta C_Y = 1.0 \times 10^{-2}$ mol dm^{-3}.

The theoretical precision of back-titrations with the indicators considered here can be calculated by means of equation (16), with [N] values corresponding to $pN = pN_e + 1 = \frac{1}{2}\log K_{NY} + \frac{1}{2}\log\Delta C_Y + 1$ and to $pN = pN_e - 1 = \frac{1}{2}\log K_{NY} + \frac{1}{2}\log\Delta C_Y^{-1}$. It can easily be shown that for pairs of stability constants lying on the straight line in Fig. 1, the precision range is from +0.01 to −0.02 at the lower end, and eventually becomes from 0.00 to −0.01.

Logarithmic Diagrams for Back-titrations

In order to represent correctly and conveniently by means of logarithmic diagrams[6] equilibrium (or conditional) concentrations in back-titrated solutions, $pN \equiv -\log[N]$ has to be chosen as the master variable (diagrams with pY as the abscissa[10] cannot account for the variation in the total amount of N, the titrant metal, during the titration). Such diagrams can be drawn in a simple manner with the usual precision (*i.e.*, within 0.02 of a logarithmic

unit, which corresponds to $\pm 5\%$ of equilibrium concentrations) only provided that the analyte remains nearly completely complexed. In fact, when $[M] < 0.05\ C_Y$, the equation

$$\Delta C_Y = [Y] + [NY] - [M] \quad .. \quad .. \quad .. \quad .. \quad (27)$$

which is obtained by subtracting the mass balance equation (8) from (10), can be simplified to

$$\Delta C_Y = [Y] + [NY] \quad .. \quad .. \quad .. \quad .. \quad (28)$$

Combining equation (28) with the expression for K_{NY}, the following equations are obtained for log[Y] and log[NY] against pN, respectively:

$$\log[Y] = \log \Delta C_Y - \log (1 + K_{NY}\ 10^{-pN}) \quad .. \quad .. \quad .. \quad (29)$$

$$\log[NY] = \log \Delta C_Y - \log \left(1 + \frac{10^{pN}}{K_{NY}}\right) \quad .. \quad .. \quad .. \quad (30)$$

On the basis of these equations, graphs of log[Y] and log[NY] against pN, together with the log[N] straight line, can be drawn by the usual procedure,[10] as for the titration of pure Y solution at a concentration equal to ΔC_Y.

Moreover, when $[M] < 0.05\ C_M$, *i.e.*, $[MY] = C_M$, the equilibrium concentration of the complex MY is represented by the horizontal line

$$\log[MY] = \log C_M \quad .. \quad .. \quad .. \quad .. \quad .. \quad (31)$$

In addition, the following expression for log[M] is obtained from equation (2):

$$\log[M] = \log C_M + \log K_{N,M} - \log[NY] - pN \quad .. \quad .. \quad (32)$$

Substituting equation (30) for log[NY], we finally obtain

$$\log[M] = \log C_M - \log \Delta C_Y + \log K_{N,M} + \log \left(1 + \frac{10^{pN}}{K_{NY}}\right) - pN \quad .. \quad (33)$$

For $pN > \log K_{NY} + 1.3$, equation (33) reduces to

$$\log[M] = \log C_M - \log \Delta C_Y - \log K_{MY} \quad .. \quad .. \quad .. \quad (34)$$

so that log[M] can be represented against pN as a horizontal line. In contrast, for $pN < \log K_{NY} - 1.3$, equation (33) reduces to

$$\log[M] = \log C_M - \log \Delta C_Y + \log K_{N,M} - pN \quad .. \quad .. \quad (35)$$

according to which log[M] is represented by a straight line having a slope of -1, and intersecting the horizontal line [equation (34)] at $pN = \log K_{NY}$. The lower pN limit for the validity of all equations above is met, according to the initial hypotheses, when the log[M] graph reaches either $\log C_M - 1.3$ or $\log \Delta C_Y - 1.3$ as the ordinate value.

As an example, the logarithmic diagram for the titration with N, $K_{NY} = 10^{8.0}$, of a solution containing 1.0×10^{-2} mol dm^{-3} of M, $K_{MY} = 10^{12.0}$, and 1.5×10^{-2} mol dm^{-3} of Y is represented in Fig. 2. Point A, at pN = 5.15, marks the equivalence condition, $\epsilon = 0$, *i.e.*, $[M] + [N] \approx [N] = [Y]$ [see equation (11)]. It can be seen that the pN value at the equivalence point is virtually identical with that for the titration of the excess amount of Y alone [see equation (3)]. The transition range of the optimum indicator is shown by vertical dashed lines; their intersections with lines Y, M and N give, using equation (11), the intrinsic precision range of the back-titrations as $+0.007 > \epsilon > -0.007$.

Fig. 3 refers to the case where $K_{NY} = 10^{12.0}$, $K_{MY} = 10^{8.0}$ and the M and Y total concentrations are the same as above. The equivalence condition is met at point B (pN = 9.3), where $[M] + [N] \approx [M] = [Y]$. The intrinsic precision of the back-titration is ± 0.01.

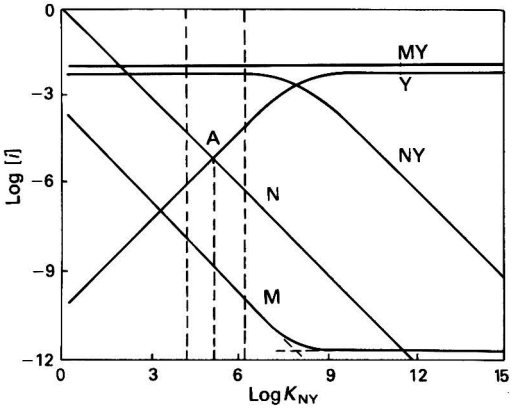

Fig. 2. Logarithmic diagrams of equilibrium concentrations for a back-titration system with $C_M = 10^{-2.00}$, $\Delta C_Y = 10^{-2.30}$ mol dm^{-3}, $K_{MY} = 10^{12.0}$ and $K_{NY} = 10^{8.0}$.

Considering the titration of the excess of Y alone, the log[Y] graph would extend as shown by the broken line in Fig. 3 and point C at pN = 7.15 would correspond to the equivalence point; if an indicator having its transition point at this pN value was to be employed for the back-titration, it would give a systematic error much worse than −5%.

Fig. 4 represents a system with K_{NY} and K_{MY} both equal to 10^8 (the concentrations are the same as above). Point D, at pN = 5.4, characterises[11] the equivalence condition, [M] + [N] = [Y]. The intrinsic precision is ±0.013. Point E, at pN = 5.15, which corresponds to the equivalence point of the titration of pure Y at C_Y, is very near to D; therefore, an indicator suitable for the direct titration of N can be used, with a low systematic error ($-0.02 < \epsilon < +0.007$).

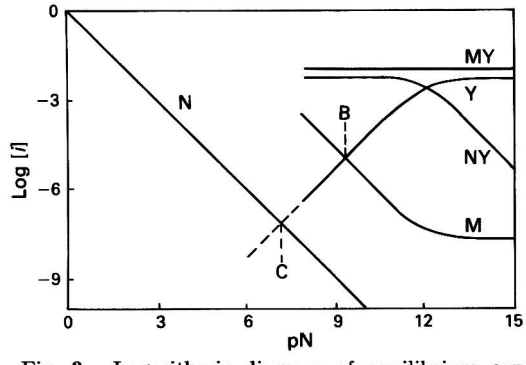

Fig. 3. Logarithmic diagram of equilibrium concentrations for a back-titration system with $C_M = 10^{-2.00}$, $\Delta C_Y = 10^{-2.30}$ mol dm^{-3}, $K_{MY} = 10^{8.0}$ and $K_{NY} = 10^{12.0}$.

A comparison between the examples above shows that a change in $K_{N,M}$ from 10^{-4} to 10^4, provided that the *lower* stability constant remains unchanged, does not substantially alter the theoretical precision of the back-titration. On the contrary, the choice of the indicator is critical.

A decrease in C_Y causes a decrease in the log[Y] graph and a concomitant increase in the log[M] graph to the same extent [see equations (29) and (33)], so that for the system in Fig. 3 neither the ordinate of point B nor the intrinsic precision change. In Fig. 2, as the log[N] graph stays unchanged, point A is lowered, so that the theoretical precision of the back-titration is slightly better: therefore, it appears to be convenient to add as small an excess of chelating agent as possible.

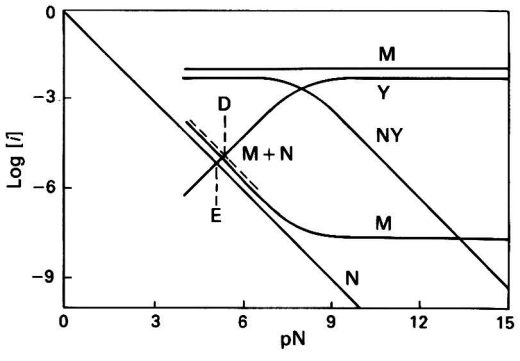

Fig. 4. Logarithmic diagram of equilibrium concentrations for a back-titration system with $C_M = 10^{-2.00}$, $\Delta C_Y = 10^{-2.30}$ mol dm^{-3}, $K_{MY} = 10^{8.0}$ and $K_{NY} = 10^{8.0}$. At pN <6.7, as log[M] = log[N] + 0.3 = log2[N], log([M] + [N]) = log3[N] = log[N] + 0.48 (broken line).[11]

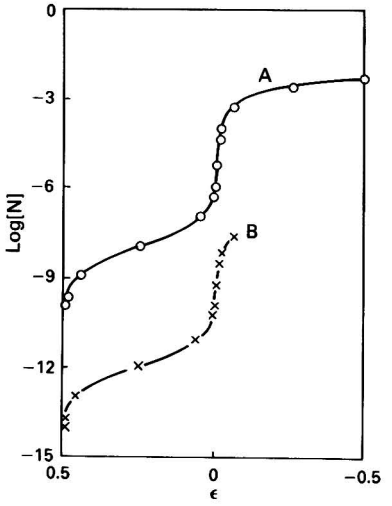

Fig. 5. pN *versus* error plots for the titration by N of a solution containing 1.00×10^{-2} mol dm^{-3} of M and 1.50×10^{-2} mol dm^{-3} of EDTA. A, $K_{MY} = 10^{12.0}$, $K_{NY} = 10^{8.0}$ (see Fig. 2); and B, $K_{MY} = 10^{8.0}$, $K_{NY} = 10^{12.0}$ (see Fig. 3).

Titration curves of log[N] *versus* ϵ or pN *versus* ϵ are easily obtained either by means of equation (16) or by inserting in equation (11) series of sets of equilibrium concentrations, drawn from the relevant logarithmic diagram at appropriate pN values. For the initial part of the titration curve, equation (36) is more conveniently used:

$$\epsilon = \frac{\Delta C_Y}{C_M} - \frac{[NY] + [N]}{C_M} \quad .. \quad .. \quad .. \quad .. \quad (36)$$

As an example, Fig. 5 shows titration curves for the systems represented in Fig. 2 (curve A) and in Fig. 3 (curve B). Both curves show a definite break at the equivalence point, and confirm that in both instances potentiometric titrations are theoretically feasible.

Practical Examples

Several practical examples of back-titrations, which confirm the theoretical findings reported so far, can be found in the existing literature.

For instance, the back-titration of aluminium(III) is presented in a leading textbook[3] as a student experiment. A hot solution containing an excess of EDTA and PAN as indicator, buffered at pH 5 with acetic acid - acetate, is titrated with a copper(II) standard solution. Conditional constants (calculated at 25 °C according to Ringbom[12]) are $K'_{AlY} = 10^{9.6}$, $K'_{CuY} = 10^{11.3}$ and $K'_{CuIn} = 10^{7.9}$. The theoretical error, calculated by means of equation (17) for a solution containing $10^{-2.00}$ mol dm^{-3} of aluminium and $10^{-1.70}$ mol dm^{-3} of EDTA ($\Delta C_Y = 10^{-2.00}$), is $\epsilon = +0.4\%$ at pCu = 8.9 (*i.e.*, at the beginning of the colour transition of PAN) and $\epsilon = -0.06\%$ at pCu = 6.9 (the end of the colour transition); therefore, the titration should have a more than acceptable precision, notwithstanding the high value of $K'_{Cu,Al}$ (= $10^{1.7}$). This prediction is confirmed by laboratory practice.

As regards potentiometric titrations, Khalifa *et al.*[13] performed potentiometric back-titrations of calcium + EDTA with mercury(II) and a mercury electrode in ammonia buffers at various pH values. The potential break they found at the equivalence point becomes more and more abrupt as the pH increases from 8.0 (where, in 0.1 M buffer, $K'_{CaY} = 10^{8.3}$ and $K'_{HgY} = 10^{10.8}$)[14] to 11.0 (where $K'_{CaY} = 10^{10.6}$ and $K'_{HgY} = 10^{10.8}$).[13] This fact has been attributed to the decrease in $K'_{Hg,Ca}$ (from $10^{2.5}$ to $10^{0.2}$); in contrast, in the light of the present discussion, it appears to be due to the increase in the stability constant of the calcium complex.

Conclusions

The simple criterion for evaluating the feasibility of back-titrations that we have developed, making use either of simplified equations or of logarithmic diagrams, allows us to state that a back-titration is theoretically feasible when both the analyte and the titrant metal ions give stable complexes (*i.e.*, both K'_{NY} and K'_{MY} have to be higher than $10^6/C_M$), independent of the ratio between their stability constants. When K'_{NY} is not higher than K'_{MY}, the indicator most suitable for the direct titration of N can be used; in contrast, when K'_{NY} is much higher than K'_{MY}, an indicator having a larger K'_{NIn} value has to be chosen.

It must be concluded that the possibility and the convenience of performing back-titrations, which probably have been frequently overlooked owing to erroneous beliefs, should be reconsidered.

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SHORT PAPERS

Determination of Fluorine in Environmental Standard Reference Materials with a Fluoride Ion-selective Electrode

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Keywords: Fluorine determination; environmental standards; rapid combustion; fluoride ion-selective electrode

Fluorine is one of several trace elements in coal receiving much attention owing to its possible harmful ecological effects. This element is essential to both plant and animal life at low concentrations, but is toxic at higher concentrations.

The potential toxicity of fluorine has been classified as "high" for terrestrial life and "low" for aquatic life. Although fluorine is present in very small amounts in coal, its high volatility and increasing world consumption may result in quantitatively significant emissions. In 1973 Goldberg[1] estimated, for instance, that about 21% (31112 tons) of the total fluorine emission in the USA originated from coal combustion. This percentage is likely to increase significantly when it is considered that, according to the Council on Environmental Quality,[2] coal production in the USA is expected to increase 5-fold between 1975 and 2000, and that about 50% of mined coal will be burned directly in power stations.

From this point of view, and considering that none of the NBS Environmental Standard Reference Materials gives a stated concentration for fluoride (the only published data available are those reported by Gladney[3] for NBS 1632 and NBS 1633), it became particularly important to compare the results obtained by different workers and to establish an accurate, precise and rapid method for the determination of this element.

Most published methods involve distillation,[4] pyrohydrolysis,[5–7] alkaline fusion[8–12] or decomposition in a bomb,[10] followed by spectrophotometric or potentiometric measurement. In some instances these methods are complex and time consuming (distillation techniques), give incomplete breakdown of the fluoride-containing components in coal and losses of fluoride during de-pressurisation of the bomb (decomposition in a combustion bomb), or lead to a loss of the element by volatilisation (alkaline fusion) if the fusion temperature is not closely and accurately controlled.

In this paper, a rapid method for the determination of fluoride in various inorganic materials is described. The procedure is based on sample combustion in a high-frequency induction furnace, collection in an alkaline solution of the fluoride liberated and subsequent measurement of the element by means of an ion-selective electrode. Standard Reference Materials NBS 1633 and 1633a (Trace Elements in Coal Fly Ash), NBS 1632 and 1635 (Trace Elements in Coals), NBS 1645 (River Sediment) and Eurostandard 681–1 (Iron Ore) were analysed by using the standard additions method. In addition, the method described here and the conventional alkaline fusion method were compared in the analysis of NBS 1632 and NBS 1633.

Experimental

Apparatus

The high-frequency induction furnace (Leco, Laboratory Equipment Corporation, Model 632–000) employed in all the experiments was equipped with a high-frequency generator, a heating chamber and a voltage regulator.

The heating chamber (Fig. 1) consisted of a high-frequency induction coil, a fusion crucible and a quartz tube through which flowed an oxygen flux, previously dried in a Leco Purifying Train No. 516–000. Under the best working conditions, the highest possible temperature was about 1400–1500 °C.

Once the combustion was completed, the gases were carried to the collection system, consisting of two bubblers each containing 50 ml of 0.05 N sodium hydroxide solution. In order to prevent possible condensation of the gases on leaving the combustion furnace, the collecting line was heated by means of a Variac transformer. The assembly is shown in Fig. 2.

A Mettler DL 40 Memotitrator was used as a digital millivolt - pH meter in conjunction with an Orion 96–09 combination fluoride electrode. The reference electrode compartment was filled with Orion 90–00–01 filling solution containing sodium, potassium, nitrate and chloride ions and wash saturated with silver(I) ions.

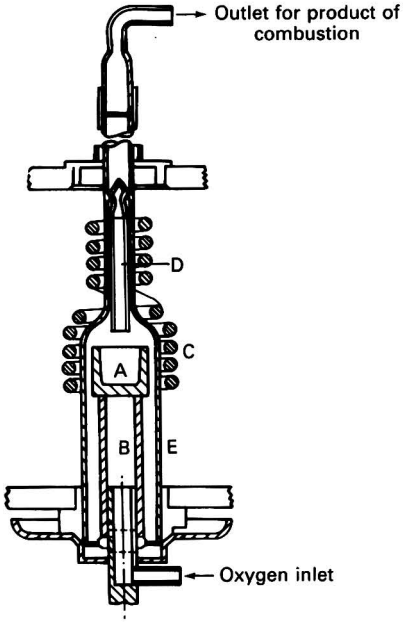

Fig. 1. The high-frequency induction furnace: A, crucible; B, ceramic pedestal; C, high-frequency induction coil; D, igniter; and E, quartz combustion tube.

Reagents

All reagents were of analytical-reagent grade. De-ionised, distilled water was used for the preparation of all standard solutions.

Standard fluoride solutions were prepared from Orion 94–09–07 sodium fluoride standardising solution containing 100 μg ml^{-1} of fluoride. The pH of the solutions (initially about 13) was adjusted to 5.0–5.5 by adding Tisab III buffer solution (Orion 94–09–11). The alkaline collecting solution was prepared dissolving 2.0 g of sodium hydroxide in 1 l of water.

Procedure

Sample combustion

Mix 0.5–1.0 g of sample, previously dried to a constant mass, with about 1.0 g of Leco Iron Chip Accelerator (and 1.0 g of magnesium oxide with coal samples; see below) in a porcelain crucible, cleaned by heating to 1000 °C, and place it in the combustion furnace. With an oxygen flux into the quartz tube of 10 ml s^{-1}, heat for the time necessary (about 6 min) to ensure complete sample combustion.

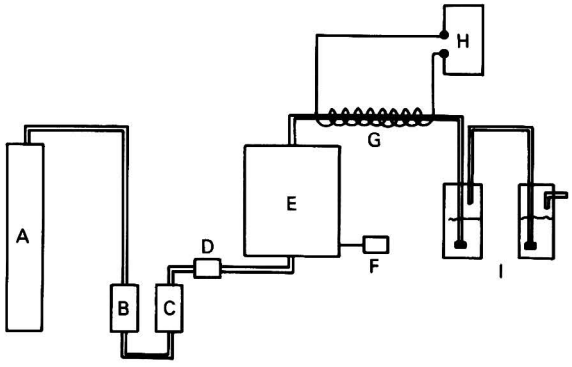

Fig. 2. Measurement assembly: A, oxygen gas supply; B, purifying train; C, flow meter; D, pressure regulator; E, heating unit; F, timer; G, heating coil; H, Variac transformer; and I, bubblers containing the adsorbent solution.

Fluoride measurement

Add 2.0 ml of Tisab III buffer to 20.0 ml of sample solution in a polythene beaker, ensure the contents of the beaker are uniformly at room temperature by using a magnetic stirrer, dip the electrode into the solution and read the potential after 5 min.

Prepare standard solutions with fluoride concentrations between 0.1 and 10.0 μg ml^{-1} and containing sodium hydroxide and Tisab III in the same amounts as in the sample solution. Read the potentials of the standards and samples alternately. When the concentrations under examination are lower than about 1 μg ml^{-1} the response of the electrode is sluggish, the potential readings slowly drift upwards and the equilibrium time of the electrode system may be considerably longer than 5 min.

This situation can be greatly improved if the analysis of fluoride in the solutions is carried out by the standard additions method, adding for instance 1 μg ml^{-1} of fluoride to both sample and blank solutions. All of the results reported were obtained by the standard additions method, which gives a greater accuracy than direct potentiometric measurement.

Results and Discussion

The best conditions were obtained measuring the fluoride recovery in a standard sample with a known concentration [Eurostandard 681–1 (Iron Ore)]. With a flow-rate of 10 ml s^{-1} of oxygen and a heating time of 6 min the recoveries for this sample ranged between 96 and 100%.

For coal samples some workers have experienced problems (possibility of explosive ignition) of over-rapid combustion when using oxygen as the purge gas.[13] This disadvantage has been overcome by adding to the fusion crucible about 1.0 g of magnesium oxide in which the sample to be analysed is dispersed. It was then possible to analyse up to 1.0 g of coal without explosive ignition.

For each standard, amounts of 0.5 g of sample (for NBS 1633, 1633a, 1635 and 1632) or 0.020 g of sample (for NBS 1645 and Eurostandard 681–1) were analysed in accordance with the above-mentioned procedure. The average fluoride concentrations and the relative standard deviations for 6–10 repeated measurements are reported in Table I. The relative standard deviations were on average less than 10%; the highest values were found for samples having the lowest fluoride concentrations. There was good agreement between the certified value (for Eurostandard 681–1) or values in the literature (for NBS 1632 and 1633) and the results obtained in this work.

To confirm the quantitative recoveries of fluoride by the rapid combustion method, an alkaline fusion method[10] was used to decompose the NBS 1632 and 1633 standards. The fluoride results obtained by the two methods are given in Table I. The agreement is satisfactory, which gives further evidence of the validity of the proposed procedure.

TABLE I

RESULTS FOR DETERMINATION OF FLUORINE ($\mu g\ g^{-1}$) IN NBS AND EUROSTANDARD REFERENCE MATERIALS

Sample	No. of Samples	Present method	Fusion method*	Literature values†
NBS 1633	6	20 ± 2	19, 22	20
NBS 1633a	6	23 ± 2	—	—
NBS 1645	8	1336 ± 97	—	—
NBS 1632	10	91 ± 5	88, 92	90
NBS 1635	6	63 ± 4	—	—
Eurostandard 681–1	6	1918 ± 64	—	1940 ± 148‡

* Alkali fusion method described by Thomas and Gluskoter.[10]
† Values reported by Gladney.[3]
‡ Certified value.

By using the proposed method the analysis time is reduced to a few minutes (15–20 min per sample), compared with the many hours required by the fusion method. In addition, the alkaline collecting solution does not contain common interferents such as Al^{3+}, Fe^{3+}, Ca^{2+}, Mg^{2+} and Ti^{2+} that are present in the samples, and therefore the subsequent potentiometric reading is virtually devoid of interferences.

I thank F. Stragliati for valuable technical assistance.

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Determination of Toluene-2,4-diisocyanate in Rubber Fumes

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Keywords: *Toluene-2,4-diisocyanate determination*; *high-performance liquid chromatography*; *workplace atmosphere*; *rubber fumes*

One of the newer cross-linking reagents developed by The Malaysian Rubber Producers' Research Association[1–3] now being commercially used in several rubber factories, contains an adduct of 2,6-dimethyl-4-nitrosophenol and toluene-2,4-diisocyanate dimer (TDI), and is sold

as Novor 924. The adduct contains no free isocyanate group, but at natural rubber vulcanisation temperatures (150–200 °C) it dissociates giving rise to the free nitrosophenol and TDI. The free nitrosophenol combines via a cyclo-addition reaction giving rise to aminophenol pendant groups on the rubber chain that react with the TDI to give cross-linked rubber. Theoretically, excess of TDI may be generated to react with the pendant groups; however, as free TDI is fairly reactive some may be lost by reaction at other sites within the rubber and, owing to an appreciable volatility, some may even escape to the atmosphere with the vulcanisation fumes. Novor 924 is often used in conjunction with small amounts of sulphur, and its usual accelerators (*e.g.*, cyclohexylbenzthiazolyl sulphenamide, CBS), but the same considerations apply.

As the level of TDI in the working environment has strict limits[4] imposed [at present the time-weighted average - short-term exposure limit (TWA–STEL) value is 0.14 mg m^{-3}] it must be determined as accurately as possible and preferably by the simplest methods available. Meddle and co-workers[5,6] and the Health and Safety Executive Booklet No. 20[7] give modifications to the method originally published by Marcali.[8]

In the first instance, the simpler of the two procedures recommended in ref. 4 was investigated to check its applicability to the determination of TDI in rubber fumes. The method is based on the reaction of an isocyanate with an acid. Hydrolysis of the isocyanate group gives rise to an aromatic amine, which, by diazotisation and coupling reactions, gives a coloured solution, the colour intensity of which can then be measured using a comparator. The simplest method fails in the presence of rubber fumes as aromatic amines are often present in vulcanisates in the form of antioxidants. As all aromatic amines give a similar colour reaction falsely high levels of TDI result.

It was thought that the second method in the Health and Safety Executive Booklet No. 20[7] should overcome this problem as it uses two absorber tubes; in one tube the isocyanate is inactivated by reaction with an aliphatic amine and only the interfering aromatic amine should be measured; in the other tube both the amine and the isocyanate are determined; the level of TDI is then determined by difference. However, even when sampling the fumes from vulcanisation of natural rubber using a sulphur cure without Novor 924 addition, positive levels of TDI were found (see Table I). Hence, even this method appears unsatisfactory for use in the presence of rubber fumes.

TABLE I

APPARENT LEVELS OF TDI FOUND IN NON-NOVOR CURES BY HEALTH AND SAFETY EXECUTIVE (HSE) AND HPLC METHODS

Sampling position	Procedure	Method	Apparent TDI/mg m^{-3}
Press daylight*	HSE	II	0.14
	HPLC		N.d.†
Closed container	HSE	II	0.70
	HPLC		N.d.

* Press daylight is a term used in the rubber industry to describe the gap between the heated platens of the moulding press.
† N.d. = not detected.

The third method investigated was the high-performance liquid chromatographic (HPLC) method of Bagon and Hardy[9] in which the TDI is trapped in ethanol to form the ethyl urethane derivative that is determined by HPLC. As well as any TDI a certain amount of vulcanisation fumes are also inevitably trapped in the ethanol and some modifications to the published conditions were required in order to separate those peaks produced by the vulcanisation fumes from the peak due to TDI. No TDI was found in the sulphur-containing vulcanisates and only low levels when Novor 924 cures were used; the results are shown in Table II.

Experimental

Apparatus

The sampling device was a 4 mm i.d. stainless-steel tube 100 mm long dipping into the absorbent via a side-arm in a B24 short form test-tube. The lower end of the tube was closed by a fine stainless-steel mesh to break up the gas stream and at the upper end a metal-deflector head was placed to direct the vulcanisation fumes to the sample tube entrance. A

TABLE II

LEVELS OF TDI FROM SULPHUR - NOVOR CURES BY HPLC

All cures were carried out at 200 °C; at a lower temperature (153 °C) slightly higher levels were found, *e.g.*, 0.009 mg m^{-3} at press daylights with 2.4 parts of Novor. These results have been corrected for a 75% recovery found at the 0.01 mg m^{-3} level.

Novor level, p.p.h.*	Sampling position	TDI/mg m^{-3}
4.2	Over mill	0.012
2.4	Over mill	0.013
4.2	Press daylight	N.d.†
2.4	Press daylight	0.004
4.2	Closed container	N.d.

* p.p.h. = parts per hundred of rubber by mass.
† N.d. = not detected; the limit of detection was 0.002 mg m^{-3}.

plug of cotton-wool was placed at the tube entrance to remove any particulate matter present in the atmosphere. The amount of cotton-wool used was a small plug of approximately 10 mg. The experiment has been performed with both cotton-wool dried at 100 °C and with cotton-wool straight from the packet. No significant difference has been observed between the results of these experiments. The gas flow through the sampling device was controlled via a Casella pump unit fitted with an integral flow-meter and timer. A 25-ml round-bottomed flask, B24, was cleaned in chromic acid, water and ethanol, washed and dried in an oven at 70 °C.

For liquid chromatography, a Perkin-Elmer LC 601 instrument was used with a Partisil 10 ODS-2 column, using ethanol - water (40 + 60) as the mobile phase. The flow-rate was 1.5 ml min^{-1} and the column temperature was 50 °C. The urethane derivative was detected using a Perkin-Elmer LC 55 spectrophotometer set at 240 nm.

Procedure

Ethanol (10 ml) was placed in the sampling device and the outlet connected via flexible tubing to the Casella pump unit; the pumping rate was adjusted to 1 l min^{-1} before any sampling began. At the end of the 10-min sampling period the ethanol was transferred into the 25-ml round-bottomed flask together with the trap washings, which did not include the cotton-wool plug, as it was the purpose of the plug to ensure that particulate matter is not included in the determination, and allowed to stand for at least 1 h. The combined solution was evaporated almost to dryness using a rotary evaporator with the solution being maintained at 40 °C in a water-bath. The residue was transferred quantitatively into a 1-ml Reacti-Vial using two 100-μl aliquots of ethanol from a syringe and the volume adjusted to the 100-μl mark by blowing nitrogen over the surface of the solution. Samples of 20 μl of this solution were used for HPLC analyses.

Standard Solutions

Two methods of preparation were used; in the first a bulk sample of the ethyl TDI urethane was made, recrystallised from an ethanol - water mixture and dried under vacuum. Its composition was checked by NMR and IR spectroscopy and elemental analysis which gave C 58.9, H 6.7 and N 10.7% (theory requires C 58.7, H 6.8 and N 10.5%). An accurately weighed amount of this material was used. The second method was to weigh accurately TDI monomer from a micro-syringe directly into a known volume of ethanol.

Results

In order to test the over-all recovery of the TDI in an air atmosphere a dynamic system was used. A 0.5-ml aliquot of TDI in dichloromethane was placed in a platinum boat at the exit end of a quartz tube capable of being electrically heated at 200 °C. The outlet was directly connected to the inlet tube of the sampling device, it having been shown that any appreciable length of glass or plastic tube adsorbed the TDI. Heating of the quartz tube and sampling of the air were started simultaneously and continued whilst a 10-l sample of laboratory air was drawn through the tube and trap. The HPLC determination was carried

out on the ethanol solution as described under Procedure. When aliquots of the standard TDI solution at levels equivalent to 0.1 and 0.15 mg m^{-3}, close to the STEL value, were tested by this method, recoveries of 90% were found. The recovery dropped to 80 $\pm$ 5 and 75 $\pm$ 5% when the TDI levels were equivalent to 0.05 and 0.01 mg m^{-3}, respectively. No attempts were made to increase the yield at this stage by using dried air or nitrogen, as this would not be practicable when sampling from a factory environment. There was no detectable carryover of TDI into a second trap connected in series with the first trap when TDI at a level of 1.0 mg m^{-3} was used, and no loss of TDI was observed at the 0.15 mg m^{-3} level in the presence of the cotton-wool plug. The method was found to be capable of determining TDI in the environment down to a level of 0.002 mg m^{-3}.

Atmospheric testing was carried out routinely at the mixing stage of the compound on a mill, at a height of 1500 mm above floor level and 100 mm from the press daylight during cure and in a closed container half filled with the hot, but cooling, vulcanisate.

Discussion

From the results shown in Table I it can be seen that Method II of the Health and Safety Executive Booklet No. 20 is not satisfactory in the presence of rubber fumes. Unidentified components of the fumes given off during vulcanisation give rise to greater colour formation in the tube that should give amine plus hydrolysed TDI, than in the reference tube where the TDI is inactivated. This gives rise to an apparent level of TDI in the fumes from a normal sulphur - CBS curing system, even though TDI is known to be absent. Other curing systems and antioxidants were tried but all gave falsely high TDI levels. The insertion of a pre-washing tube was also tried but the interference could not be completely eliminated.

The HPLC method was tried next, and it was found that the vulcanisation fumes that were trapped in the ethanol could be tolerated, as the peaks arising did not coincide with the peak due to the TDI derivative. In order to establish whether TDI was reacting with vulcanisation fumes in the ethanol, a sample of the TDI standard, generated in a heated tube as previously described, was passed into an ethanolic solution of vapours collected from a sulphur - CBS vulcanisate; again there was an 80% recovery of the TDI showing that the presence of vulcanisation fumes in the ethanol was not causing loss of TDI during the determination. The TDI recovery experiments involving the sulphur - CBS vulcanisate vapours have been repeated using the cotton-wool plug. The recovery levels of TDI were found to be 75 $\pm$ 5%. These agree with the previously found levels without the plug of 80% within experimental error. It is therefore concluded that there is no catalysis of the reaction between TDI and cotton-wool by adsorbed rubber vulcanisation fumes.

The level of TDI first found at the mill mixing stage was surprisingly high (0.074 mg m^{-3}) as the mixes were only carried out on cold mill rolls with the rubber temperature rising only to 45 °C. However, during the addition of Novor 924 on the mill it was observed that a very small amount of the powder was blown into the atmosphere and as the entrance to the sampling device did not contain a filter this particulate matter could be entering the absorbing solution. Support for this hypothesis was shown by the observation of a noticeable yellow colour in one instance. A known amount of Novor 924 was placed in ethanol for 1 h and analysed by HPLC for TDI, when it was found that 4.7% of the Novor 924 did indeed react as though it was TDI. A small cotton-wool plug was placed in the entrance to the stainless-steel tube in order to filter out any particulate matter; analysis of the sampled air showed 0.010 mg m^{-3} of TDI whereas the figure of 0.034 mg m^{-3} TDI was the equivalent figure obtained by analysis of the cotton-wool plug.

Conclusion

There are interferences in the Health and Safety Executive Booklet No. 20 methods, causing falsely positive TDI values. The HPLC method described offers a reliable method for the determination of TDI in the atmosphere in, *e.g.*, the rubber industry working environment. The results show that only very small amounts (well below the present regulation levels) of TDI are found at the vulcanisation stage and no detectable amounts when the vulcanisates are cooling down.

Thanks are due to the Board of MRPRA for permission to publish this work.

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Determination of Fluorometholone Purity by Very High-performance Liquid Chromatography

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Keywords: *High-performance liquid chromatography*; *fluorometholone determination*; *steroids*

Very high-performance liquid chromatography (VHPLC) represents a major advance in liquid chromatographic technology in that the chromatographic performance is significantly improved.[1] Many separations can be completed in a few minutes, with over 10000 theoretical plates.[2]

Fluorometholone (**I**) is used extensively as an anti-inflammatory local steroid in pharmaceutical products. The analysis of this drug is an important quality control step for the raw material[3] and for commercial finished products. The use of convential HPLC for the determination of **I** has been demonstrated previously.[4,5]

In this paper, the application of VHPLC to the determination of **I** is described, both with and without the use of internal standardisation, and with a rapid sample work-up procedure.

Experimental and Results

Methanol was of HPLC grade (Merck) and was used without further purification.

Water was obtained from a Millipore installation and its resistivity was at least 18 MΩ cm^{-1}. It was filtered through a 0.22-μm inert filter (Sartorius).

The procedure is shown schematically in Fig. 1.

	Time required/min
Weigh sample	1
↓	
HPLC	5
↓	
Data reduction and report generation	0.5
	Total: 6.5 (time per sample)

Fig. 1. Scheme of the procedure for the determination of fluorometholone.

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Liquid-chromatographic Conditions

The analysis was performed on a liquid chromatograph (Perkin-Elmer) connected to an automatic programmable integrator (Perkin-Elmer Sigma 10). To avoid possible integrator errors associated with negative base-line disturbances caused by the solvent front, a delay time of 3.5 min was programmed. Injection was performed through a septumless injector equipped with a 10-μl sample loop (Rheodyne, Model 71.25).

The analysis was performed on a Perkin-Elmer HS 5 C 8 column. The elution solvent was methanol - water (58 + 42) and the flow-rate was 2.8 ml min^{-1}.

Ultraviolet detection was effected at 236 nm with a sensitivity of 0.1 a.u.f.s., using an LC 85 variable-wavelength absorbance detector (Perkin-Elmer) equipped with a 2.4-μl flow cell and used in the fast response mode.

Sample Work-up and Analysis

The use of a 5-μm high-speed column, coupled with appropriate instrumentation, permitted a three-fold reduction in analysis time with a resolution equivalent to or greater than those previously obtained[4,5] using conventional columns and instrumentation.

Internal Standard Preparation

A 10-mg sample of fluoxymesterone was weighed accurately into a 10-ml calibrated flask. Methanol was added and the flask was placed in an ultrasonic bath until the compound had dissolved. The solution was made up to volume with methanol. A 5-ml volume of this solution was pipetted into a 200-ml calibrated flask and the volume was adjusted with methanol.

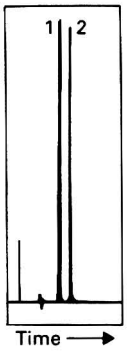

Fig. 2. Typical chromatogram obtained with the VHPLC method. 1, Fluorometholone; and 2, fluoxymesterone.

Fluorometholone Standard Preparation

A 25-mg sample of fluorometholone (USP Standard) was weighed accurately into a 25-ml calibrated flask, 15 ml of methanol were added and the flask was placed in an ultrasonic bath until the compound had dissolved. The solution was made up to volume with methanol.

Sample Preparation

A 25-mg sample of fluorometholone was weighed accurately into a 25-ml calibrated flask, 15 ml of methanol were added and the flask was placed in an ultrasonic bath until the compound had dissolved. The solution was made up to volume with methanol.

Preparation of solutions for analysis

A 1-ml volume of the standard solution was pipetted into a 20-ml calibrated flask and made up to volume with the internal standard solution.

A 1-ml volume of the sample solution was pipetted into a 20-ml calibrated flask and made up to volume with the internal standard solution.

Preparation of Eluent

A 580-ml volume of methanol was mixed with 420 ml of water and stirred with a magnetic stirrer for 15 min. The solution was then filtered through a 0.22-μm filter (Sartorius) and de-gassed with helium for 10 min.

Method of Quantitation

A typical chromatogram is shown in Fig. 2. The peak areas of fluorometholone and fluoxymesterone were measured using a computer (Perkin-Elmer Sigma 10). A calibration

TABLE I

LINEARITY AND REPRODUCIBILITY STUDY BETWEEN THE AREA DETECTED AT 236 nm AND THE AMOUNT OF FLUOROMETHOLONE INJECTED

Fluorometholone injected/μg	Fluoxymesterone injected/μg	Fluorometholone peak area (S_1)	Fluoxymesterone peak area (S_2)	$R = S_1/S_2$	Mean value of R	Standard deviation, σ	Relative standard deviation, %
0.25	0.5	1748	6003	0.2911	0.2942	4.6×10^{-3}	0.5
		1746	6024	0.2898			
		1768	6048	0.2923			
		1845	6111	0.3019			
		1832	6051	0.2997			
		1792	6053	0.2961			
		1784	6090	0.2947			
		1819	6029	0.2986			
		1750	6019	0.2902			
		1735	6111	0.2882			
0.5	0.5	3899	6017	0.6479	0.6424	2.8×10^{-3}	0.4
		3795	5926	0.6043			
		3801	5931	0.6408			
		3809	5947	0.6404			
		3843	5996	0.6409			
		3850	5993	0.6424			
		3932	6098	0.6448			
		3949	6117	0.6455			
		3839	6010	0.6387			
		3873	6026	0.6427			
1	0.5	8074	5951	1.3567	1.3569	0.0129	0.9
		7994	5885	1.3583			
		8038	5912	1.3596			
		8152	6170	1.3212			
		7968	5847	1.3627			
		7918	5797	1.3658			
		7967	5833	1.3658			
		8092	5951	1.3597			
		8130	5978	1.3599			
		8179	6016	1.3595			
1.5	0.5	12168	6163	1.9743	2.0193	0.0427	2.1
		12177	6177	1.9713			
		12164	6193	1.9641			
		11933	5783	2.0634			
		11992	5841	2.0530			
		12095	5897	2.0510			
		12136	5944	2.0417			
		12190	5969	2.0422			
		12249	5854	1.9718			
		12063	5854	2.0606			
2	0.5	16270	5811	2.7998	2.7896	0.0165	0.6
		16233	5749	2.8236			
		16307	5818	2.8028			
		16354	5858	2.7917			
		16350	5836	2.8015			
		16468	5929	2.7775			
		16512	5933	2.7830			
		16414	5874	2.7943			
		16500	5926	2.7843			
		16549	5975	2.7697			

Correlation coefficient = 0.999474.

graph was prepared by plotting peak areas for a series of known concentrations. Values for unknown concentrations in samples were calculated by computer from the standard peak areas.

Reproducibility and Linearity Study

The reproducibility was calculated by repeating the analysis of a standard ten times. The relative standard deviation was 0.9% (Table I).

Accuracy Study

The accuracy of the procedure was calculated by preparing and running four consecutive samples, each sample being injected five times. The relative standard deviation was 0.52% (Table II).

TABLE II

ACCURACY OF PEAK AREAS

Sample No.	Peak area detected for fluorometholone (S_1)	Peak area detected for fluoxymesterone (S_2)	$R = S_1/S_2$	Mean value of R	Relative standard deviation, %
1	8074	5951	1.3567	1.3517	1.2
	7994	5885	1.3583		
	8038	5912	1.3596		
	8152	6170	1.3212		
	7968	5847	1.3627		
2	7918	5797	1.3658	1.3621	0.24
	7967	5833	1.3658		
	8092	5951	1.3597		
	8130	5978	1.3599		
	8179	6016	1.3595		
3	7865	5736	1.3711	1.3697	0.37
	7909	5802	1.3631		
	7917	5796	1.3659		
	7860	5718	1.3742		
	7855	5716	1.3746		
4	7866	5734	1.3718	1.3677	0.28
	7856	5726	1.3719		
	7868	5756	1.3669		
	7915	5806	1.3632		
	7858	5756	1.3651		

Mean value of R = 1.3628.
Mean relative standard deviation = 0.52%.

Detection Limit

The detection limit was 0.5 ng injected.

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Determination of Noscapine and Papaverine in Mixtures

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Keywords: *Noscapine determination*; *papaverine determination*; *titrimetry*

The two opium alkaloids papaverine and noscapine (narcotine) normally appear together during the isolation process because of the similarity of their chemical structures and the closeness of their pK_a values. It is therefore necessary to develop a convenient method for their individual determination in their mixtures.

Noscapine and papaverine in mixtures have been separated by ion-exchange chromatography[1–3] and filtration as bases, which is a time-consuming method. Brauniger and Borgwardt[4] reported a method for their separation and determination in milligram amounts with the use of 1 M citric acid buffer. Another method is the formation of their reineckate complexes.[5,6] In all instances the accuracy is low. Precipitation of acidic solutions of the alkaloids with bismuth - EDTA and potassium iodide is complicated and time consuming and has a low accuracy ($\pm$1.5% error).[7] Paper chromatography has an error of $\pm$1.5–2.5%.[8,9] Other methods used include gas chromatography, high-performance liquid chromatography, spectrofluorimetry, spectrophotometry and NMR spectroscopy.[10–18] These methods have the merit of being microanalytical but in all instances the precision is $\pm$1–1.9%.

A method for the determination of noscapine and papaverine in papaveretum has been described in the BPC,[19] which involves treatment of the mixture with alcoholic alkali and then gravimetric determination of the individual alkaloids after separation from the mixture. This method is also time consuming and cumbersome.

In this work the conditions of the BPC method have been re-examined. The modified method proposed here is quicker and more convenient. The operations of drying, etc., required in the gravimetric method have been replaced with a simple non-aqueous titrimetric method.

Experimental

Preparation of Solutions

Alcoholic sodium or potassium hydroxide solution. An approximately 10% *m*/*V* solution of sodium or potassium hydroxide was prepared in absolute ethanol or methanol by dissolving 1.5 g of the alkali in 15 ml of ethanol or methanol.

Perchloric acid, 0.1 N. An 8.5-ml volume of perchloric acid (70%) was added very cautiously and dropwise to 900 ml of glacial acetic acid, 30 ml of acetic anhydride were added and the volume was made up to 1000 ml with glacial acetic acid.[20]

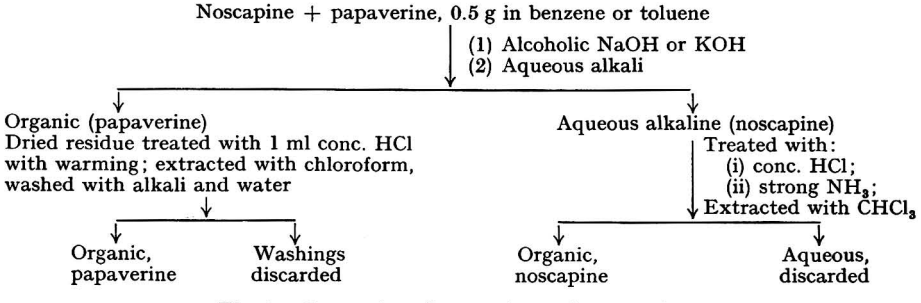

Fig. 1. Separation of noscapine and papaverine.

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Procedure

The procedure is outlined schematically in Fig. 1.

About 0.5 g, accurately weighed, of a mixture of noscapine and papaverine was dissolved in 20 ml of benzene or toluene and shaken with 15 ml of 10% alcoholic sodium or potassium hydroxide solution in a separating funnel for 2 min, and worked up for analysis of noscapine and papaverine as described below.

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

Determination of noscapine

Noscapine was extracted with 20-, 10-, 10- and 5-ml portions of 4% sodium hydroxide solution. To the combined aqueous solution were added 10 ml of concentrated hydrochloric acid and the resulting acidic solution was transferred into a separating funnel and then rinsed with three successive 5-ml portions of water. About 10 ml of concentrated ammonia solution (27.0–30.0% *m*/*V* of ammonia, *ca.* 0.896 g ml^{-1}) were added and the mixture was extracted with successive 20-, 10-, 10- and 5-ml portions of chloroform, washing each extract with the same 10 ml of water. The chloroform extract was dried over anhydrous sodium sulphate, filtered, the sodium sulphate washed twice with 5 ml of chloroform, the washings were added to the extract, the solvent was distilled off and the contents were evaporated to dryness on a water-bath. The residue was dissolved in 10 ml of glacial acetic acid, 5 ml of acetic anhydride were added and the solution was titrated against 0.1 N perchloric acid using crystal violet as the indicator, the colour change being from violet to green. Each 1 ml of 0.1 N perchloric acid is equivalent to 0.041 34 g of noscapine.

Determination of papaverine

The benzene or toluene extract was dried over anhydrous sodium sulphate, filtered, the sodium sulphate washed with solvent and the total extract evaporated. The residue was treated with 1 ml of concentrated hydrochloric acid and heated on a water-bath for 15 min. The solution was transferred into a separating funnel with 20 ml of water and extracted with successive 20-, 10-, 10- and 5-ml volumes of chloroform, washing each extract in turn first with the same 10 ml of 4% sodium hydroxide solution and then with the same 10 ml of

TABLE I

RESULTS FOR DETERMINATION OF NOSCAPINE AND PAPAVERINE IN MIXTURES (TOTAL COMPOSITION = 100%)

Noscapine			Papaverine		
Taken, %	Found, %	Relative difference, %	Taken, %	Found, %	Relative difference, %
84.87	84.37	0.59	15.13	15.22	0.59
84.86	84.40	0.54	15.14	15.16	0.13
84.82	84.82	0.00	15.18	15.10	0.53
84.50	84.50	0.00	15.50	15.50	0.00
79.82	79.45	0.46	20.18	20.05	0.64
74.02	74.98	0.05	25.98	25.92	0.23
70.08	70.01	0.10	29.92	30.06	0.47
64.89	64.73	0.25	35.11	35.05	0.17
59.96	59.73	0.38	40.04	39.95	0.22
55.02	54.64	0.69	44.98	44.85	0.29
50.10	50.34	0.48	49.90	49.66	0.48
49.88	49.78	0.20	50.12	49.91	0.42
49.61	49.50	0.22	50.39	50.30	0.18
44.91	44.86	0.11	55.09	55.08	0.02
40.09	39.75	0.85	59.91	59.89	0.03
35.17	35.11	0.17	64.83	64.55	0.43
30.05	30.02	0.10	69.95	69.73	0.31
25.13	25.14	0.04	74.87	74.90	0.04
20.03	20.09	0.30	79.97	79.98	0.01
15.01	15.04	0.20	84.99	84.55	0.52
Mean, %		0.29			0.29
Standard deviation, % ..		0.24			0.21

water. For the determination of papaverine the chloroform extract was treated as for noscapine. Each 1 ml of 0.1 N perchloric acid is equivalent to 0.03394 g of papaverine.

The results are summarised in Table I.

In another set of experiments after the treatment with 10% alcoholic sodium or potassium hydroxide solution, noscapine was extracted with 20, 10, 10 and 5 ml of water instead of 4% sodium hydroxide solution, without affecting the accuracy of results (Table II).

TABLE II

RESULTS OBTAINED USING EXTRACTION WITH WATER

Noscapine			Papaverine		
Taken, %	Found, %	Relative difference, %	Taken, %	Found, %	Relative difference, %
14.15	14.21	0.42	85.85	85.61	0.28
15.72	15.84	0.76	84.28	84.12	0.19
24.99	24.84	0.60	75.01	74.68	0.44
25.20	25.16	0.16	75.80	75.68	0.16
49.77	49.58	0.39	50.23	50.09	0.28
50.10	50.05	0.10	49.90	49.82	0.16
84.56	84.77	0.25	15.44	15.30	0.91
85.63	85.81	0.21	14.37	14.28	0.63
Mean, %		0.36			0.38
Standard deviation, % ..		0.21			0.25

Results and Discussion

Noscapine is an isoquinoline alkaloid present in opium and is always extracted together with papaverine, which is structurally similar except for the presence of a lactone ring in the former. When a mixture of noscapine and papaverine is dissolved in benzene or toluene and the solution is treated with strong alkali (the alkaline solution is prepared in an alcohol to ensure homogeneity of the reaction medium), noscapine is converted into noscapinate while papaverine remains unaffected. Water or aqueous alkali is then added to the reaction mixture and the noscapinate formed is dissolved in the aqueous layer and separated from papaverine, which remains in the organic layer. The aqueous layer is then treated with strong acid when relactonisation takes place and noscapinate is reconverted into noscapine. Ring opening in the presence of a strong alkali and its simultaneous closure in the presence of a strong acid are both instantaneous and quantitative reactions. Papaverine does not interfere in the noscapine - alkali reaction, thereby ensuring a complete separation of the two alkaloids.

The proposed method presented is a modification of the BPC method.[19] In our hands the BPC method was found to be less satisfactory and more time consuming. The time factor may be very important in process control work as the subsequent steps await analytical results obtained in the previous step. For this reason, there was a need for a rapid and more reliable method for the determination of the individual alkaloids.

Various parameters of the BPC method such as time, temperature, amount and nature of the alkali and the reaction medium were thoroughly investigated, and the results may be summarised as follows.

The reaction of noscapine in alcoholic alkali was complete in less than 2 min whereas the BPC recommended a reaction time of 40 min. We also concluded after comparative studies that sodium hydroxide and potassium hydroxide give identical results, and the same applies to methanol and ethanol. We feel that the reaction of noscapine with alcoholic alkali is so fast that the nature of the alkali or alcohol does not make any detectable difference.

Similarly, during the course of the determination of noscapine, instantaneous relactonisation is achieved on addition of concentrated hydrochloric acid, whereas in the BPC method heating at 95 °C is recommended, which has been found experimentally to be superfluous. Further, as a comparison of the results in Tables I and II shows, the extraction with 4% alkali solution is not necessary. Instead, extraction with water alone gives equally good results (Table II).

The gravimetric method for the determination of the alkaloids is replaced here by a non-aqueous titrimetric method. The BP[21] prescribes a potentiometric titration for non-aqueous

media. We have simplified the method by taking that change in the colour of the indicator which corresponds to the maximum value of dE/dV (where E is the electromotive force and V the volume of the titrant) as the end-point, thereby obviating the need for potentiometry for regular titrations.[21]

As is evident from Tables I and II, the proposed method covers a wide range of compositions of the mixture, whereas in the BPC method the composition range is narrow and the precision of the results is poorer.

The help and encouragement received from the authorities of the Government Opium and Alkaloid Works Undertaking and the provision of the necessary facilities are gratefully acknowledged.

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Communication

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

Very Rapid and Simple Method for Preparing a Glucose Sensor of Good Quality

Keywords: *Glucose sensor*

In a previous paper,[1] a convenient method for preparing a glucose sensor was described. However, the calibration graph was linear only for the range 0–3 mM whereas the normal level of glucose in human blood is in the range 3.8–5.6 mM. We therefore attempted to increase the linear relationship to levels above 5.6 mM.

Glucose oxidase (E.C. 1.1.3.4 from *Aspergillus niger*, 130 IU mg^{-1}; Wako Pure Chemicals, Osaka, Japan) and catalase (E.C. 1.11.1.6 from bovine liver, 2500 IU mg^{-1}; Sigma, St. Louis, MO, USA) were co-immobilised simply by adsorption as follows. A porous acetylcellulose membrane (Millipore, Type HA, pore diameter 0.45 μm, 140 μm thick, total diameter 25 mm) was soaked in 5 ml of an enzyme solution (0.006 M Na_2HPO_4 - KH_2PO_4 buffer, pH 7.2, containing 2.5 mg of each enzyme) and was then dried in a vacuum desiccator (10^{-2} mmHg). This membrane was directly attached to the PTFE membrane of an oxygen electrode (Ishikawa Manufacturing Co. Ltd., Tokyo, Japan) and covered with a dialysis membrane (Visking Co., Chicago, IL, 20 μm thick, 25 mm diameter). The glucose sensor thus prepared was stored in 0.066 M phosphate buffer at 4 °C.

A glass gauging cell was filled with 50 ml of the oxygen-saturated buffer solution at 37 $\pm$ 0.2 °C. Oxygen saturation was achieved by bubbling oxygen in place of air through solution by means of a sintered-glass disc. In addition, the solution was stirred magnetically with a 20-mm PTFE bar during the experiment. When the output of the sensor had reached a steady state, an appropriate volume of 1.6 M glucose in the buffer solution was injected into the buffer solution in the glass cell. The decrease in current was displayed directly together with the dI/dt signal.[2]

The graph of $(-dI/dt)_{max}$ *versus* glucose concentration was linear in the range 0–13 mM and passed through the origin. The response time was only about 15 s. The sensor could be used repeatedly for at least 2 months with little deterioration in response.

We have therefore developed a very simple and rapid method for preparing a glucose sensor of good quality.

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Book Reviews

PYROLYSIS MASS SPECTROMETRY OF RECENT AND FOSSIL BIOMATERIALS. COMPENDIUM AND ATLAS. By HENK L. C. MEUZELAAR, JOHAN HAVERKAMP and FRED D. HILEMAN. *Techniques and Instrumentation in Analytical Chemistry, Volume* 3. Pp. xiv + 293. Elsevier. 1982. Price $67.50; Dfl145. ISBN 0 444 42099 1.

Pyrolysis mass spectroscopy (Py-MS) is not a new technique, having been with us for some 30 years or so. However, it is only in more recent times that the development of new and improved methods of pyrolysis, coupled with parallel improvement in mass spectrometer design, have allowed Py-MS to develop into the important analytical tool it is today. In this context, the authors of this text have found an opportune moment to publish a thorough appraisal of the field with specific attention being given to the study of macromolecular biomaterials. A generous selection of pyrolysis mass spectra, which comprise more than half of the book, are included. They are mainly of biologically important materials, but a useful section on plastics materials is also included. The presentation of the text and figures is clear and concise and the biography extensive, the main areas covered being the origin and development of Py-MS (particularly experimental methods), structural investigations, pyrolysis mechanism and data analysis procedures.

All in all, a most valuable addition to the literature in this field, and the authors are to be congratulated on producing a text which should become the standard introduction for anyone considering entering this rapidly expanding area of analytical chemistry. W. J. CRIDDLE

BENZODIAZEPINES. A HANDBOOK. BASIC DATA, ANALYTICAL METHODS, PHARMACOKINETICS AND COMPREHENSIVE LITERATURE. By HARALD SCHUTZ. Pp. xii + 439. Springer-Verlag. 1982. Price DM198; $88. ISBN 3 540 11270 7; 0 387 11270 7.

"Benzodiazepines" is a handbook containing basic data (*e.g.*, formulae, relative molecular masses, melting-points, biotransformation routes, operating conditions and retention times for the application of TLC and GLC techniques and IR, UV and mass spectra). It presents a selection of analytical methods for the determination of benzodiazepines individually and in mixtures, pharmacokinetic data, such as blood, serum and plasma levels at varying times following administration and a survey of benzodiazepine literature, which are of particular importance to those concerned with the detection and determination of these drugs and their metabolites in biological materials. Nineteen commercially available 1,4- and 1,5-benzodiazepines, together with 23 important metabolites and 18 hydrolysis derivatives to which benzodiazepines are degraded prior to the application of certain analytical techniques, such as gas chromatography of the resulting benzophenones and visible spectrophotometry of the coloured derivatives produced by diazotisation of the amino groups in these benzophenones followed by their coupling with *N*-(1-naphthylethylenediamine) are surveyed in this manner.

The TLC data were collected on silica gel 60 F_{25} with three solvent systems, chloroform - acetone (90 + 10 *V*/*V*), benzene and benzene - propan-2-ol - 25% ammonia solution (85 + 15 + 1 *V*/*V*) and GLC was carried out on the stationary phases 3% SE-30 and 3% OV-17 on Chromosorb G AW DMCS (80–100 mesh) at three column temperatures, 220, 250 and 280 °C. IR and mass spectra are presented very clearly and only a little information is given on the allocation of the observed IR spectral bands to specific bonds in the molecules and on the MS fragmentation patterns, respectively. The UV data are presented in both tabular and graphic forms and in three media, absolute ethanol, 0.1 N hydrochloric acid and 0.1 N sodium hydroxide solution.

Presentation of selected analytical methods is in two sections: one for the detection and determination of benzodiazepines in mixtures with each other and other drugs and the other for the determination of a single "special" benzodiazepine. These presentations take the form of tables with headings that include analytical technique used, literature reference, volume of sample required, extraction procedure, concentration range analysable, minimum detectable amount, day-to-day precision and percentage recovery. Twenty methods are presented for the determination of chlordiazepoxide (Librium) in mixtures using GLC, TLC, differential-pulse polarography, radioimmunoassay and HPLC techniques, but no critique is given on the relative merits of the analytical methods. The same is true of the ten methods listed for its "special" determination.

Pharmacokinetic data are collected from the literature from pages 205 to 244, in tabular form under the major headings of drug administered, by what route and whether or not it was a single or multiple dose study. Sub-headings in this section include nature and volume of samples analysed, value and time of peak concentration and number of subjects studied in the survey. The ranges of maximum concentrations of selected benzodiazepines after oral administration are also presented graphically.

The literature survey is extensive, covering pages 245 to 278, and is collected under 35 sub-headings. The volume is completed by several alphabetical listings of references, no doubt trying to keep pace with the rapidly increasing literature on this subject, and finally a subject index.

This publication is valuable as an up-to-date reference work on benzodiazepine literature and on the relevant chemical, analytical and pharmacological data contained therein. It merits a place in the laboratories and libraries of those institutions where analytical, pharmacological, toxicological and clinical investigations are taking place.

W. F. SMYTH

INTRODUCTION TO HIGH-SPEED LIQUID CHROMATOGRAPHY. By J. L. DICESARE, M. W. DONG and L. S. ETTRE. Pp. vi + 106. Perkin-Elmer. 1981. Price £6.

This short booklet falls somewhere between a genuine independent monograph and an advertising booklet for specific instrumentation, in this instance the new Perkin-Elmer equipment for fast liquid chromatography. Over the last 10 years or so it has become obvious that existing commercial HPLC equipment is seriously inadequate in respect of extra-column dispersion and detector time constant. This has been particularly demonstrated by the recent work on narrow-bore packed columns. It is therefore a little galling to have these long required improvements being advanced as "the greatest thing since sliced bread." Nevertheless, the improvements that can be realised by using an adequate detector - injector - coupling system of low dispersion and low time constant are substantial and should certainly be publicised. Basically, what Perkin-Elmer have done is to provide a system whose detector, at least, gives a dispersive standard deviation of around 10 μl, which is about one quarter of that obtained using more conventional equipment. The authors devote much time and space and give numerous examples to show how the performance of columns packed with 3-μm particles can be more fully exploited using the new system. Unfortunately, they also demonstrate with somewhat naive and presumably inadvertent clarity that their total system is far from good enough to exploit their advantages to the full! In their Fig. 6, for example, they show a plot of plate number, N, against k' for a 3-μm particle column. For $k' = 0$, $N \approx 2000$, while for $k' = 7$, $N \approx 14000$. Everyone knows that for a good analytical column N should be almost independent of k' or indeed should actually be largest when $k' = 0$. The discrepancy is entirely an instrumental effect. From the figure it is readily deduced that the dispersive standard deviation of the equipment is about 25 μl, not 10 μl as claimed, without a column. For the full capability of the column to be realised the equipment would actually need to have a dispersive standard deviation of around 3 μl. Hence there is still some way to go and the manufacturer will have to look into this carefully.

The book contains many elegant chromatograms, including gradient separations. It is aimed at the moderately experienced liquid chromatographer and explains in a simple way how separations can be optimised. It is a good advertisement for Perkin-Elmer's new equipment, even if it also points clearly to its inadequacies.

JOHN H. KNOX

CHROMATOGRAPHIC SEPARATION AND EXTRACTION WITH FOAMED PLASTICS AND RUBBERS. By G. J. MOODY and J. D. R. THOMAS. *Chromatographic Science Series, Volume* 21. Pp. viii + 139. Marcel Dekker. 1982. SwFr89. ISBN 0 8247 1549 7.

This is Volume 21 in the *Chromatographic Science Series* and deals with the properties and uses of the ubiquitous polyurethane foams in the inorganic and organic fields of separation and extraction in analytical science.

Five chapters go to make up the book, the first on the nature and properties of polyurethanes,

followed by inorganic applications of the foams in aqueous media, concentration of organic compounds by this means from dilute samples, and concluding with foam chromatography of organic compounds.

The authors clearly have made a detailed study of the subject and know a lot about it—a review by them having appeared in *The Analyst* in 1979, from which this volume seems to be a natural progression.

There is discussion of both loaded and unloaded foams, the latter for example being employed as a means of recovery of precious metals from dilute solutions, mention also being made of the recovery of gold, antimony and tin. Loaded foams are described, particular note being made of tributyl phosphate as loading in the separation of various inorganic elements, including gold, zinc, iron and antimony. Di(2-ethylhexyl)phosphoric acid and silicone rubber are both indicated to be loadings that offer promise in the inorganic field.

In the chapter on the concentration of organic compounds from dilute solutions and air, pesticides feature prominently—a rather interesting experiment mentioned being that of a field test for polychlorobiphenyls. Phthalate plasticisers and nicotine are among those compounds concentrated by means of such foams.

The incorporation of stationary phases into foams for use in gas - liquid chromatography and open-pore polyurethane and modified foams for liquid - liquid and gas - solid chromatography are described in the final chapter.

The volume as a whole contains 128 pages of text, tables, etc. (together with 147 references and an index), on a somewhat specialised topic; however, it is interesting and informative reading and raises the question as to why the techniques described are not used more widely.

D. SIMPSON

STABLE ISOTOPES. PROCEEDINGS OF THE 4TH INTERNATIONAL CONFERENCE, JÜLICH, MARCH 23–26, 1981. Edited by H.-L. Schmidt, H. Förstel and K. Heinzinger. *Analytical Chemistry Symposium Series, Volume* 11. Pp. xviii + 775. Elsevier. 1982. Price $127.75; Dfl275. ISBN 0 444 42076 2.

With the development of increasingly sensitive and selective methods of analysis, stable isotopes are becoming extensively used for investigations covering a broad range of scientific research. Not only have measurements of natural isotopic abundance ratios led to advances in subjects as diverse as geology and botany, but with the ever decreasing cost of materials labelled with these isotopes they are now being used as tracers and probes for investigations of, for example, metabolic pathways. Perhaps even more significant is their use in medicine for drug distribution and metabolism studies where, in many instances, they can replace radiolabelled materials and thus overcome the hazards associated with even low doses of radiation. The multi-disciplinary nature of research with stable isotopes is well reflected in this book, which contains 100 papers presented at the fourth International Conference on Stable Isotopes, held in Jülich in March, 1981. Both reviews and original papers are included, but with the reproduction of 100 articles these are, out of necessity, brief. Production, which is of a high standard, is by photographic reproduction of authors' typewritten originals.

Section 1 of the book is concerned with the theory and consequences of isotope effects and contains 11 papers on topics such as isotopic fractionation in biological systems and the effects of isotopes on various enzymes. Geochemistry and cosmochemistry are covered in Section 2 (13 papers) and the reports are mainly concerned with the measurement of natural isotopic ratios in rock and water samples. Many of the papers have a historical theme with discussions on the origins of the solar system, the Earth's crust and life. A third of the book is devoted to biomedical applications with sub-sections on pharmacology and drug metabolism, clinical diagnosis and breath tests. Here one is dealing mainly with the administration of labelled forms of a drug or biochemical as tracers or with the use of stable isotopes as standards for quantification. Measurements of bioavailability, studies on the pharmacokinetics of drugs during chronic treatment using the pulse labelling technique and various aspects of metabolic switching are all discussed. Section 4 (19 papers) deals with applications to agriculture and environmental research with studies on the measurement of nitrogen balance and on the possibility of using stable isotopes to control food products. Experimental methods are covered in Section 5. There is heavy emphasis both here and in the rest of the book on mass-spectrometric techniques, although one of the reviews (N. A. Matwiyoff) is

concerned exclusively with ^{13}C NMR spectroscopy. Nine papers are devoted to isotopic separation and the synthesis of labelled compounds. The book concludes with both author and subject indexes.

This book is basically the record of a meeting and, although the subject matter is multi-disciplinary, the use of stable isotopes is still a specialised subject. The relatively high cost of the volume will almost certainly restrict its circulation to libraries. However, this book brings together a useful collection of reviews and applications, which, as the organisers of the meeting hope, should stimulate further research into the applications of these increasingly useful isotopes.

D. J. HARVEY

ANALYSIS OF POLYMER SYSTEMS. Edited by L. S. BARK and N. S. ALLEN. Pp. xii + 311. Applied Science Publishers. 1982. Price £28. ISBN 0 85334 122 2.

This is a book of eight chapters by eight contributors. The aim of the book is to create an awareness in polymer analysts of the more important applications and developments that have been made in this field. Each chapter deals with different techniques, some new and some well established. The whole book is in the nature of a series of reviews rather than practical worked examples. The emphasis throughout is on synthetic polymers with only the occasional mention of natural polymers. Although the title is "Analysis of Polymer Systems" there is only brief reference to plasticisers, stabilisers, pigments, etc.

The book starts with a general introduction to polymers with some useful tables on classification according to elemental analysis, behaviour on heating and solubility. This is followed by a chapter on microstructural analysis of synthetic polymers by high-resolution NMR. This review is comprehensive covering ^{1}H and ^{13}C NMR with 215 references. Two of the more established techniques, IR and Raman spectroscopy, are then briefly illustrated with a few examples. Wisely, reference is made to a number of other publications where this subject can be studied in greater detail. The relatively new technique of luminescence (fluorescence and phosphorescence) spectroscopy is discussed in Chapter 4. Sections on the theory and measurement of polymer luminescence are very readable and help to understand the applications of the technique. Mass spectrometry (MS) is usually considered a technique for analysis of low relative molecular mass organic compounds; however, the chapter on the analysis of polymers by MS demonstrates with many good examples the progress that has been achieved recently in polymer characterisation by MS; 89 references back up this review.

Chapter 6, on the now routine thermoanalytical techniques, I found heavy going. The range of techniques is amply described and the theory for each developed mathematically. For those wishing to delve deeply into the subject there are 112 references. Following thermal methods of analysis there is a very readable chapter on relative molecular mass determination by ebullioscopic methods (boiling-point elevation). This review may have been more useful had other relative molecular mass determination methods been compared, *e.g.*, vapour pressure osmometry. The final chapter on gel permeation chromatography is an excellent review with 472 references. Theory and practice are explained and there is an abundance of application illustrations. Reference to HPLC, which is now often side by side with GPC, is scant.

Overall this book is very readable. Attention is given to the theory, practice and application of eight important techniques. The book will be of interest to analytical chemists in industry, government laboratories and academic institutions who are concerned with the analysis of synthetic polymers.

R. M. CLARKE

ENVIRONMENTAL CARCINOGENS SELECTED METHODS OF ANALYSIS. Volume 4. SOME AROMATIC AMINES AND AZO DYES IN THE GENERAL AND INDUSTRIAL ENVIRONMENT. Edited by H. EGAN, L. FISHBEIN, M. CATEGNARO, I. K. O'NEILL and H. BARTSCH. *IARC Publications* No. 40. Pp. xiv + 347. International Agency for Research on Cancer (distributed by the World Health Organization). 1981. Price SwFr60; $30. ISBN 92 8 321140 5.

The International Agency for Research on Cancer (IARC) regards publication of carefully selected analytical methods as an important part of their drive to encourage the generation of truly reliable data on carcinogens. This book forms part of a series in which IARC collects together analytical

methods for specific groups of compounds. Like other parts of the series, this volume on aromatic amines and azo dyes has been the subject of extensive scrutiny by eminent editorial and review boards prior to publication. While the bulk of this volume is devoted to the presentation of detailed analytical methods, there are a number of background chapters which I found both interesting and readable. The first two chapters, for instance, are collected together under the umbrella of "Biological Activity." They give a useful short history of the recognition of aromatic amines as carcinogens and present a comprehensive tabulation of compounds evaluated by IARC, classified according to the current knowledge on their carcinogenicity. The second chapter introduces the important concept of metabolic activation and its role in the reaction of carcinogens with large biological molecules such as DNA.

In a series of three chapters brought together under the theme of "Occurrence and Monitoring in Industry," there is a refreshingly straightforward collection of thumb-nail sketches of the various short-term predictive tests for carcinogenicity. The ways in which these biological procedures can be used to monitor human exposure to aromatic amines are also illustrated.

Over one-third of the book is devoted to the publication of analytical methods for the determination of aromatic amines of industrial importance in a variety of media including air, soil, groundwater, laboratory animal diet and human urine. Methods are presented in the ISO format and consequently are split into readily digestible segments which I found particularly easy to follow. Each method is divided into short sections including scope and field of application, the principle, hazards, reagents, sampling procedure and calculation of results. Literature references, frequently to the authors' own published work on the subject, are given with each method. Despite the fact that each author includes a short paragraph on the hazards of the compound under investigation, I noted that several methods still involve the use of benzene as a solvent.

The remainder of the book is devoted to non-industrial exposures. The subjects range from the formation of heterocyclic amines in cooked foods through drugs and food colours to laboratory wastes. Again there are useful introductory descriptions followed later by detailed analytical methods. In this section I was surprised to see paper chromatography being recommended as an analytical method for food colours.

In general terms I found this a useful book which collected together a mass of information which otherwise would take the reader a great deal of time and effort to assemble. I would strongly recommend this book as a starting point for anyone who is contemplating the analysis of aromatic amines.

G. T. Steel

Spectrophotometric Study of the Ruthenium(III) - 2-Thiobarbituric Acid System

Ruthenium(III) forms a 1:2 complex with 2-thiobarbituric acid, with an absorption maximum at 338 nm; the molar absorptivity is 1.1×10^4 l mol^{-1} cm^{-1} and the Sandell's sensitivity of the reaction is 0.0091 μg cm^{-2} per 0.001 absorbance unit. Beer's law is obeyed for up to 28 μg ml^{-1} of ruthenium. The molar composition of the complex was determined by the molar ratio method and confirmed by means of elemental analysis. Co-ordinate bonding involving the sulphur atom of 2-thiobarbituric acid was proved by infrared spectroscopy. The tolerance of the system to platinum metals and other common cations is reported.

A statistical evaluation of the proposed method has been undertaken and a comparison with important spectrophotometric reagents for ruthenium(III) used in recent years is presented.

Keywords: *Ruthenium determination*; *2-thiobarbituric acid*; *spectrophotometry*

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Analyst, 1983, **108**, 386–394.

Complexometric Back-titrations: a Theoretical Restatement

The requirements for complexometric titrations to be theoretically feasible are examined, employing a simple criterion based on the evaluation of the titration error in a given range of the measured variable around the equivalence point. It is shown that the feasibility of a back-titration is essentially conditioned by the complex formed by the chelating agent either with the analyte or the titrant metal ion, having the lower stability constant. In contrast, the ratio between the stability constants of such complexes is not so important as is commonly believed; however, it determines the choice of a suitable indicator.

The same conclusions are reached by using newly developed logarithmic diagrams of equilibrium concentrations for back-titration systems.

Keywords: *Complexometric titrations*; *back-titrations*; *titration error*; *logarithmic diagrams*; *potentiometric titrations*

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Analyst, 1983, **108**, 395–403.

Determination of Fluorine in Environmental Standard Reference Materials with a Fluoride Ion-selective Electrode

Short Paper

Keywords: *Fluorine determination*; *environmental standards*; *rapid combustion*; *fluoride ion-selective electrode*

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Analyst, 1983, **108**, 404–407.

Determination of Toluene-2,4-diisocyanate in Rubber Fumes

Short Paper

Keywords: *Toluene-2,4-diisocyanate determination*; *high-performance liquid chromatography*; *workplace atmosphere*; *rubber fumes*

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Analyst, 1983, **108**, 407–411.

Determination of Fluorometholone Purity by Very High-performance Liquid Chromatography

Short Paper

Keywords: *High-performance liquid chromatography*; *fluorometholone determination*; *steroids*

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Analyst, 1983, **108**, 411–414.

Determination of Noscapine and Papaverine in Mixtures

Short Paper

Keywords: *Noscapine determination*; *papaverine determination*; *titrimetry*

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Research and Development Laboratory, Government Opium and Alkaloid Works Undertaking, Neemuch 458 441 (M.P.), India.

Analyst, 1983, **108**, 415–418.

Very Rapid and Simple Method for Preparing a Glucose Sensor of Good Quality

Communication

Keywords: *Glucose sensor*

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Analyst, 1983, **108**, 419.

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