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Quantitative Determination of 2-Propanol in Sulphonamide Tablets using Headspace Analysis

Synopsis

Volatile components were desorbed from sulphonamide tablets under controlled conditions and trapped cryogenically at the inlet of a packed GC column. Temperature programming was used to separate the components and the content of 2-Propanol was determined with a repeatability of 0.05% (2.7σ).

Key Words

(Residual) solvents/Tablets/Headspace.

Introduction

The analysis of the gaseous phase allowed to equilibrate above a sample, "headspace analysis", is particularly useful in the food and flavouring industries, as a more representative picture of the volatile components, (the aroma), can often be obtained than from the main sample. The food industry also uses the technique to analyse for taints in products and as a check on the quality of packaging materials.

In the present case, headspace analysis is used to measure the residual 2-Propanol in sulphonamide tablets not completely removed in the manufacturing process.

Three samples of tablets were analysed using the Pye Unicam PU4750 Headspace Analyser. This unit mounts on the injection port of the gas chromatograph and is constructed so that sample contamination of the inner surfaces is reduced to a minimum. The front section contains a three-way valve which both allows the headspace itself to be flushed on to the column and allows the lines to be backflushed to prevent cross contamination while the sample chamber is isolated from the column.

The sample chamber is located in the rear, demountable, section of the unit, together with the heaters and thermostat which control the sample temperature.

Duplicate samples from each of the three batches of tablets A, B and C were equilibrated in the sample chamber for twenty minutes at 130°C. The sample chamber was then flushed with argon for eight minutes, volatile materials being trapped on a short section of the column which was cooled with a jet of liquid carbon dioxide. After this time, the temperature of this section was raised to oven temperature and the sample swept on to the main part of the column for analysis using the conditions listed below.

Instrumentation

Pye Unicam series 304 Gas Chromatograph; CDP4 Data Processing Unit Column: 2.1 m × 4 mm 3% OV11 on Chromosorb WHP 100–120 Column temperature: 40–120°C at 20°C/min then 120°–240°C at 10°C/min
Injector temperature: 230°C
Detector: single FID
Detector temperature: 240°C
Carrier gas: argon 50ml/min
Attenuation: × 64 (range on FID amplifier 10^2 and 10^3)

Results and Discussion

The following table summarizes weights of each sample before and after headspace analysis, giving lost volatiles as actual weight and % of sample weight. The area of the 2-Propanol peak is calculated by the CDP4 as percentage of total eluent peaks and 2—Propanol thus calculated back to % of original sample.

Low/medium polar stationary phase OV-11 was used instead of Carbowax or porapak types and relatively long equilibration and flushing times were selected because it was not known what other volatiles might be present. A number of such compounds were shown to be present but were not identified.

Further Information

Please contact I. S. Gilkison, Pye Unicam Ltd, York Street, Cambridge, Great Britain CB1 2PX [Telephone (0223) 358866].

TABLE

Sample	Weight	Weight after headspace analysis	Loss of weight g (*)	Loss of weight related to sample weight (%)	Area of 2-Propanol (%)	Weight of 2-Propanol related to sample weight (%)
A	2.9500	2.9352	0.0148	0.50	21.02	0.11
A	2.9835	2.9686	0.0149	0.50	18.21	0.09
B	2.4363	2.4180	0.0183	0.75	87.85	0.66
B	2.4478	2.4288	0.0190	0.78	86.20	0.67
C	2.3086	2.2961	0.0125	0.54	97.66	0.53
C	2.2891	2.2755	0.0136	0.59	85.59	0.51

(*) Loss of weight includes water loss which is not detected by FID but which is small enough not materially to affect the result.

Methods for the Determination of Carbon in Soils and Sediments. A Review

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Summary of Contents

Keywords: *Review; carbon determination; soils; sediments*

Introduction

Numerous research areas in the chemical and biological sciences (*e.g.*, limnology, zoology, geochemistry and agronomy) utilise carbon measurements of total, inorganic and/or organic carbon. Examples of such research include studies of fluxes in aquatic ecosystems,[1–3] the behaviour of organic compounds in water,[4,5] the sorption properties of sediments and soils,[6–9] sedimentation rates in lakes,[10,11] organic - inorganic interactions in waters,[12] diagenetic processes in sediments[13] and studies that characterise sediments and soils.[14,15] These topics represent a small portion of the studies that involve carbon measurements but serve to indicate the diversity of research to which these measurements are applied.

Unfortunately, one method is not used consistently between similar research projects, so limitations with respect to cross-comparisons of data are likely to arise. This occurrence has already been observed by some investigators.[16,17] Information regarding methods used for carbon determination and their comparative differences is thus crucial when choosing a method and interpreting the resulting data. This review presents information on the determination of carbon in sediments and soils. Many workers have examined these methods on an individual or a comparative basis.[18–28] Emphasis is therefore placed on presenting pertinent information rather than summarising all the literature.

Analytical methods for determining carbon in soils and sediments rely on the combustion of the carbonaceous materials and indirect or direct measurement of the CO_2 evolved. Two common procedures used for this analysis are dry combustion and wet oxidation. Dry combustion methods involve combustion of the sample at high temperatures (>900 °C) with subsequent measurement of the evolved CO_2 either photometrically, gravimetrically, volumetrically, conductimetrically or titrimetrically. A summary of these procedures is presented in Table 1. In wet oxidation methods, either the CO_2 evolved or the amount of oxidising agent used in the process is measured. Techniques used for the determination of the CO_2 evolved are the same as for dry combustion methods. The measurement of the oxidising agent consumed generally involves back-titration of the oxidising agent. Both of these methods can be used to determine the percentage total, inorganic and organic carbon. Differentiation between inorganic and organic carbon can be achieved by acid digestion to remove the carbonate carbon, and the use of selective reagents to inhibit organic carbon oxidation or the use of temperatures specific for non-carbonate or carbonate carbon decomposition.[22,24,28–34]

Another method used to give an estimate of organic matter is the mass loss on ignition, which involves combustion of the sample in a furnace and measurement of the mass before and after combustion. The mass loss determined at the appropriate temperatures (350–1000 °C) is a measure of the non-carbonate and carbonate carbon content of the sample. Large sources of error are inherent in this method and are generally attributed to mass losses of non-organic materials, such as carbonates and clay minerals, at high temperatures.[35] Hence loss on ignition will not be considered at length in this comparative study but will only be mentioned whenever measurements for estimates of carbon are compared.

Methods

Dry Combustion

In this method, the sample is combusted in a stream of CO_2-free O_2 or an inert carrier gas. The combustion gases are then passed through a series of traps to remove any interferences (*e.g.*, particulates, sulphur, halogens, nitrogen oxides and/or water vapour), through a catalyst furnace to ensure complete oxidation and finally to the appropriate detection point. The catalyst furnace is generally used with

Table 1. Techniques for measuring CO_2

Type of analysis	Common means of analysis	Type of measurement
Photometric . .	Infrared analyser	Measures differential absorption of infrared energy between CO_2 and reference gas
Gravimetric . .	Sorption trap	Measures mass gain in trap after combustion
Volumetric . .	Gas burette	Measures volume of CO_2 released after combustion or acid digestion
Conductimetric . .	Thermal conductivity detector	Measures differential resistance between CO_2 and reference gas
Titrimetric . .	Non-aqueous titration	Measures amount of CO_2 consumed in solution by back-titration with standard solution

medium-temperature furnaces and the detection system is dependent on the type of analysis chosen. A simplified schematic diagram of a combustion train is presented in Fig. 1.

The types of furnaces used to achieve combustion are the resistance, induction or tube type. In resistance furnaces the heat is derived from high-resistant materials. In medium-temperature furnaces (900–1000 °C) Nichrome is often used for the heating element, whereas in high-temperature furnaces (1400–1600 °C) silicon carbide is the popular choice of material. These materials surround a combustion tube within which the sample is contained. Accelerators such as tin or copper(II) oxide can be mixed with the sample to aid the combustion. Induction furnaces are heated by high-frequency electromagnetic radiation and reach temperatures exceeding 1500 °C. Soils and sediments do not heat by induction and therefore analysis of these samples requires the use of a susceptor in the form of tin or iron chips, siliceous earth bed and/or quartz-enclosed crucibles.[20,29] Tube-type furnaces can also be used and are commonly operated at 900–1100 °C; their construction has been described.[32,36–39]

Several studies have been made of the efficiency of dry combustion methods and a summary is given in Table 2. The average mean recovery is 95.86% with a relative standard deviation of 2.05%. Examination of the range of recoveries should be treated with caution, as low values of 33, 50 and 67% were obtained in the analysis of two or three samples. The recovery of 33% was obtained only once in the analysis of ingot iron. Other recoveries obtained from this sample were 125 and 108%.[40] In the analysis of seawater, a 50% recovery was reported for heptanoic acid. This compound is volatile and therefore low recoveries are expected.[17]

Dry combustion techniques can be used to measure total, inorganic or organic carbon if the sample is combusted at different temperatures or pre-treated by acid washing or digestion (Table 3). The different composition temperatures of inorganic or organic carbon can be used for the determination of these components. Such techniques are often used in conjunction with commercial analysers that employ photometric or conductimetric detectors in which the sample is combusted at 650–720 °C and 950–1100 °C.[27,44,45] The lower temperature range combusts the organic carbon and the higher temperature range combusts this fraction plus the inorganic carbon. Inorganic carbon is determined by difference. Inaccurate measurements of the organic carbon, however, can occur as a result of carbonate decomposition and/or incomplete organic carbon oxidation at the lower temperatures.[44,46] The inorganic carbon can also be determined by combustion of the sample at different oxidation intervals and extrapolation of the data to zero time.[45] Alternatively, the organic carbon may be removed by baking at 500 °C and the inorganic carbon measured in the remaining sample. Errors associated with this method can be caused by carbonate decomposition.[47] Total, organic and/or inorganic carbon measurements can also be made by analysing the sample before, after or upon acid treatment. The measurements before and after acid treatment are used to calculate the total and organic carbon, respectively. The inorganic carbon can then be determined by difference or by measuring the CO_2 evolved on acid digestion of the sample.

Procedures that are easily adapted to such analyses involve volumetric (gasometric) measurements. This type of determination is made in the presence of an antioxidant (*e.g.*, $FeSO_4$) to reduce organic carbon decomposition. Accurate quantitation by this method requires that the sample be completely dried before analysis and completely digested by the acid. This method therefore should not be used to analyse samples that contain compounds such as iron sulphide that interfere with gas production.[48–50] Organic carbon can be determined by the difference between the total and inorganic carbon or by analysis of the acid-treated residue. Acid treatment to remove carbonates may involve the use of a separate digestion apparatus, acid washing the sample or bubbling sulphur dioxide through a suspension of the sample.[30–34] Errors associated with these analyses stem from the subtraction of one large number from another when analysing high-carbonate samples,[51,52] and removal of organic carbon during acid washing or digestion.[13,22,52] Another method for the determination of organic carbon minimises these errors.[51] In this method the residue and supernatant are analysed for organic carbon after treatment with phosphoric acid, sonication and centrifugation of the mixture.

Wet Oxidation

In these procedures an oxidising agent, generally potassium dichromate or potassium persulphate in an acid, is used to oxidise the carbonaceous materials in the sample. The methods differ in the oxidising solution, digestion temperature, digestion time used and the means for measuring

Table 2. Recoveries with dry combustion methods

Reference	Materials analysed	Recovery, % Range	Average
40	NBS steel standards	33–125	89
41	Thiourea	96–103	100
41	Benzoic acid	100–101	100
41	Five organic compounds	100–104	101
42	Seventeen organic standard solutions	98–100	99
43	NBS carbonate standards, Na_2CO_3, $CaCO_3$	99–100	100
17	Five organic compounds spiked in seawater	50–104	88

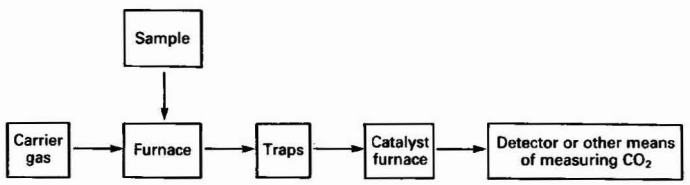

Fig. 1. Schematic diagram of the combustion process. Types of furnaces: induction; resistance; tube-type. Types of traps: dust to remove particulates (metal oxides); MnO_2, to remove sulphur oxides; halogens and nitrogen oxides; sulphuric acid, to remove water vapour; and copper(I) oxide to reduce nitrogen oxides to N_2

the amount of carbon. A summary of these methods is presented in Table 4.

The general reaction and oxidation potential for dichromate oxidation in the presence of free acids is represented by the following equations:

$$Cr_2O_7^{2-} + 14H^+ + 6e \rightarrow 2Cr^{3+} + 7H_2O \quad . . \quad (1)$$

$$E = E_0 + \frac{0.0001983T}{6} \log \left(\frac{[H^+]^{14}[Cr_2O_7^{2-}]}{[Cr^{3+}]^2} \right) . . \quad (2)$$

where E is the potential (volts) of the indicator electrode, E_0 is the standard electrode potential (volts) and T is the temperature (K). As is evident from equation (2), the Nernst equation, this reaction is affected by the temperature and pH of the reaction and the dichromate and chromate ion concentrations. Both the rate of the reaction and the decomposition of dichromate increase with increasing temperature. A balance must be achieved between incomplete oxidation at low temperatures and loss of dichromate at high temperatures. Temperatures between 125 and 150 °C are recommended. A 2 : 1 acid to dichromate ratio (by volume) is used to prevent precipitation of the dichromate ion, which occurs at high concentrations, and high digestion temperatures are used for extended periods at low concentrations. Finally, with 0.008–0.33 M dichromate solution an acceptable amount of dichromate is reduced (<50%) and the solubility of this reagent is maintained.[61]

Similarly to dichromate, persulphate also decomposes at an increasing rate with increasing temperature. The decomposition is explained by reaction kinetics. Two decomposition reactions occur simultaneously that affect the reaction rate. One is an uncatalysed reaction and the other is catalysed by hydrogen ion. These reactions and their kinetics are as follows:

Uncatalysed reaction:

$$S_2O_8^{2-} \rightarrow 2SO_4^{2-} \quad . . \quad . . \quad . . \quad (3)$$

$$2SO_4^{2-} + 2H_2O \rightarrow 2HSO_4^- + 2HO^- \quad . . \quad (4)$$

$$2HO^- \rightarrow H_2O + \tfrac{1}{2}O_2 \quad . . \quad . . \quad (5)$$

Hydrogen ion catalysed reaction:

$$S_2O_8^{2-} + H^+ \rightarrow H_2SO_8^- \quad . . \quad . . \quad (6)$$

$$H_2SO_8^- \rightarrow SO_4^{2-} + HSO_4^- \quad . . \quad . . \quad (7)$$

$$SO_4^- \rightarrow SO_3^{2-} + \tfrac{1}{2}O_2 \quad . . \quad . . \quad (8)$$

Reaction kinetics:

$$-\frac{d[A]}{dt} = k_0[A] + k_3 n[A] \quad . . \quad . . \quad . . \quad (9)$$

$$-\frac{d[B]}{dt} = k_3[A](-d[CO_2]/dt) \quad . . \quad . . \quad . . \quad (10)$$

$$-\frac{d[A]}{dt} = k_0[A] + k_4 n[A][B] \quad . . \quad . . \quad . . \quad (11)$$

$$-\frac{d[B]}{dt} = k_4[A][B] \quad . . \quad . . \quad . . \quad (12)$$

where [A] is the concentration of the persulphate, [B] is the concentration of the organic compound and n is the number of moles of persulphate required to oxidise the organic compound per mole of carbon. Equations (3)–(5) represent the thermal decomposition of persulphate and equations (6)–(8) represent the oxidation of the organic compounds. The operational parameters that affect persulphate oxidation are the temperature and pH of the system. As the persulphate decomposes, the resulting hydrogen sulphate ions cause a decrease in the pH of the solution. It is then necessary to use a buffer (*e.g.*, KH_2PO_4) to maintain the optimum reaction pH of 3–6. Under these conditions maintenance of a temperature of 100 °C for 8 min results in complete oxidation of the organic compounds. Use of higher temperatures result in incomplete oxidation.[62,63] Most persulphate oxidation methods do not employ these conditions.

Wet oxidation methods used solely for the determination of organic carbon commonly measure the amount of oxidising agent consumed by back-titration. Such techniques only detect the easily oxidisable carbon present and are popular as inorganic carbon need not be removed prior to analysis. Other wet oxidation procedures determine the CO_2 evolved and can be used to determine total, inorganic and organic carbon. Organic carbon determination generally involves acid treatment to remove the inorganic carbon. Inorganic carbon can be measured by adding $FeSO_4$ as an antioxidant prior to acid digestion.[31,53–58,64,65] Hence total carbon can be determined by wet oxidation methods using the sum of the

Table 3. Measurements of total, inorganic and organic carbon

Total	Inorganic	Organic
Dry combustion of sample	Total organic	Dry combustion after acid treatment of sample (HCl, H_2SO_4, H_3PO_4)
Inorganic and organic	Measure CO_2 evolved volumetrically on acid digestion	Wet oxidation of sample
Inorganic and organic	Wet oxidation of sample in presence of $FeSO_4$	Wet oxidation of sample
Inorganic and organic	Measure CO_2 evolved volumetrically on acid digestion	Dry combustion of sample after acid treatment

Table 4. Summary of wet oxidation methods

Reference	Published method used	Oxidising agent	Digestion temperature/°C	Digestion time/min	Means of measuring CO_2
53	Shaw	Dichromate	—	10–15	Gravimetric
54	Allison	Dichromate	210	10	Gravimetric
54, 55	Walkley - Black	Dichromate	120	30	Back-titration
56	Meibus	Dichromate	—	30	Back-titration
55, 57	Tinsley	Dichromate	—	120	Back-titration
	Schollenberger 1	Dichromate	175	1.5	Back-titration
	Schollenberger 2	Dichromate	155	20–25	Back-titration
58	Nommik	Dichromate	155–160	5–10	Non-aqueous titration
59	Menzel and Vacaro	Persulphate	130	30	IR analyser
60	Strickland and Parsons	Persulphate	130	40	IR analyser
22	Walkley - Black	Dichromate	120	30	Spectrophotometric

organic carbon content obtained by these methods and the inorganic carbon content measured in the presence of $FeSO_4$ (Table 3).

Historically, in the 1930s and 1960s wet oxidation methods were developed to alleviate the instrumentation costs associated with dry combustion methods, and in the 1970s microcombustion methods were developed to overcome the limitations of wet oxidation and other dry combustion methods. Numerous studies therefore exist that compare wet oxidation and dry combustion methods.[19,23,26,28,66] Most of these studies addressed the determination of organic carbon. Concerning the dichromate oxidation methods, Allison,[54] Metson *et al.*[19] and Wagersky[66] reviewed recovery studies using the dichromate titration methods. This discussion, therefore, will concentrate on the literature after this time. A summary of the publications reviewed comparing dichromate oxidation and dry combustion methods is presented in Table 5. The recoveries were calculated by comparing the wet oxidation values with those obtained by dry combustion (% carbon measured by wet oxidation/% carbon measured by dry combustion). This approach was adopted as most workers acknowledge that the dry combustion methods are the more accurate and standard procedures. In general, methods that measured the CO_2 evolved either titrimetrically or gravimetrically gave the highest recoveries (see Table 5). This was attributed to the incomplete oxidation of the carbonaceous materials by chromic acid and/or the oxidation level of these compounds. The dichromate titration methods assume that two oxygen atoms per carbon atom are needed for the complete oxidation of a compound. This assumption is true only for elemental carbon and for organic compounds such as carbohydrates in which carbon has a valency of zero. Lignins require more than two oxygen atoms, and proteins require fewer.[55,61] Therefore, depending on the compounds present in the sample, this assumption will be a source of error. Dichromate does not completely oxidise all compounds and so the degree of oxidation is also dependent on the sample. The efficiency of dichromate oxidation is greatest for carbohydrates and strongly hydrated or hydroxylated compounds. Lipids and crude proteins are oxidised more slowly and less completely, owing to the immiscibility of these compounds and the formation of acetic acid from protein oxidation (acetic acid is resistant to oxidation).[61] The error due to incomplete oxidation can be reduced by the inclusion of a correction factor. In general, this approach has not been successful owing to sample heterogeneity.[54]

Other sources of error in these oxidation techniques are associated with the presence of substances that react with dichromate. Oxidisable inorganic compounds (*e.g.*, chlorides) cause high results, whereas the presence of reduced compounds [*e.g.*, iron(II), manganese oxides] produce low results. These sources of error can be eliminated or reduced by adding silver sulphate or mercury(II) oxide to precipitate chlorides, using a correction factor for chlorides, pre-drying the soil to oxidise the iron, and/or adding iron(II) sulphate to precipitate the manganese.[30,32,34,61] The procedures that measure the CO_2 evolved are not subject to the errors associated with the oxidation level of the compound. They are, however, subject to the other sources of error mentioned. These methods therefore require the use of traps to remove chlorides, nitrogen and sulphur oxides and acid fumes from the carrier gas stream.[53,54]

Fewer studies have been published that compare persulphate oxidation and dry combustion methods. Table 6 compares some of these methods. In enriched seawater the recovery of a range of organic compounds was 98–102%.[59] In the analysis of natural samples, a greater variability in these values was observed. A range of 48–255% was reported for the recovery of organic carbon in seawater, sediments, particulates and clay samples. A low recovery (average 78%) for seawater samples was partly attributed to incomplete oxidation of the colloidal organic carbon.[16] Low recoveries can also be due to incomplete oxidation of the organic carbon.[2] High recoveries can be attributed to the presence of water[45] or the use of different types of filters in the analysis of particulate samples.[2]

Conclusion

From this review it is apparent that differences exist between the common methods used to determine carbon in soils and sediments. Dry combustion methods have long been recognised to produce the most accurate results.[59,60,64,68,69] The criticisms of dry combustion techniques in the past were largely directed at their cost, convenience and applicability to certain areas. For example, agronomists believed that dichromate oxidation of the organic carbon provided a better measurement of the chemically active fraction.[34] This belief, however, was based on the false assumption that these methods did not oxidise inert carbonaceous material in the soil.[53,57] This method therefore does not provide the selectivity that agronomists desired. Another criticism of dry combustion methods concerned their long analysis time. As a result of the development of microcombustion techniques, numerous commercial analysers are available today that are easy to use and provide rapid analysis. The expense of these instruments, however, may still be a limiting factor.

Table 5. Recoveries of organic carbon using various dichromate methods

Reference	Type of dichromate oxidation method used	Sample type	Recovery, % Range	Recovery, % Average
53	Shaw	Soil and plant material	98–100	98
54	Allison	Soil	98–101	99
55	Walkley - Black*	Soil	78–86	82
57	Walkley - Black*	Carbonised materials	0–83	19
55	Shaw	Soil	98–101	100
57	Shaw	Carbonised materials	86–135	107
55	Tinsley	Soil	94–101	95
57	Tinsley	Carbonised materials	61–128	97
55	Schollenberger 1*	Carbonised materials	1–126	68
57	Schollenberger 2*	Carbonised materials	0–83	19
56	Meibus	Soil	96–103	100
44	Meibus	Lake sediment	98–124	108
43	Allison	Soils	98–101	100
58	Nommick	Soils	89–126	99
36	Ferris and Jepson	Clay (95% kaolinite)	—	72

* Uncorrected values were used to calculate the recovery.

Table 6. Recoveries of organic carbon using various persulphate oxidation methods

Reference	Type of dichromate oxidation method used	Sample type	Recovery, % Range	Average
1, 70	Menzel and Vacaro	Particulates in seawater	82–255	126
36	Ferris and Jepson	Clay (95% kaolinite)	—	71
16	Modified Strickland and Parsons	Seawater	48–149	78
67	Modified Menzel and Vacaro	Sediments	81–148	111

Although dry combustion methods are the preferred method with respect to quantitation, other considerations may lead the analyst to use one of the other methods. The limitations of these methods should be recognised and sources of error associated with these techniques should be minimised. In addition, care should be taken in interpreting the data obtained by these methods, as they can be dependent on the analytical method employed.

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Determination of B_2 Vitamers in the Body Fluid of Plankton Using High-performance Liquid Chromatography with Fluorescence Detection

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A sensitive method, using high-performance liquid chromatography (HPLC) with fluorescence detection, has been developed for the separation and determination of B_2 vitamers in the body fluid of plankton. B_2 vitamers are liberated from the protein moiety in the body fluid by non-hydrolytic extraction with trichloroacetic acid and analysed by the proposed HPLC method. The major B_2 vitamer in the body fluid of plankton is riboflavin, although there is also evidence to suggest the presence of endogenous enzymes that can decompose flavin mononucleotide and flavin adenine dinucleotide.

Keywords: *B_2 vitamers determination; high-performance liquid chromatography; fluorescence detection; plankton body fluid analysis*

Vitamin B_2 is a biologically important substance, as it plays a central role as a coenzyme in biochemical redox reactions. It is ubiquitous in nature, especially in the living body where it takes the form of flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD). As plants, bacteria and moulds can synthesise vitamin B_2 while animals cannot, it is dietetically indispensable for animals. Therefore, much work has been carried out on the determination of vitamin B_2 in food and beverages.

Recently, high-performance liquid chromatography (HPLC), which is both a selective and rapid method of analysis, has been increasingly used for the separation and determination of B_2 vitamers in the above fields[1-5] and also biochemistry and clinical chemistry.[6] Recently, we have developed an HPLC method with fluorimetric detection for the separation and determination of riboflavin (RF), FMN and FAD in the serum of fish. As fish feed on plankton, which are at the origin of the food chain involving marine organisms, the serum of fish is closely related to the body fluid of plankton. Therefore, establishment of a sensitive and selective analytical method for the determination of B_2 vitamers in the body fluid of plankton was considered to be of great significance to the study of the described food chain.

This paper describes an HPLC method for the determination of each B_2 vitamer in the body fluid of plankton and thus offers a comparison with the constitution of the serum of some fish.

Experimental

Apparatus

The HPLC measurements were made with a Shimadzu Model LC-4A liquid chromatograph equipped with a Shimadzu Model RF-510LC fluorescence spectrophotometer and a Shimadzu Model C-R1A(S) Chromatopac computing integrator. The excitation and emission spectra were measured on a Shimadzu Model RF-502 double monochromator spectrofluorimeter. A Tomy Seiko Model SD-30N automatic high-speed autoclave and Yamato Model BT-45 bath incubator were used for the acid and enzymatic hydrolyses, respectively.

Reagents and Standards

Each standard stock solution of RF (37.6 μg ml^{-1} ≡ 100 μM), FMN (137 μg ml^{-1} ≡ 300 μM) or FAD (236 μg ml^{-1} ≡ 300 μM) was prepared in water and stored in a freezer at −10 °C. A mixed standard solution of RF, FMN and FAD of 0.376, 1.37 and 2.36 μg ml^{-1}, respectively, as a final concentration, was prepared daily by mixing each standard stock solution in a mixture of 400 μl of 10% trichloroacetic acid (TCA) solution, 400 μl of 2 M sodium acetate buffer and 200 μl of water.

Each enzyme solution containing 5% α-amylase (recrystallised four times, E.C. 3.2.1.1) and 10% papain (recrystallised twice, E.C. 3.4.22.2) (Sigma Chemicals, Poole, Dorset) was freshly prepared before use.

Chromatographic Conditions

As B_2 vitamers are water soluble, a Zorbax-NH_2 column, which has both reversed-phase and ion-exchange modes, was used to separate RF, FMN and FAD from each other and from other components. A mixture of 10% methanol and 90% sodium dihydrogen phosphate (0.2 M) - phosphoric acid (added for the purpose of pH control) buffer was used as the mobile phase, as this produced the optimum separation and retention time.

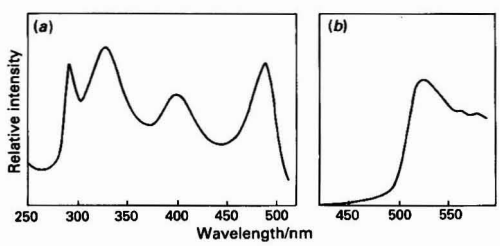

Fig. 1. (*a*) Excitation ($\lambda_{em.}$ = 526 nm) and (*b*) emission ($\lambda_{em.}$ = 328 nm) spectra of FMN in water

Table 1. Experimental conditions for HPLC

Stationary phase	Zorbax-NH_2
Column size	150 mm × 4.6 mm i.d.
Mobile phase	10% MeOH - 90% NaH_2PO_4 buffer (pH 3.0, 0.02 M)
Flow-rate	1.3 ml min^{-1}
Column temperature	50 °C
Detector	Fluorescence spectrophotometer $\lambda_{ex.}$ 328 nm (band width 20 nm), $\lambda_{em.}$ 526 nm (40 nm)
Injection volume	100 μl

Table 2. Effect of incubation of body fluid of plankton

Sample	Added/μM	Found/μM RF	FMN	FAD
Body fluid only, not incubated	—	0.845	0.082	0.491
Body fluid only, incubated	—	1.41	N.d.†	N.d.
Body fluid + RF, incubated	1.00	2.31	N.d.	N.d.
Body fluid + FMN, incubated	1.00	2.49	N.d.	N.d.
Body fluid + FAD, incubated	5.00	6.02	N.d.	N.d.
Fish serum* only, not incubated	—	0.971	0.094	0.367
Fish serum* only, incubated	—	1.13	N.d.	0.370

* Serum obtained from black carp.
† Not detected.

Table 3. Results of the determination of B_2 vitamers in plankton body fluid by treatment with TCA only and enzymes

B_2 vitamer	Found/μM* TCA only	Enzymes
RF	1.74	2.83
FMN	0.24	N.d.
FAD	1.04	N.d.
Total	3.02	2.83

* Concentration of B_2 vitamers in the sample solution.

Fluorescence Properties

Excitation and emission spectra of aqueous solutions of each B_2 vitamer were measured in order to determine the excitation and emission wavelengths for the detector. The results for FMN are shown in Fig. 1, and the peak positions in the excitation and emission spectra of the other B_2 vitamers were similar. From the maxima of the peaks, 328 and 526 nm were chosen as the excitation and emission wavelengths, respectively.

It is known that the fluorescence intensity of B_2 vitamers is pH dependent. The maximum value for RF and FMN was obtained at pH 3.5–7.5 and for FAD at pH 2.7–3.1.[8,9] The pH of the mobile phase was fixed at 3.0 where the fluorescence intensity of FAD was a maximum (this is because the fluorescence quantum yield of FAD is much smaller than those of RF and FMN).

Procedure

Where possible the procedure was carried out in the dark as B_2 vitamers are light sensitive.

Preparation of sample solution

About 50 mg of plankton, from which the water was drained by standing overnight on a Toyo filter-paper (No. 5c), was weighed exactly as a wet mass in a glass homogeniser (volume 1 ml, Wheaton Co., Millville, NJ, USA). After the addition of 1 ml of water, the sample was homogenised at 0 °C. The homogeneous solution was transferred into a centrifuge tube and centrifuged at 3000 rev min^{-1} for 5 min. The supernatant was stored in a freezer at −10 °C until just before use.

Acid hydrolysis. A 200-μl volume of the sample solution was taken in a small brown bottle and 200 μl of 0.1 N hydrochloric acid were added. After the bottle had been sealed, the sample solution was hydrolysed at 121 °C in an autoclave for 1 h.

Enzymatic hydrolysis. The pH of the acid-hydrolysed sample solution was adjusted to 4.0–4.5 with about 35 μl of 1 M sodium acetate solution. When the sample solution was subjected directly to enzymatic hydrolysis, 235 μl of 1 M sodium acetate buffer (pH 4.2) were added to 200 μl of the sample solution. To these pH-adjusted solutions, 50 μl of each aqueous enzyme solution (5% α-amylase and 10% papain) were added and the sample solution was incubated at 42–45 °C for 4 h.

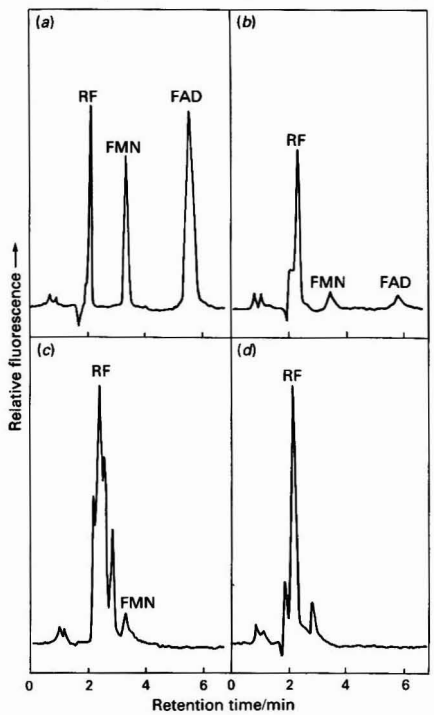

Fig. 2. Chromatograms of the B_2 vitamers in (*a*) standard solution; (*b*) sample solution treated with TCA only; (*c*) sample solution hydrolysed with acid; and (*d*) sample solution hydrolysed with enzymes. Sample: plankton body fluid

Extraction. To precipitate the proteins, 65 μl of 50% TCA solution were added to the hydrolysed sample solution with acid or enzymes. When the sample solution (200 μl) was used directly, 400 μl of 10% TCA solution were added for non-hydrolytic extraction. The TCA sample solution was allowed to stand for about 30 min in the dark at 0 °C and then 400 μl of 2 M sodium acetate buffer (pH 3.0) were added. After centrifuging at 1200 *g* and 4 °C for 3 min, the supernatant was filtered through a Millipore filter (pore size 0.45 μm).

An aliquot of the sample filtrate was analysed by HPLC under the conditions shown in Table 1.

Results and Discussion

Comparison of Different Sample Preparation Techniques

Because FMN and FAD are protein-bound and cannot be analysed without liberation, it is necessary to hydrolyse the

Table 4. Seasonal variation of the RF contents in the body fluid of plankton

Sampling date*	Water temperature/ °C	pH	DO, †%	RF content, p.p.m.‡ Salinity, ‰	A§	B§	C§
30.1.1984	3.8	7.95	94	33.8	7.79	5.65	—
31.7.1984	30.8	8.43	144	28.3	—	10.88	8.54
28.8.1984	29.8	8.38	139	27.0	6.13	5.46	3.88
5.9.1984	30.6	8.27	129	28.5	7.45	5.12	—

* The sample was taken by drawing a plankton net horizontally at a depth of 0–1 m near the centre of Lake Hamana (water depth about 12 m).
† Relative percentage of dissolved oxygen in brackish water compared with a brackish water saturated with air.
‡ p.p.m. as wet mass.
§ Mesh size of plankton net: A, 20 μm; B, 40 μm; and C, 90 μm.

Table 5. Contents of B_2 vitamers in the serum of different fish

Sample	RF/ μg ml^{-1}	FMN/ μg ml^{-1}	FAD/ μg ml^{-1}	Total/ μg ml^{-1}
Black carp ..	0.372	0.040	0.307	0.719
Gibel	0.205	N.d.	0.442	0.647
Eel	0.267	0.049	0.259	0.575

Table 6. Recoveries and detection limits of B_2 vitamers in body fluid of plankton by standard additions

B_2 vitamer	Recovery (n = 3),* %	Detection limit, p.p.m.
RF	106.7	0.10
FMN	106.9	0.18
FAD	97.3	1.46

* Recoveries of each B_2 vitamer were determined by adding 0.376 μg ml^{-1} of RF, 0.456 μg ml^{-1} of FMN and 2.36 μg ml^{-1} of FAD to a sample solution originally containing 0.531 μg ml^{-1} of RF.

sample with acid or enzymes. However, when the acid and enzymes adopted in common preparation techniques[1–5] are used, FMN and FAD are hydrolysed to RF. Therefore, a sample preparation technique as used previously,[7] utilising TCA for the non-hydrolytic liberation of FMN and FAD from the protein moiety, was attempted; as before, this technique was also compared with three other techniques, *i.e.*, hydrolysis with acid and each of two enzymes and then with both acid and enzymes.

Fig. 2 shows an example of the chromatograms of B_2 vitamers in the sample solution treated by different techniques. The result of the hydrolysis using both acid and enzymes is not shown, because it was the same as that of the acid hydrolysis. For the sample solution treated with TCA only [Fig. 2(*b*)] the three B_2 vitamers of RF, FMN and FAD were detected, but in that of the sample solution hydrolysed with the acid, RF and FMN were detected while FAD was not, presumably because FAD was almost entirely hydrolysed to RF or FMN. RF was not clearly separated from other interfering components as can be seen in Fig. 2(*c*), although the mobile phase conditions were varied.

For the sample solution hydrolysed with the enzymes, FMN and FAD were almost entirely hydrolysed. Meanwhile, the standard solution was also hydrolysed with the enzymes in a similar way, but FMN and FAD were not hydrolysed at all.[7]

From these observations, it was hypothesised that the body fluid of plankton contains some endogenous enzymes that can decompose FMN and FAD (over the pH range used in this incubation), which are hydrolysed by the acid.

In order to explain the above observations, the body fluid of plankton to which RF, FMN and FAD had been added was incubated under the same conditions as in the enzymatic hydrolysis but without α-amylase and papain. The results, given in Table 2, show that FMN and FAD were almost entirely hydrolysed to RF. However, it was concluded that the serum of black carp does not contain these enzymes as FAD (presumably in the bound form) was detected under these incubation conditions.

The results for the determination of B_2 vitamers in the sample solution treated with TCA only were compared with those of the sample solution hydrolysed with enzymes. As shown in Table 3, the total concentration of B_2 vitamers in the sample solution treated with TCA only is nearly equal to the RF concentration in the sample solution hydrolysed with the enzymes. Therefore, in this work, it was decided to use the sample preparation technique with TCA only, which allows the determination of each B_2 vitamer.

Analysis of Real Samples

The results for the determination of B_2 vitamers in the body fluid of plankton taken from Lake Hamana (Japan) are shown in Table 4. RF was the only one of the three B_2 vitamers detected, as both FMN and FAD in most of the samples were present below the detection limits. It can be seen that the concentration of RF decreased with increasing mesh size of the plankton net used. Further, the content of RF in the body fluid of plankton taken on July 31 was approximately twice as large as on other occasions, perhaps because the dominant species of plankton at this time were different. In contrast, FMN and FAD as well as RF were detected in the serum of different fish, as shown in Table 5.

Precision, Accuracy and Detection Limits

The precision of the proposed method for the determination of RF in plankton and fish was assessed by analysing one sample six times. The coefficients of variation (CV) were 4.8 and 10.8%, respectively. The CV of RF in the body fluid of plankton was smaller than that in fish serum and this is thought to be because the FMN and FAD levels in plankton were very low, and therefore the variation of the measured RF value, based on the conversion of FMN and FAD into RF in the extraction process with TCA, would not effectively occur in comparison with the serum samples.

The accuracy of the method was assessed by additional experiments where RF, FMN and FAD were added to the sample solution originally containing 0.531 μg ml^{-1} (1.4 μM) of RF to give concentrations of 0.376 μg ml^{-1} (1 μM), 0.456 μg ml^{-1} (1 μM) and 2.36 μg ml^{-1} (3 μM), respectively. The detection limits of each B_2 vitamer were estimated assuming that the signal to noise ratio should be at least 3. As shown in Table 6, the recoveries of the three B_2 vitamers were satisfactory and the detection limit of FAD was much higher than those of the other B_2 vitamers because FAD has a lower fluorescence quantum yield.

Conclusion

A reliable and sensitive detection of B_2 vitamers in the body fluid of plankton was obtained using the proposed method, and it was found that the major B_2 vitamer was RF in the body fluid of plankton compared with both RF and FAD in fish

serum. In addition, it would appear that the body fluid of plankton contains some endogenous enzymes that can hydrolyse free and bound FMN and FAD under the described incubation conditions, whereas fish serum does not.

It is anticipated that the proposed method will also be useful for the study of B_2 vitamers in the blood and body fluids of both humans and animals.

We thank Emeritus Professor Dr. Hidehiro Gotô of Tohoku University for his kind and unfailing guidance and Professor Dr. G. Schwedt of the Institute for Food Chemistry of the University of Stuttgart (FRG) for many discussions about HPLC techniques for vitamin B_2 determination.

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Determination of Alkylamines by Indirect Photometric Chromatography

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A method has been developed for the direct determination of some alkylamines in aqueous solution using the indirect photometric chromatography technique. The method uses 3-cm columns packed with high-capacity resins and copper sulphate solution as the eluent. The detection limits range from 0.5 to 2 μg ml^{-1} using a 1-ml sample loop.

Keywords: *Alkylamine determination; photometric chromatography; aqueous solution*

Aliphatic amines have no functional groups that can be easily exploited in their determination. They are difficult to determine by gas chromatography because of frequent tailing that results from sample adsorption on to the chromatographic support or adsorbent. For aqueous samples water tends to be a problem in the analysis because it reduces the response of the ionisation detector or alters the retention times of the amines.[1] Most analytical techniques for amines are long as they involve extraction and derivatisation.[2,3] We were interested in the determination of amines in aqueous samples because of experiments in which the speciation of amines on environmental particulates in model systems was being studied.[4] For this we required a simple and rapid method of determination.

Aliphatic amines are protonated in solution at pH less than 8 and hence are amenable to analysis by cation exchange. Ion chromatography with a conductivity detector has been used to determine amines.[5] The conductivity detector is a poor detector for amines because the suppressor column also neutralises the amines and lowers the sensitivity.[5] Small and Miller[6] have used indirect photometric chromatography to separate sodium, potassium and ammonium ions by ion exchange with a copper sulphate solution as the eluent. Indirect photometric detection is based on the principle that the ion content of the eluent and sample in equilibrium with the resin is fixed. A schematic chromatogram is shown in Fig. 1 for a protonated amine (RH^+) analyte on a copper-saturated resin with a copper sulphate solution as the eluent. When the sample is injected the analyte amine ions adsorb on to the resin displacing an equivalent amount of copper(II) ions. If the amount of RH^+ added is greater than the copper(II) ion concentration in the eluent, then an increased absorbance is obtained (as shown in Fig. 1). When the amine ions elute from the column the amine ions in the sample replace an equivalent amount of copper(II) ions in the eluent and the absorbance of the eluent decreases. This method is very useful for non-absorbing ions provided an absorbing eluent can be used. We have developed a procedure for the determination of amines in aqueous samples using this method.

Experimental

Reagents

Amines. Obtained from Fisher Scientific. Make up stock solutions (1000 μg ml^{-1}) in distilled water except for dibutylamine (40% ethanol). Dilute with distilled water to make solutions of 0.1–10 μg ml^{-1}.

Resins. Dowex 50 (100–200 mesh), Aminex A-9 (11 μm, Bio-Rad) and Aminex A-8 (7 μm, Bio-Rad) were used. Saturate with copper ions by stirring in 1 M copper sulphate solution overnight. Pack into 3 × 0.41 cm stainless-steel columns using a high-pressure column packing apparatus.

Eluent. Copper sulphate solution (0.01 M).

Liquid Chromatography

A Constametric III pump (Laboratory Data Control, Riviera Beach, FL), coupled to a home-made pulse dampener, a Rheodyne Model 7125 sample injector with a 1.0-ml sample loop and a Bio-Rad Model 1305 variable wavelength detector with a deuterium lamp (Bio-Rad, Richmond, CA) set at 240 nm, or a Waters Model 6000A pump with a Model LC 25 UV - visible detector, were used.

Treatment of Soil Samples

Extract volatile amines from soil following a modified form of the procedure developed by Golovnya *et al.*[7] Place 500 g of a loamy soil (collected from the Ottawa area in this work) into a 2-l distillation flask. Add 300 ml of distilled water and 200 ml of pH 9.5 buffer solution (0.1 M sodium hydroxide - sodium tetraborate) and soak for 15 min. Distil on an oil-bath at 150 °C and collect 200 ml of the distillate into a flask containing 50

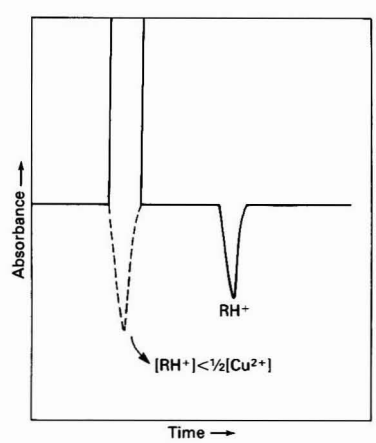

Fig. 1. Principle of indirect photometric chromatography

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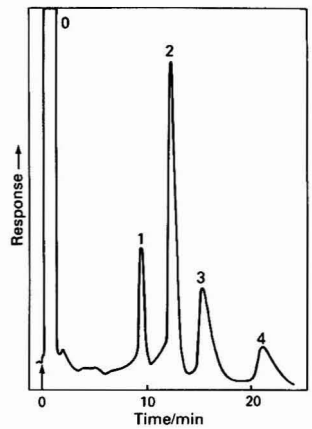

Fig. 2. Ion chromatogram of alkylamines (20 μg of each). 0, Solvent; 1, diethanolamine; 2, dimethylamine; 3, diethylamine; and 4, triethylamine

Table 1. Determination of amines by indirect photometric chromatography

Amine	Retention time/min	Detection limit/μg ml^{-1}
Ethylamine	15.2	1
Diethylamine	17.6	1
Triethylamine	23.2	2
Ethanolamine	11.2	1
Diethanolamine	11.2	1
Triethanolamine	11.2	1
Methylamine	12.4	1
Dimethylamine	14.4	0.5
Trimethylamine	16.4	1
sec-Butylamine	19.0	1
Dibutylamine	Not detected	—
Diisopropylamine	15	Very broad peak

Table 2. Retention times of inorganic cations on Aminex A-8

Cation	Retention time/min
Sodium	4.6
Ammonium	11.8
Potassium	14.0
Calcium	Not detected

ml of 0.05 M hydrochloric acid. Reduce the distillate volume to about 20 ml using a rotary evaporator at 40 °C. Quantitatively transfer the solution into a 50-ml flask and make up to volume using the eluent.

Recoveries

Heat the soil overnight at 170 °C to remove amines present in the soil, which may interfere in the recovery studies. Spike the soil with 10 ml of a solution containing 20 μg ml^{-1} each of diethylamine, dimethylamine, diethanolamine and triethylamine and mix well. Proceed to extract the amines as described above.

Results and Discussion

Ion chromatographic procedures usually employ low-capacity resins (0.016–0.024 mequiv. g^{-1}) for the analytical separation. This study, however, used high-capacity resins with capacities

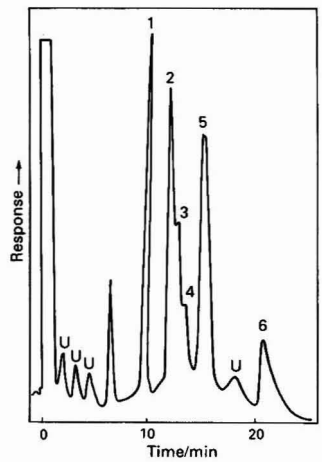

Fig. 3. Ion chromatogram of volatile amines detected in soil. 1, Methylamine; 2, dimethylamine; 3, ethylamine; 4, trimethylamine; 5, diethylamine; 6, triethylamine; and U, unknown

Table 3. Mean recoveries of amines from spiked soil (six samples)

Amine	Mean recovery, %	Standard deviation, %
Dimethylamine	86	20
Diethanolamine	91	10
Diethylamine	88	15
Triethylamine	72	16

of 2–5 mequiv. g^{-1}. The small particle size Aminex A-8 gave adequate resolution using a 3.0 × 0.41 cm column. A flow-rate of 1.0 ml min^{-1} gave a back pressure of 1500 lb in^{-2} and a typical four-component chromatogram is shown in Fig. 2. The column and flow-rate combination gave about 27 600 plates m^{-1}.

The retention times for several amines are shown in Table 1 and for inorganic cations in Table 2. The two tables suggest that it should be possible to use the system in model studies of selected amine mixtures. The inorganic cations sodium and calcium would not interfere, hence one can use sodium salts for buffers and both sodium and calcium forms of the environmental particulates (clay and humic acid). Dibutylamine could not be detected, probably because of its strong adsorption on cationic surfaces[4] and, also, because of its low solubility in aqueous systems. In general, the elution times increased with increase in molecular complexity within each homologous series, except for the ethanolamines all of which eluted at the same retention time. The reason for this is not clear. The calibration graphs were linear in the 0.1–10.0 μg ml^{-1} concentration range studied. The detection limits (twice the background noise) using a 1.0 ml injection of sample in eluent were 2 μg ml^{-1} for triethylamine, 0.5 μg ml^{-1} for dimethylamine and 1 μg ml^{-1} for the other amines studied. If samples were injected in distilled water a large positive peak was observed to interfere with some of the amines. Chromatographic determinations of amines in the model systems used 0.1 ml of 1 M copper sulphate solution to 10 ml of sample to remove the interference. When used on a regular basis the column performance deteriorated after about 2 weeks. Injection of 1 M copper sulphate solution at a low flow-rate (0.2 ml min^{-1}) restored the column performance to its original state. The accuracy of the method was determined by analysing individual secondary amines by differential-pulse polarography after derivatisation of the amines to nitros-

amines using Lund's method.[8] The two methods agreed within a 5% relative standard deviation error margin.

The method was tested in the determination of alkylamines in soil. Fig. 3 shows the amines that were detected in a soil sample collected from the Ottawa area. Among the amines detected (based on retention times) were methylamine (8 μg g^{-1}), dimethylamine (2 μg g^{-1}), trimethylamine (1 μg g^{-1}), ethylamine (3 μg g^{-1}), diethylamine (6 μg g^{-1}) and triethylamine (5 μg g^{-1}). The unidentified peaks could be due to other amines that were not present in the standard mixtures. Table 3 shows that the recoveries were good for the diethanolamine, dimethylamine, diethylamine and triethylamine spikes at the 400 μg kg^{-1} level. Recoveries were variable if the spikes were added to soil that had not been pre-heated to remove the volatile amines.

In conclusion, simple aqueous amine mixtures can be determined directly at low levels by the indirect photometric chromatography technique.

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Determination of Paraquat Residues in Soil by an Enzyme Linked Immunosorbent Assay

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A rapid, convenient and accurate method, based upon an enzyme linked immunosorbent assay (ELISA), is described for the determination of paraquat residues in soil. Polystyrene plates, coated with paraquat - keyhole limpet haemocyanin (KLH) conjugate, are incubated with the test samples and a known amount of monoclonal antibody. Residual antibody that has not reacted with free paraquat in the sample combines with paraquat - KLH on the plate. The determination of the fixed antibody is achieved by the addition of peroxidase labelled rabbit anti-mouse immunoglobulin G followed by reaction with a chromogenic substrate. The enzyme activity of the solid phase is determined from the absorbance measurements, which are inversely proportional to the concentration of paraquat. The method shows high specificity and correlates well with the traditional ion exchange - spectrophotometric method for the determination of paraquat.

Keywords: *Paraquat determination; enzyme linked immunosorbent assay; residues; soil analysis*

Immunological techniques are widely used in medicine for the quantitative determination of trace amounts of drugs, hormones and other compounds. In recent years the potential applications of immunology in other scientific fields, especially pesticide residue analysis, have been recognised.[1-5]

Both a radioimmunoassay (RIA)[6] and an enzyme linked immunosorbent assay (ELISA)[7] are available for the determination of the herbicide paraquat (1) in serum and urine. However, environmental studies of paraquat residues in crops and soils have previously relied on conventional analytical techniques, in particular a spectrophotometric procedure,[8] that require a time consuming ion-exchange chromatographic clean-up stage.[8,9] In contrast, an immunological method, which makes use of a highly specific antibody reaction, should be suitable for the analysis of crude extracts of plants and soil. The application of the paraquat ELISA to soil samples was investigated with the aim of achieving a rapid, efficient and sensitive analytical method, able to handle large numbers of samples simultaneously. The optimum experimental conditions for the determination and the sensitivity, precision and specificity of the method are discussed.

Experimental

Enzyme Linked Immunosorbent Assay

Apparatus

The assay was carried out on polystyrene microtitre plates (Nunc Immunoplate I). Absorbance measurements were recorded on a Titertek Multiskan MC vertical light path spectrophotometer (Flow Laboratories) interfaced with a Commodore PET computer programmed to perform the necessary calculations and to print the results.

Reagents and buffer solutions

All reagents used for the preparation of buffer solutions were of analytical-reagent grade.

Coating buffer, pH 9.6. Prepared by adding 1.59 g of Na_2CO_3, 2.93 g of $NaHCO_3$ and 0.2 g of NaN_3 to 1 l of distilled water.

Phosphate-buffered saline with 0.05% *Tween* 20 (*PBS - T*), *pH* 7.4. Prepared by adding 8 g of NaCl, 0.2 g of KH_2PO_4, 2.9 g of $Na_2HPO_4.12H_2O$ and 0.5 ml of Tween 20 to 1 l of distilled water.

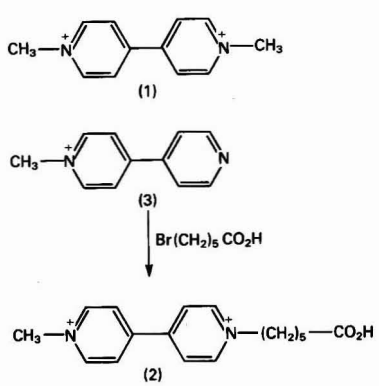
Incubation buffer. Prepared by dissolving 0.1 g of gelatine in 50 ml of hot (*ca.* 60 °C) PBS - T of twice the normal concentration, followed by the addition of 50 ml of saturated sodium sulphate solution. (Quoted as 50% sodium sulphate buffer.)

Citrate - phosphate buffer, pH 5.0. Prepared by adding 5.0 g of citric acid and 17.0 g of $Na_2HPO_4.12H_2O$ to 1 l of distilled water.

Substrate solution. Prepared by adding 80 mg of *o*-phenylenediamine (Sigma Chemicals, London) and 20 mg of urea hydrogen peroxide (BDH Chemicals, Poole, Dorset) to 100 ml of pH 5.0 citrate - phosphate buffer. The solution was made up immediately prior to use.

Preparation of antigens

Keyhole limpet haemocyanin (KLH, Calbiochem, La Jolla, CA) and bovine serum albumin (BSA, BDH Chemicals) were coupled to the adduct (2), derived from 6-bromohexanoic acid (BSA) and monoquat (3), via a carbodiimide reaction, as reported previously by Niewola *et al.*[7] The resulting conjugates contained 662 mol of paraquat per mole of KLH and 15 mol of paraquat per mole of BSA. The amount of paraquat bound to the protein was determined by a spectrophotometric dithionite assay for paraquat and the protein concentration was established by a standard Lowry test.



Preparation of monoclonal antibody coupled to paraquat - BSA

The procedure described by Niewola *et al.*[10] was used. Spleen cells from Balb/c mice, which had been immunised with paraquat - BSA, were fused with the NS.1 myeloma cell line using a protocol based on the method of Kohler and Milstein.[11] Antibody secreting clones were detected using an immunoradiometric assay and paraquat specific hybrids were cloned three times by limiting dilution. Large amounts of monoclonal antibody were produced by injecting 5×10^6 hybrid cells intraperitoneally into mice that had been primed with pristane (2,6,10,14-tetramethylpentadecane) 10 d earlier. After 10–14 d the ascites were collected, separated from cells and stored at −20 °C until required.

Extraction and assay

A homogeneous representative 10-g sample of air-dried soil was refluxed with 40 ml of 6 M sulphuric acid for 5 h. After the mixture had cooled, 10 ml of water were added. A 5-ml aliquot of the supernatant solution was treated with 0.5 ml of a 5% *m/V* solution of ethylenediaminetetraacetic acid and the pH adjusted to 6.0–7.0 with 12.5 M sodium hydroxide solution. The solution was finally made up to 10 ml with de-ionised water, giving an extract of concentration equivalent to 0.1 g of soil per ml of solution. The extracts were analysed using the ELISA method, which is represented diagrammatically in Fig. 1.

Wells of microtitre plates were coated by passive adsorption from 200 µl of a 1 µg ml^{-1} solution of paraquat - KLH in coating buffer. After standing for 2 h at room temperature the plates were washed three times for 3 min with PBS - T. Aliquots of 100 µl of incubation buffer were added to each well. Test samples and 1 µg ml^{-1} paraquat standards (100 µl) were applied to wells in the top row of the plate, mixed thoroughly with buffer and subjected to a two-fold serial dilution down the plate. Four test samples and one standard were run in duplicate on each plate (columns 2–11). The ascites containing monoclonal antibody were subjected to a 1 + 1000 dilution in PBS - T and 100 µl of the solution were added to each well. Following a 30-min incubation at room temperature the plates were washed as before and 200-µl aliquots of a dilution (1 + 4000) of horse radish peroxidase labelled rabbit anti-mouse IgG (Miles, Yeda, Israel) in PBS - T containing 0.5% *m/V* of BSA were added. After standing for 1 h at room temperature the plates were washed and finally developed by addition of 200 µl of substrate solution. The colour reaction was allowed to proceed for 10 min and was then quenched with 50 µl of 0.5 M citric acid. The absorbance

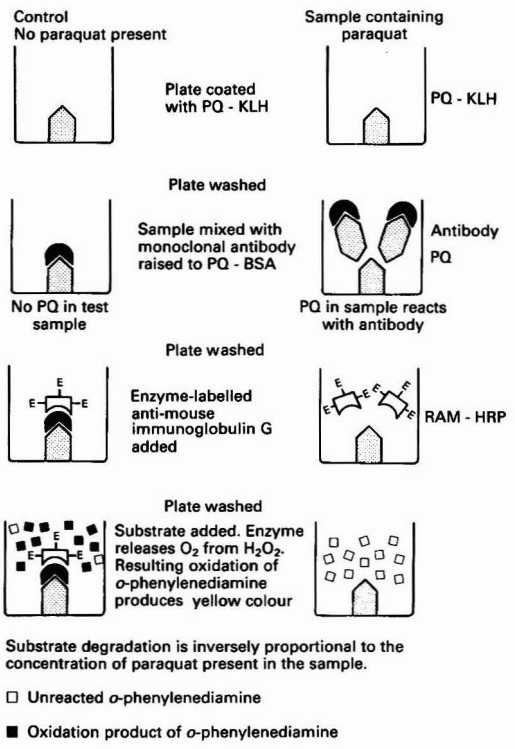


Fig. 1. Diagrammatic representation of stages in the paraquat ELISA. PQ = paraquat

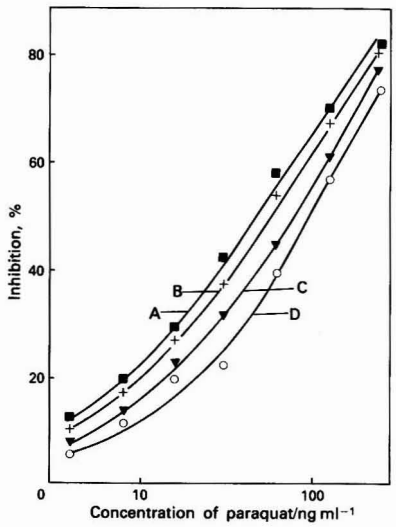


Fig. 2. Effect of sodium sulphate on the calibration graph. Plot of percentage inhibition *versus* paraquat concentration for solutions of incubation buffer containing: A, 0; B, 6.25; C, 25; and D, 50% of sodium sulphate

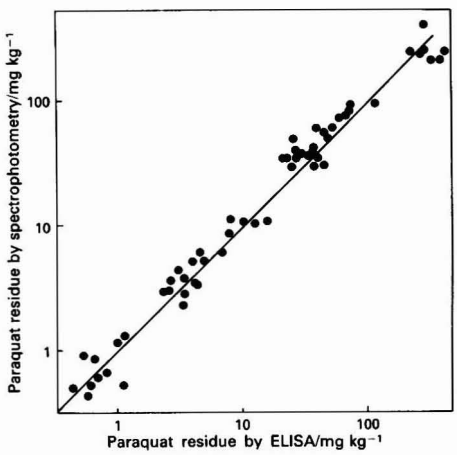
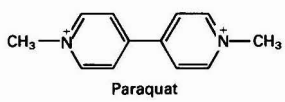
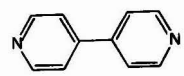
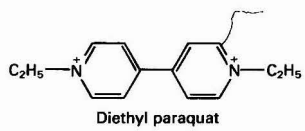
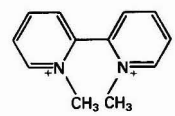
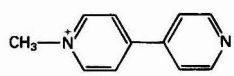
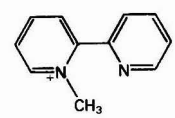
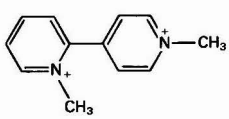
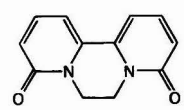
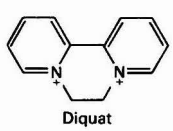
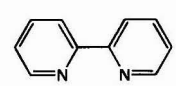
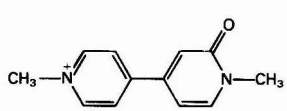
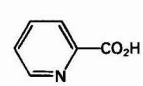
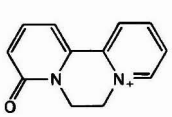
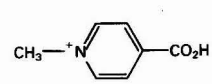
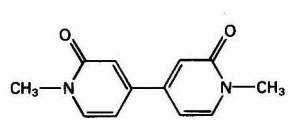
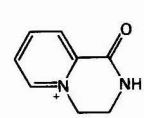


Fig. 3. Comparison of ELISA and spectrophotometric methods for the determination of paraquat

Table 1. Analysis of fortified soil extracts using the ELISA

	Paraquat in soil/mg kg^{-1}								
Added	0	10	20	50	100	150	200	250	300
Found	0	8.5	20	37	90	141	204	247	287

Table 2. Cross-reactivity of the monoclonal antibody

Compound	Cross-reactivity*, %	Compound	Cross-reactivity*, %
 Paraquat	100		<0.001
 Diethyl paraquat	113		<0.001
 Monoquat	5.7		<0.001
	0.016		<0.001
 Diquat	0.007		<0.001
	0.002		<0.001
	0.002		<0.001
	<0.001		<0.001

* Cross-reactivity (%) is calculated from the molar concentration of compound required to produce 50% inhibition in the binding of antibody to paraquat - KLH conjugate relative to paraquat.

of each well was measured at 450 nm and the paraquat concentration, which was inversely proportional to the absorbance, was calculated with the aid of a computer program, relative to a reference standard on each plate. Calculation of the level of paraquat present in each sample was based on data taken from a calibration graph, which plots the logarithm of $I/(1 - I)$ (where I = the inhibition of antibody calculated from the expression below) *versus* the logarithm of the concentration of paraquat:

$$I = \frac{A_{Ab} - A_{PQ}}{A_{Ab}}$$

($0 < I < 1$) where A_{Ab} is the absorbance with antibody alone (zero standards) (column 12 of plate) and A_{PQ} is the absorbance with antibody and added paraquat (column x, x = 2–11, test samples and standards). The absorbance values were first corrected (automatically) for background (coated wells incubated with enzyme labelled conjugate and substrate, *i.e.*, reagent blanks, column 1).

Ion Exchange - Spectrophotometric Method for the Determination of Paraquat[8,9]

Procedure

A 25-g homogeneous representative sample of air-dried soil was refluxed for 5 h with 100 ml of 6 M sulphuric acid. The digest was filtered and percolated through a column of cation-exchange resin (Duolite C225-SRC14), which retained the paraquat and some of the natural soil constituents. The column was washed successively with 2 M hydrochloric acid, 2.5% *m/V* ammonium chloride solution and water and the paraquat was finally eluted with saturated ammonium chloride solution. A portion of the column eluate was reacted with sodium dithionite in alkali. The quantitative measurement of paraquat residues was achieved by differential spectrophotometric measurement of light absorption over the range 430–360 nm by the single electron free radical derived from paraquat, and subsequent comparison with a calibration graph. In this method recovery values were typically in the range 85–100% and where necessary the raw data were corrected for recovery.

Results and Discussion

Validation, Accuracy and Precision of ELISA

The suitability of the ELISA for soil analysis was initially tested by assaying a number of control soil samples, fortified after extraction and neutralisation with paraquat in the range 10–300 mg kg^{-1}. The results in Table 1 were close to the expected values and thus confirmed that natural soil components did not interfere with the determination. These results justified the further refinement of the method for soil analysis.

Subsequent studies under the optimum assay conditions, in conjunction with extraction, measured the accuracy and precision of the ELISA. Control soil samples, which had been fortified before extraction with known concentrations of paraquat, typically 1.0 and 10 mg of paraquat per kg of soil, were extracted, neutralised and assayed on consecutive days. A coefficient of variation of 14.5% was achieved at the 1.0 mg kg^{-1} level (n = 13, mean = 0.97, s.d. = 0.14); at the 10 mg kg^{-1} level it was 15.7% (n = 51, mean = 9.6, s.d. = 1.5). At least one untreated control sample and several accurately fortified recovery samples were analysed alongside each batch of test soils. Where necessary, the results generated for the unknowns were then corrected for the mean batch recovery value. Corrections were made whenever the mean recovery was <100%.

Sensitivity of Paraquat ELISA for Soil Analysis

The limit of detection of the assay for soil extracts is at present 0.02 μg ml^{-1}, which corresponds to 0.2 mg kg^{-1} of paraquat in soil for a typical extraction procedure. This level of sensitivity is satisfactory for virtually all determinations of paraquat in soil and lower limits of detection are rarely required.

The neutral soil extracts contained high concentrations of sodium sulphate and the effects of this on the calibration graph were evaluated. Paraquat standards were diluted in incubation buffer containing varying concentrations of sodium sulphate and it was shown that an increase in sodium sulphate concentration produced a parallel shift of the calibration graph to the right (Fig. 2), thus resulting in a decrease in assay sensitivity. On the basis of this observation, 50% of saturated sodium sulphate was included in the incubation buffer to optimise the accuracy of the method. For the analysis of water samples or body fluids, sodium sulphate is omitted from the incubation buffer, thus giving enhanced sensitivity.

Specificity of the Paraquat ELISA

A series of compounds, which were structurally related to paraquat, were diluted with incubation buffer. The cross-reactivity of the monoclonal antibody for each compound was measured as the concentration of derivative required to produce 50% inhibition of binding of the antibody to the solid-phase paraquat - KLH conjugate in the ELISA. The results are summarised in Table 2. Cross-reactivity with diethyl paraquat was very high (>100%) but was much less with monoquat. Diquat cross-reactivity was only 0.007%. Many of the other compounds tested exhibited little or no binding to the antibody (cross-reactivity <0.001%).

Comparison of ELISA and Spectrophotometric Methods of Paraquat Determination

Fifty-seven soil samples containing paraquat residues in the range 0–300 mg kg^{-1} were extracted and independently analysed by the two methods. The results from the ELISA were corrected for a recovery factor of 95%. The spectrophotometric results were corrected on an individual batch basis. There was a high degree of agreement between the two methods with a correlation coefficient of the individual values of 0.97 (Fig. 3). The mean spectrophotometric to ELISA ratio was 1.06, which was not statistically significantly different from 1.

Conclusions

The paraquat ELISA provides an accurate method for the determination of residues in soil. It correlates well with the spectrophotometric procedure and offers a number of advantages over the traditional method. The speed and efficiency of the assay allow a rapid throughput of extracts and the method is particularly useful for the simultaneous analysis of large numbers of samples. The appreciable saving in time and manpower offers considerable advantages over alternative methods.

The specificity of the monoclonal antibody permits direct analysis of crude extracts and the lack of cross-reactivity between the antibody and diquat is also advantageous. Diquat is used extensively in agriculture, either on its own or as a mixture with paraquat and the ELISA is of considerable value for the determination of paraquat in paraquat - diquat mixtures. Traditional atomic absorption spectrometry is not amenable to the measurement of paraquat in paraquat - diquat mixtures owing to significant overlap of the spectra generated by the two compounds.

The ELISA may be performed on very small volumes of extracts and, in theory, it should be possible to analyse minute amounts of soil. For practical purposes, however, at least 10 g of homogeneous soil are recommended to ensure representative sampling. The ELISA method for paraquat determination should prove to be a powerful analytical tool in environmental studies and there is clearly scope for the development of similar assays for many common pesticides.

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Instrumental Determination of Some Trace Elements in Biological Materials by Epithermal and Thermal Neutron Activation Analysis

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The possibilities of increasing the utility of instrumental neutron activation analysis (NAA) of biomedical and environmental samples have been examined by introducing an epithermal neutron activation technique. The major advantage, in comparison with classical neutron activation methods, is found to be the reduction of interfering matrix activities, which allows the rapid instrumental determination of some trace elements of interest with minimum delay. The technique was evaluated using standard biological materials and the accuracy, precision, detection limits and the time scales of analysis were compared with those obtained using conventional thermal neutron activation. Results of the analysis of a mixture of plasma and erythrocytes are given to assess the method. The method can be applied to determine As, Br, Cd, Fe, Mn, Mo, Ni, Rb, Se, Sr and Zn in biological materials without chemical separation.

Keywords: *Trace element determination; neutron activation analysis; epithermal neutron activation; biological materials analysis*

The role of neutron activation analysis (NAA) in the determination of trace elements in biomedical and environmental samples has steadily increased in importance[1] mainly because of its high sensitivity,[2] multi-element capability[3] and the fact that the method is free of blank values from reagents if these are added after activation. The instrumental form of NAA based on the γ-ray counting of radioactive nuclides produced by the (n, γ) radiative neutron capture and similar neutron-induced reactions is particularly attractive as it involves an absolute minimum of sample handling and is therefore less prone to errors.[4] However, a serious limitation of instrumental NAA is that the relatively more abundant matrix elements become strongly activated during irradiation and their intense activities obscure the γ-rays of those elements at lower concentrations. To determine the latter by instrumental means, long waiting times, sometimes up to 3 months,[5] are necessary to allow interfering activities to decay to a sufficiently low level. In addition, long counting times are needed in order to obtain good precision. Such long waiting times preclude the detection of radionuclides with half-lives shorter than or comparable to those of interfering activities and generally reduce the analytical usefulness of instrumental neutron activation analysis (INAA), especially for application in clinical investigations.

In biological materials, ^{24}Na, ^{32}P, ^{38}Cl and ^{82}Br produce the bulk of interfering activities and consequently their half-lives determine the speed of INAA. In some instances, INAA can be particularly rapid if short-lived isotopes are utilised and fast pneumatic transfer facilities are available.[6] The main disadvantage here is that the problems of rapidly changing analyser dead time and pulse pile-up effects generally reduce the accuracy of determinations[7] unless elaborate electronics or computational procedures are used.[8]

In fission reactors, the neutron energy spectrum is conveniently divided into three energy intervals with decreasing flux intensities as follows: thermal neutrons, *i.e.*, neutrons with energies comparable to thermal energies of the moderator atoms, usually equal to 0.0253 eV, epithermal neutrons, *i.e.*, neutrons with energies between 1 eV and 1 keV, and fast neutrons whose average energy is about 2 MeV. In the usual activation analysis, the total spectrum of neutrons is used, but samples are mainly activated by the (n, γ) reaction induced by thermal neutrons owing to their higher flux and larger cross-section (probability to induce the reaction).

Essentially, the rate at which radionuclides are produced by nuclear reactions depends on the energy distribution of the neutron flux and the way in which the cross-section varies with the neutron energy. For many elements, the variation of the cross-section is inversely proportional to the neutron velocity (the $1/v$ law) and these are strongly activated by slow (*i.e.*, thermal) neutrons. In contrast, other elements possess resonance cross-sections in the epithermal region, which are usually larger than thermal cross-sections by several orders of magnitude. Therefore, if a sample is irradiated with epithermal neutrons, it is expected that the activation yield of the "resonance" elements will be enhanced relative to those interfering nuclides which are activated mainly by thermal neutrons. In reactor activation, this technique, called epithermal neutron activation analysis (ENAA), is performed by enclosing samples in thermal neutron filters such as cadmium or boron, which remove thermal neutrons from the reactor neutron spectrum. It has been applied to a variety of sample matrices including geological[9–11] and biological materials.[12–14] The advantages of the technique are generally given in terms of the "advantage factor,"[15] a quantity obtained from the ratio of the cadmium or boron ratios between the interfering nuclide and the desired nuclide. This quantity, however, does not give the true picture of the usefulness of ENAA, as has been illustrated.[16,17] In many instances, the advantage factors based on single-element interference are different from those obtained in the actual sample[18] and also they depend very much on the type of filter used and how efficiently thermal neutrons are removed,[19] so that realistic benefits of using ENAA can only be evaluated by the results obtained under the actual given analytical conditions.

In this paper, an epithermal irradiation scheme is presented to improve the turnover and elemental information for the instrumental determination of some trace elements in biological materials using radionuclides with half-lives greater than 1 h. The technique of ENAA is compared with the conventional NAA in terms of accuracy, precision, detection limits and the time scales of analysis obtained from the analysis of standard biological materials. Results of the analysis of several samples of biological fluids are also given. In the following, the conventional form of activation will be referred to either as total reactor spectrum (RNAA) or simply thermal neutron activation (TNAA).

Experimental

Preparation of Samples and Standards

Samples of standard reference materials, NBS SRM-1577 (bovine liver) and NBS SRM-1566 (oyster tissue) were

weighed in high-purity quartz ampoules (Heraeus Suprasil), which were cleaned as described elsewhere,[5] dried in an oven at 50 °C to a constant mass of 50–100 mg and heat-sealed. Standard solutions of elements to be determined were prepared from Merck Titrisol standard solutions by pipetting aliquots of 0.2 ml into clean quartz ampoules and evaporating to dryness before heat sealing. Several samples of IAEA-H8 (horse kidney) material submitted for inter-comparison analysis were prepared in the same manner. Finally, several samples of a mixture of 70 µl of plasma and erythrocytes were heat sealed in quartz ampoules after lyophilisation.

Irradiation and γ-Spectrometry

All irradiations were carried out in the BER II 5 MW reactor in two irradiation facilities referred to as the HV and RBK for which the thermal neutron flux amounted to 3.2×10^{12} n cm^{-2} s^{-1} and 3×10^{13} n cm^{-2} s^{-1}, respectively. Epithermal irradiations were performed by means of sintered boron carbide (B_4C) containers in the HV facility and an $AlMg_3$ sealed sleeve packed with powdered B_4C, which is permanently installed in the RBK facility with a provision for sample irradiation with and without the filter. A detailed description of the filters is given elsewhere.[20] The estimated epithermal neutron flux was 2.9×10^{10} n cm^{-2} s^{-1} in the HV (Φ_{epi}/Φ_{th} = 0.9%) and 5.3×10^{11} n cm^{-2} s^{-1} in the RBK (Φ_{epi}/Φ_{th} = 1.8%). Two irradiation schemes were developed: short irradiations ($t_i \leq 1$ h) in the HV and long irradiations ($t_i \leq 60$ h) in the RBK facility. γ-Ray spectra were measured either with a well-type Ge(Li) (Princeton Gamma-Tech) or with a well-type intrinsic Ge detector (Detector Systems GmbH) with associated electronics. The element contents and detection limits were determined by means of a computer program PECI.[21] Cooling and counting times were optimised after a series of experimental test measurements for both irradiation techniques.

Results and Discussion

The most important requirement for a given element to be determined with high sensitivity by ENAA is the value of the ratio of its resonance integral to thermal neutron cross-section (RI/σ_0). These values are given in Table 1 for the radionuclides used in the analysis together with other essential data.

To assess the usefulness of ENAA relative to thermal NAA for irradiation times of the order of 1 h, NBS SRM-1566 (oyster tissue) was analysed. Table 2 shows values for the element contents and the corresponding lower limits of detection (LD), together with NBS values and the optimum cooling and counting conditions. The uncertainty shown in the results in Table 2 represents that from counting statistics.

Table 1. Nuclear data of the radionuclides used in the analysis[22]

Element	Radionuclide	Half-life	γ-Ray measured/keV	RI/σ_0
Ag	$^{110}Ag^m$	250 d	658.0	297.8
As	^{76}As	26.5 h	599.1	14.65
Br	^{82}Br	35.34 h	776.6	16.67
Cd	^{115}Cd	53.5 h	528.6	10.0
Co	^{60}Co	5.27 y	1332.5	2.0
Cr	^{51}Cr	27.0 d	320.1	0.53
Cs	^{134}Cs	2.06 y	796.3	15.50
Fe	^{59}Fe	44.60 d	1291.3	1.05
K	^{42}K	12.4 h	1524.7	0.88
Mn	^{56}Mn	2.58 h	846.6	1.05
Mo	^{99}Mo-^{99}Tc	66.7 h	141.0	53.57
Na	^{24}Na	15.03 h	1369.0	0.57
Ni	^{58}Co	70.8 d	811.0	(n,p) reaction
Rb	^{86}Rb	18.66 d	1076.6	14.0
Sc	^{46}Sc	84.0 d	889.3	0.44
Se	^{75}Se	120.0 d	264.5	9.1
Sr	$^{87}Sr^m$	2.83 h	388.0	5.7
Zn	^{65}Zn	245.0 d	1115.5	1.95
	$^{69}Zn^m$	13.8 h	438.0	3.34

Table 2. Results of the analysis of NBS SRM-1366 (oyster tissue) (µg g^{-1}) by INAA with ENAA and RNAA. Values in parentheses are recommended values

		ENAA*		RNAA†		
Element	Isotope	$\bar{x} \pm s$	LD	$\bar{x} \pm s$	LD	NBS value
As	^{76}As	11.96 ± 0.56	0.16	15.87 ± 3.5	1.80	13.49 ± 1.9
Br	^{82}Br	51.70 ± 7.1	0.62	50.57 ± 0.45	0.26	(55)
Co	^{60}Co	0.39 ± 0.06	0.08	0.44 ± 0.07	0.02	(0.4)
K	^{42}K	8600 ± 300	165	8200 ± 700	72.4	9700 ± 500
Mn	^{56}Mn	16.57 ± 0.97	0.16			17.50 ± 1.2
Na	^{24}Na	4200 ± 300	11.2	4700 ± 200	3.0	5100 ± 300
Sr	$^{87}Sr^m$	10.99 ± 0.76	1.5			10.36 ± 0.56
Zn	$^{69}Zn^m$	848.5 ± 4.5	25.6			852.0 ± 14.0
Irradiation time		1.0 h		1.0 h		
Cooling time		1.0–2.0 h		4–6 d		
Counting time		3600 s		3600 s		

* Arithmetic mean ($\bar{x}$) ± standard deviation from five parallel determinations.
† Arithmetic mean ($\bar{x}$) ± standard deviation from eight parallel determinations.

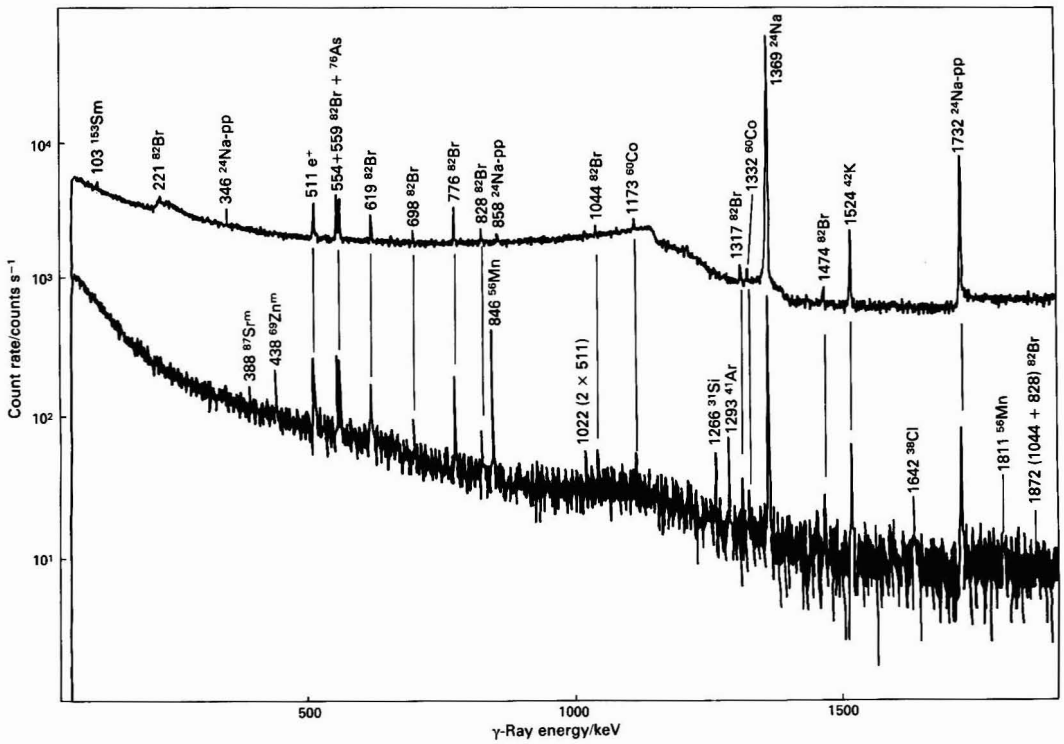

Fig. 1. γ-Ray spectrum of NBS SRM-1566 (oyster tissue) for brief irradiation. Upper spectrum: activation with total neutron spectrum, t_i = 1 h; t_w = 6 d; and t_c = 3600 s. Lower spectrum: activation with epithermal neutrons, t_i = 1 h; t_w = 2 h; and t_c = 3600 s (t_i = irradiation time; t_w = waiting time and t_c = counting time). ^{24}Na-pp = ^{24}Na gamma-ray pair-peak

Table 3. Results of the analysis of NBS SRM-1577 (bovine liver) (μg g^{-1}) by INAA with ENAA and RNAA. Values in parentheses are recommended values

Element	Isotope	ENAA* $\bar{x} \pm s$	ENAA* LD	RNAA* $\bar{x} \pm s$	RNAA* LD	NBS value
Ag	$^{110}Ag^m$	0.049 ± 0.016	0.030	0.053 ± 0.017	0.027	(0.06)
Br	^{82}Br	8.23 ± 0.45	0.14			8.70 ± 0.34
Co	^{60}Co	0.30 ± 0.07	0.008	0.21 ± 0.01	0.002	0.24 ± 0.02
Cs	^{134}Cs	0.023 ± 0.008	0.02	0.02 ± 0.005	0.001	(0.017)
Fe	^{59}Fe	287.0 ± 81	4.6	264.0 ± 44	1.48	270.0 ± 20
Mo	^{99}Mo-^{99}Tc	2.89 ± 0.45	0.34			3.10 ± 0.2
Ni	^{58}Co	0.52 ± 0.15	0.07			(0.4)
Rb	^{86}Rb	18.64 ± 0.58	0.13	17.97 ± 0.42	0.75	18.3 ± 1.0
Se	^{75}Se	1.02 ± 0.06	0.03	1.04 ± 0.03	0.012	1.10 ± 0.1
Zn	^{65}Zn	135.14 ± 6	0.26	129.0 ± 1.5	0.06	130.0 ± 10.0
Irradiation time		60 h		60 h		
Cooling time		21–30 d		70–90 d		
Counting time		7200 s		7200 s		

* All values are arithmetic means ($\bar{x}$) ± standard deviation from ten parallel determinations.

These decay times are determined essentially by the level of sample activity that can be measured with the counting detector and electronics used. With the counting system used a maximum decay period of ≥4 d was required before the γ-spectra of samples could be measured with acceptable analyser dead time in the unfiltered irradiations. This means that radionuclides with half-lives shorter than this period could not be measured. In contrast, the cooling time in epithermal activation of comparable irradiation time was essentially the time required to retrieve the sample from the reactor plus the time spent in separating the samples from the boron carbide containers, which was between 1 and 2 h. Fig. 1 shows the γ-spectrum of oyster tissue for the two irradiation modes under these conditions. In practice, it is possible to reduce the time delay in epithermal activation to a bare minimum so that even radionuclides with half-lives of the order of a few minutes can be measured.

On the basis of the results obtained, As, Br, Mn, Sr and Zn can be determined rapidly with reasonable sensitivity by ENAA. The determination of As at 559 keV is usually made

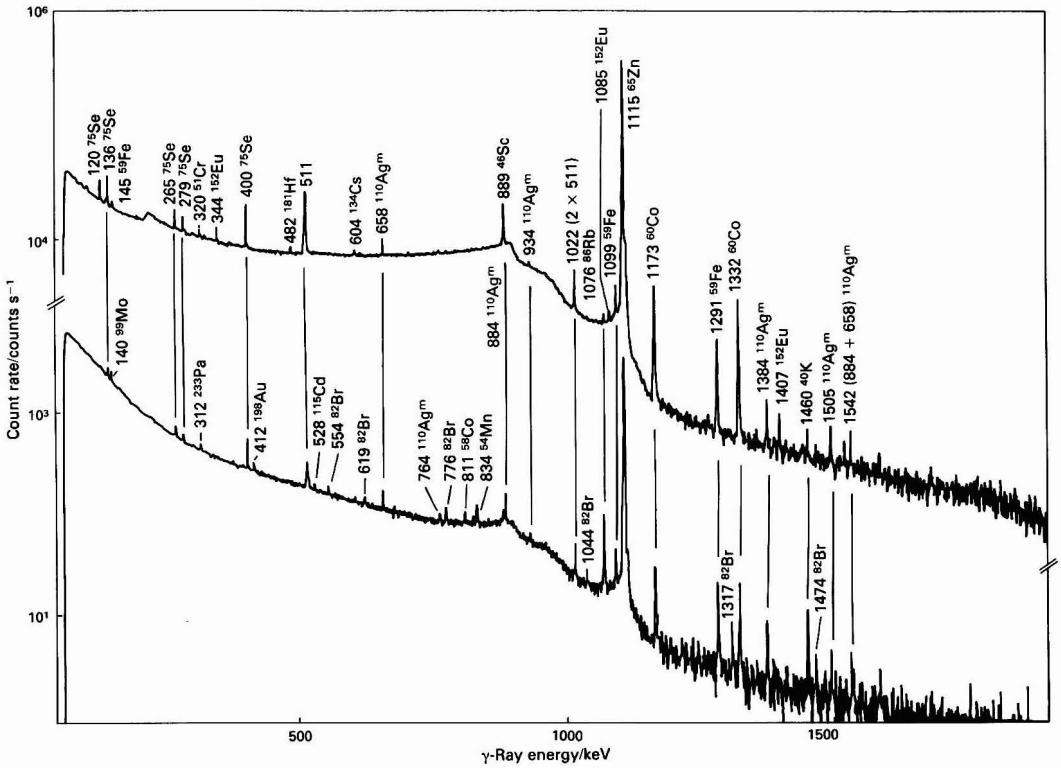

Fig. 2. γ-Ray spectrum of NBS SRM-1566 (oyster tissue) for prolonged irradiation. Upper spectrum: activation with total neutron spectrum, t_i = 48 h; t_w = 50 d; and t_c = 7200 s. Lower spectrum: activation with epithermal neutrons, t_i = 48 h; t_w = 15 d; and t_c = 7200 s

Table 4. Results of the analysis of NBS SRM-1566 (oyster tissue) (μg g⁻¹) by INAA with ENAA and RNAA

Element	Isotope	ENAA* $\bar{x} \pm s$	ENAA* LD	RNAA* $\bar{x} \pm s$	RNAA* LD	NBS value
Ag	$^{110}Ag^m$	0.86 ± 0.09	0.024	0.93 ± 0.06	0.016	0.89 ± 0.09
Br	^{82}Br	52.90 ± 3.3	1.6			(55)
Co	^{60}Co	0.42 ± 0.07	0.010	0.34 ± 0.01	0.001	(0.4)
Cr	^{51}Cr			0.75 ± 0.10	0.036	0.69 ± 0.27
Fe	^{59}Fe	212.5 ± 37.0	6.2	218.9 ± 9.0	2.8	195.0 ± 34
Mo	^{99}Mo - ^{99}Tc	0.16 ± 0.04	0.016			(≤0.2)
Ni	^{58}Co	0.98 ± 0.10	0.01			1.03 ± 0.19
Rb	^{86}Rb	5.04 ± 0.10	0.07	4.27 ± 0.19	1.2	4.45 ± 0.09
Sc	^{46}Sc			0.015 ± 0.002	0.0004	—
Se	^{75}Se	2.04 ± 0.04	0.041	2.21 ± 0.08	0.02	2.10 ± 0.5
Zn	^{65}Zn	887.60 ± 10.00	0.56	884.60 ± 17.0	0.18	852.0 ± 14.0
Irradiation time		48 h		48 h		
Cooling time		15–21 d		50–70 d		
Counting time		7200 s		7200 s		

* All values are arithmetic means ($\bar{x}$) ± standard deviation from ten parallel determinations.

difficult by the strong interference from 544 keV ^{82}Br, as can be seen in Fig. 1. Although in general the use of epithermal activation improves the situation only marginally, as both of the two isotopes have comparable RI/σ_0 ratios and half-lives, the two peaks were sufficiently resolved in epithermal activation in this instance. Co can also be determined with epithermal activation, but better sensitivity is achieved with thermal activation after 6 d of decay. An important contribution of epithermal activation in this instance was the extra elemental information obtained by the measurement of the short-lived isotopes of Mn, Sr and Zn, which could not be measured with thermal activation.

In the long irradiations of more than 24 h, the limiting factor is imposed by the Bremsstrahlung from the ^{32}P ($t_{0.5}$ = 14.3 d) activity. To study the improvements of epithermal activation relative to thermal activation, NBS SRM-1577 (bovine liver) and NBS SRM-1566 (oyster tissue) were analysed after 60 and 48 h of irradiation in the RBK facility, respectively. For

epithermal activation, the earliest time the γ-spectrum of the samples could be measured was 10 d, but the best precision was obtained after 15 d, whereas for thermal activation the earliest was after 50 d in both instances. A typical γ-ray spectrum of NBS oyster tissue measured under these conditions is shown in Fig. 2. Tables 3 and 4 give the results of determinations and the detection limits obtained under the optimum cooling and counting conditions given. The standard deviation in Tables 3 and 4 is the uncertainty from counting statistics alone and from these results it is clear that the main advantage of epithermal activation for the assay of long-lived isotopes is speed. The total analysis time is reduced by a factor of about 3–4 as a result of the reduction in the ^{32}P background activity. In general, however, lower detection limits are obtained with thermal activation particularly for long-lived radionuclides, *e.g.*, ^{60}Co, ^{59}Fe, ^{75}Se, ^{65}Zn and $^{110}Ag^{m}$. For Rb, epithermal activation is the most preferable method as its RI/σ_0 value is high and, because its half-life is comparable to that of ^{32}P, thermal activation with long waiting times is undesirable. As is evident in Tables 3 and 4, lower detection limits for Rb are obtained with epithermal activation. Epithermal activation was also found to be favourable for instrumental determinations of Mo and Ni in both materials.

This advantage was utilised for the rapid instrumental determination of cadmium through its short-lived isotope ^{115}Cd ($t_{0.5}$ = 53.5 h) in the IAEA inter-comparison H8 (horse kidney) material. The contents of Cd and other elements determined are given in Table 5 and compared with those determined in parallel analysis with thermal activation and the IAEA range of values obtained in the preliminary analysis. The results compare favourably for all elements. To assess the applicability of the technique to the analysis of biological fluids, a mixture of plasma and erythrocytes (haematocrit 20%), from a sample collection taken from an area currently being characterised for selenium levels,[23] were analysed by ENAA. Corresponding samples were analysed with the conventional thermal neutron activation. Table 6 gives the results of the elemental content of Fe, Rb, Se and Zn determined with both techniques. As can be seen in Table 6, epithermal activation provides a quick alternative for instrumental determination of these elements in biological fluids at such levels of concentration. For much lower concentrations, however, thermal neutron activation is the preferable method for most elements as the epithermal neutron flux is usually one or two orders of magnitude less than the thermal neutron flux in many irradiation facilities.

Precision and Accuracy

The reliability of the concentrations determined by the two irradiation techniques can be evaluated in terms of the precision and accuracy achieved. For this reason, accuracy is defined as the difference between the experimental arithmetic mean content ($\bar{x}$) and the certified or recommended value (given in parentheses) (x_c) expressed as relative deviation (%) and precision is given as the standard deviation (s) of N repeated determinations expressed in terms of the coefficient of variation (CV) (CV = 100 $s/\bar{x}$). Figs. 3 and 4 show the distribution of precision and accuracy for the total number of elements determined by means of the two activation techniques in NBS bovine liver and NBS oyster tissue with long irradiation conditions. For many elements, good precision is achieved with the conventional thermal neutron activation. This is simply because many elements have esentially the same response to thermal neutrons and the activation yields are generally high with little or no discrimination. In epithermal activation, however, selectivity is more pronounced and good precision is usually obtained for those elements which exhibit favourable resonance cross-section characteristics, as revealed in Figs. 3 and 4. However, in both instances, the matrix

Table 5. Results of the analysis of IAEA-H8^{-1} (horse kidney) (μg g^{-1}) by INAA with ENAA and RNAA

Element	Isotope	ENAA* $\bar{x} \pm s$	ENAA* LD	RNAA* $\bar{x} \pm s$	RNAA* LD	IAEA range
Cd	^{115}Cd	188.0 ± 14	1.34			140.2–252.67
Co	^{60}Co	0.20 ± 0.03	0.01	0.13 ± 0.01	0.002	0.096–1.39
Fe	^{59}Fe	300.0 ± 50	7.2	298.0 ± 41	2.4	144.0–329
Rb	^{86}Rb	21.55 ± 1.92	0.15	24.19 ± 2.93	0.16	19.0–26.0
Se	^{75}Se	4.84 ± 0.44	0.06	4.49 ± 1.10	0.10	2.87–6.40
Zn	^{65}Zn	193.0 ± 18	0.34	210.0 ± 21	0.22	165.0–255.0
Irradiation time		24.0 h		24.0 h		
Cooling time		15–21 d		50–70 d		
Counting time		7200 s		7200 s		

* All values are arithmetic means ($\bar{x}$) ± standard deviation from eight parallel determinations.

Table 6. Results of the analysis of a mixture of plasma and erythrocytes (haematocrit, 20%)

	Plasma + erythrocytes analysed with ENAA					Plasma + erythrocytes analysed with RNAA				
	Concentration/ μg g^{-1} (dry mass)				Detection limit/	Concentration/ μg g^{-1} (dry mass)				Detection limit/
Element	1	2	3	4	μg g^{-1}	1	2	3	4	μg g^{-1}
Fe	1940 ± 32	2006 ± 65	2141 ± 23	1699 ± 71	50.0	1920 ± 83	1984 ± 85	2155 ± 95	1664 ± 71	8.0
Rb	7.4 ± 0.2	9.0 ± 0.2	6.7 ± 0.1	10.3 ± 0.3	0.4	7.0 ± 0.5	8.8 ± 0.6	6.9 ± 0.6	9.3 ± 0.6	1.6
Se	5.4 ± 0.2	4.9 ± 0.3	4.5 ± 0.1	7.4 ± 0.2	0.3	5.3 ± 0.4	5.0 ± 0.1	4.6 ± 0.2	7.1 ± 0.2	0.1
Zn	24.7 ± 0.5	27.2 ± 0.6	28.9 ± 0.5	29.3 ± 0.7	2.0	25.3 ± 1.2	31.5 ± 1.5	29.6 ± 1.4	29.7 ± 1.3	0.1
Irradiation time	48 h					5 d				
Cooling time	25 d					3 months				
Counting time	7200 s					7200 s				

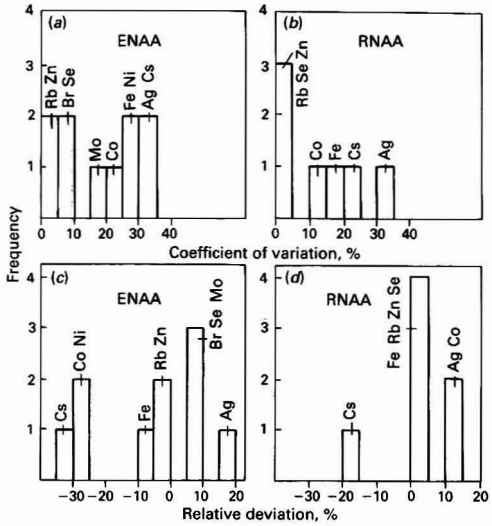

Fig. 3. Distribution of (*a*) and (*b*) precision and (*c*) and (*d*) accuracy of determinations in NBS bovine liver

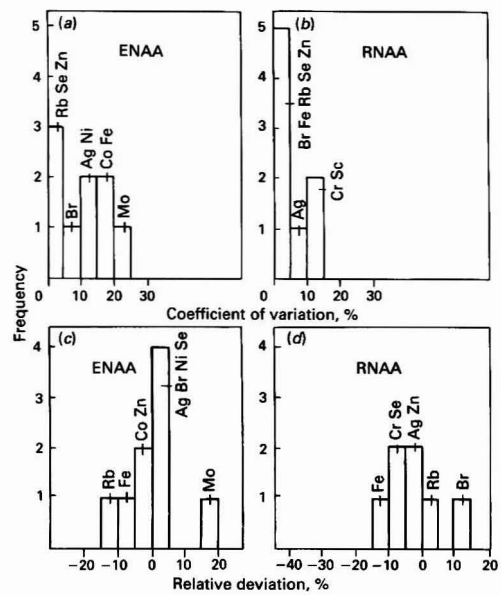

Fig. 4. Distribution of (*a*) and (*b*) precision and (*c*) and (*d*) accuracy of determinations in NBS oyster tissue

composition of the sample may influence the precision. The accuracy of determinations, on the other hand, is comparable for many elements in both activation techniques.

Conclusions

Epithermal neutron activation may be advantageously utilised for the rapid instrumental determination of trace elements in biological materials. The reduction in the time scale of analysis, in comparison with the conventional thermal neutron activation method, is of value particularly for the routine analysis of biomedical and environmental samples, where it may be necessary to obtain accurate and reliable results quickly. The technique can be applied to determine As, Br, Cd, Fe, Mn, Mo, Ni, Rb, Se, Sr and Zn in biological materials at both the upper and lower p.p.m. range without chemical separation. If it is complemented with thermal neutron activation analysis, the range of elements that can be determined instrumentally can be substantially increased. The major disadvantage of epithermal activation, however, is the need for neutron filters, which raises the relative cost of the application.

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Resonance Raman Spectroscopic Study of 2,4,6-Trinitrotoluene and its Adduct with the SO_3^{2-} Ion

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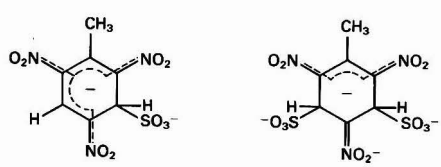
It is shown that, although 2,4,6-trinitrotoluene (TNT) itself undergoes photodegradation under resonance Raman conditions, its adduct formed in aqueous sodium sulphite is stable to this treatment. Enhancement of the (polarised) 1304 cm^{-1} band of the adduct is optimal with 457.9 nm (21 839 cm^{-1}) excitation and forms the basis of a sensitive method for the detection and identification of TNT at *ca.* 10^{-6} M in aqueous solution.

Keywords: *2,4,6-Trinitrotoluene; resonance Raman spectroscopy; sulphite adduct*

The application of spectroscopic techniques to the detection and identification of explosive materials is of considerable importance in forensic investigations. Although the use of infrared spectroscopy has been well documented,[1-3] much less attention has been paid to the complementary vibrational technique, Raman spectroscopy. In two recent papers, Carver and co-workers[4,5] reported the observation of Raman spectra of trace amounts of five explosive compounds, recorded using non-resonant laser excitation. The spectra were obtained from samples adsorbed on silica gel and activated charcoal substrates, employing conventional, *i.e.*, macro, sample illumination[4] and a Raman microprobe.[5] There do not appear to be any reports of resonance Raman studies of explosives.

The original purpose of this study was to assess the potential of resonance Raman spectroscopy for the detection of 2,4,6-trinitrotoluene (TNT), for which the longest wavelength absorption band is at 232 nm (43 100 cm^{-1}). Although it was expected that laser excitation at 257.3 nm (38 873 cm^{-1}) would produce substantial resonance enhancement of the Raman spectrum, photodecomposition occurred at this wavelength; nevertheless, pre-resonance Raman spectra could be obtained using longer wavelength excitation. Owing to the photodecomposition of TNT at 257.3 nm (38 873 cm^{-1}), it was decided to investigate the resonance Raman spectrum of the TNT: SO_3^{2-} adduct formed by dissolution of TNT in an aqueous solution of sodium sulphite. In dilute (<0.01 M) sulphite solutions there is an absorption band at 460 nm (21 740 cm^{-1}) [with a shoulder at 560 nm (17 860 cm^{-1})] attributed to a 1:1 adduct (**I**). At higher sulphite concentrations (>0.1 M) there is a broad absorption band with $\lambda_{max.}$ = 420 nm (23 810 cm^{-1}), believed to be due to a 1:2 adduct[6] (**II**); it is in this concentration region that these studies were carried out.



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Experimental

TNT was supplied by the Ministry of Defence (RARDE, Fort Halstead) and used without further purification. TNT is essentially insoluble in water, and so Raman spectra of TNT were obtained from 0.1 M solutions in acetone using a spinning cell. Solutions of the TNT: SO_3^{2-} adduct were prepared by dissolving 1 mg of TNT in 10 cm^3 of 10% aqueous sodium sulphite, the Raman spectra in this instance also being obtained using a spinning cell.

Raman spectra were recorded on a Spex Ramalog 6 spectrometer in conjunction with Coherent Radiation Model CR12 argon ion and CR3000K krypton ion lasers, and a CR590 dye laser employing stilbene 3 as the lasing medium. Excitation at 257.3 nm (38 873 cm^{-1}) was obtained by frequency-doubling the mode-locked 514.5 nm (19 435 cm^{-1}) output of the argon ion laser using a Coherent Radiation Model 468 mode locker and an Inrad 5-15 frequency doubler. Detection of the scattered radiation was achieved by standard photon counting techniques using an RCA C31034 photomultiplier. Wavenumber measurements were calibrated with the emission spectrum of neon and band intensities were determined as the products of peak heights and full-width half-maxima and corrected for the spectral response of the instrument.

Electronic spectra were recorded on a Cary 14 spectrophotometer.

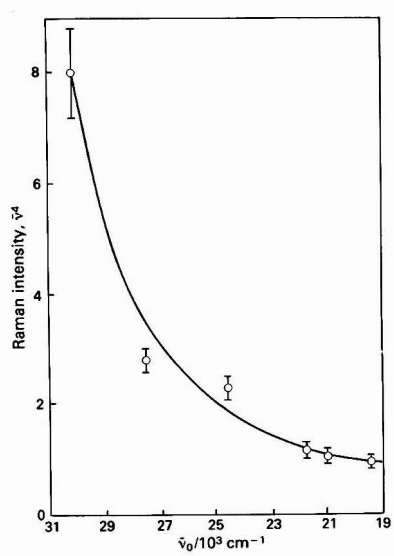

Fig. 1. Pre-resonance Raman excitation profile of the 1358 cm^{-1} band of TNT in acetone solution

Results and Discussion

TNT in Acetone Solution

Raman intensity measurements of the 1358 cm^{-1} $\nu_s(NO_2)$ band of TNT were determined for excitation in the range 337.5–514.5 nm (29 630–19 435 cm^{-1}) using the 1430 cm^{-1} band of acetone as an internal standard. The pre-resonance Raman excitation profile plotted from these measurements is shown in Fig. 1. Photolysis occurred at each of the excitation wavelengths employed, most markedly for UV excitation, resulting in a brown discolouration of the sample and an intense luminescence background. Owing to the photolysis and concomitant luminescence, the Raman band intensity measurements can be regarded as only approximate, but it is clearly evident that the intensity of the 1358 cm^{-1} band increases markedly as the excitation wavelength approaches that of the absorption band of longest wavelength [232 nm (43 100 cm^{-1})]. An attempt was made to obtain a resonance Raman spectrum of TNT using 257.3 nm (38 873 cm^{-1}) excitation. However, this was unsuccessful owing to very rapid photodecomposition of the sample and the generation of intense luminescence.

TNT in Aqueous Sulphite Solution

The orange solution formed by dissolving TNT in 0.8 M (10% *m/V*) sodium sulphite solution has a strong, broad absorption band with a peak at 420 nm (23 810 cm^{-1}). Excitation at 457.9 nm (21 839 cm^{-1}) of a solution in which the TNT concentration was *ca.* 2×10^{-4} M produced the resonance Raman spectrum shown in Fig. 2. This spectrum is characterised by a strong band at 1304 cm^{-1}, $\nu_s(NO_2)$, and several weaker bands, all of which are polarised [$\rho_\perp(\pi/2) \approx 1/3$]; the details are given in Table 1. Owing to the presence of a broad luminescence that maximises at *ca.* 18 300 cm^{-1}, the resonance Raman spectra excited with laser lines with $\lambda_0 > 457.9$ nm are of much poorer quality than that with $\lambda_0 = 457.9$ nm (21 839 cm^{-1}). Notwithstanding this problem, it was possible to monitor the intensity of the 1304 cm^{-1} band throughout the resonance region using the 980 cm^{-1} band of free SO_3^{2-} as an internal standard. The exitation profile (EP) for the 1304 cm^{-1} band is shown in Fig. 3, together with the absorption spectrum of the solution. The EP maximum is red-shifted from the absorption maximum by *ca.* 2000 cm^{-1}, which might be ascribed to interference effects. It was not possible to obtain reliable intensity data for the weaker Raman bands, but we did not observe any gross changes in the intensity distribution throughout the region of excitation, suggesting that all of the bands are due to the same molecular species.

It is estimated that the Raman intensity of the 1304 cm^{-1} band of the TNT : SO_3^{2-} adduct is enhanced for 457.9 nm (21 839 cm^{-1}) excitation by at least three orders of magnitude with respect to the 1358 cm^{-1} band of TNT itself. Additionally, there did not appear to be any photodegradation of the sample during the time scale of the Raman experiments.

The formation of adducts with sulphite ions is a reaction that is specific to aromatic trinitro compounds. Thus, resonance Raman spectroscopy of the 1 : 2 TNT : SO_3^{2-} adduct provides a sensitive analytical technique for the detection of TNT in the presence of other explosives (*e.g.*, nitroglycerine or RDX), because the Raman spectra of other such explosives present would be non-resonant at the excitation wavelengths used here and would therefore be very much weaker than the resonance Raman spectrum of the 1 : 2 TNT : SO_3^{2-} adduct. In an analytical situation one would simply dissolve the unknown material in sodium sulphite solution and record the Raman

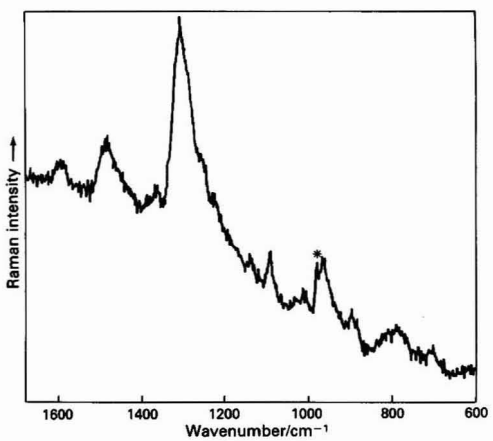

Fig. 2. Resonance Raman spectrum of 2×10^{-4} M TNT in 0.8 M sodium sulphite solution. $\lambda_0 = 457.9$ nm; power at the sample ≈100 mW; scanning speed, 1 cm^{-1} s^{-1}; spectral slit width, 3 cm^{-1} at 457.9 nm

Table 1. Details of the resonance Raman spectrum of the TNT : SO_3^{2-} adduct

Wavenumber/cm^{-1}	Intensity*	Wavenumber/cm^{-1}	Intensity*
702	vw	1030	vw
715	vw	1093	m
740	vw	1140	w
785	br, vw	1190	sh
814	vw	1258	sh
884	sh	1284	sh
897	w	1304	vs
963	w	1365	w
980			
(ν_1, Na_2SO_3)	m	1480	br, m
1010	w	1590	br, w

* Abbreviations: s = strong; m = medium; w = weak; br = broad; sh = shoulder; v = very.

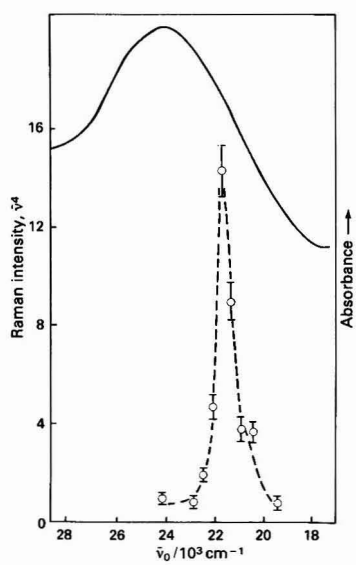

Fig. 3. Absorption spectrum of TNT in 0.8 M sodium sulphite solution, and the resonance Raman excitation profile of the 1304 cm^{-1} band

spectrum. Quantitative measurement of the amount of TNT present could be determined by comparison with solutions containing known amounts of TNT using the 980 cm^{-1} band of Na_2SO_3 as a calibration standard. Although the absorption spectrum of the solution can also be used for analysis, it is broad and featureless and may not be specific to the species under investigation. In contrast, the detailed resonance Raman spectrum, together with the excitation profile of the 1304 cm^{-1} band, provides a "fingerprint" for the 1:2 TNT:SO_3^{2-} adduct.

Conclusion

It has been shown that TNT undergoes photodecomposition when excited with laser radiation within the contour of its longest wavelength absorption band, and that it is therefore not possible to obtain the resonance Raman spectrum of TNT. Resonance Raman spectra can, however, be obtained from the TNT:SO_3^{2-} adduct formed by dissolving a small amount of TNT in sodium sulphite solution. Thus, resonance Raman spectroscopy of this adduct may be used as a sensitive method for the detection and identification of TNT at levels down to *ca.* 10^{-6} M.

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Determination of Antimony in Urine by Candoluminescence Spectrometry

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The candoluminescence emission intensities produced by antimony solutions applied to CaO - $CaSO_4$ matrices have been compared using two techniques, a matrix contained in the cavity of the head of an Allen screw and a rod of the matrix material. The optimum gas flow-rates differ slightly in the two techniques as do the matrix conditions, *i.e.*, the burner head to matrix distance and the horizontal distance into the flame.

The rod technique has the advantage that larger samples of organic solvents, *i.e.*, from a solvent extraction stage, may be applied to the matrix and this procedure provides a simple and reliable routine method for the determination of antimony in urine over the range 10–70 μg l^{-1}. The results are compared with those obtained elsewhere using atomic absorption spectrometry with electrothermal atomisation.

Keywords: *Antimony determination; candoluminescence, spectrometry; urine analysis*

There is considerable disagreement amongst workers about the toxicity of antimony[1,2] but it is generally recognised that stibine (SbH_3) is the most toxic of all antimony compounds—a short term (10 min) exposure to 0.3 p.p.m.[3] affects the central nervous system, which results in nausea and weakness. When ingested, water-soluble antimony compounds cause nausea, vomiting and diarrhoea[1,2] but the reason why very few cases of antimony poisoning have been reported is that antimony in the blood stream is rapidly excreted by the kidneys and therefore increased levels are found in urine.

Reagents that may be used for the spectrophotometric determination of antimony have been reviewed by Marczenko.[4] The Rhodamine B method[5,6] is one of the most extensively researched and is reported to be very sensitive (0–40 μg of Sb in 10 ml of solution can be determined). The colour intensity depends on the amount and order of addition of reagents. Constant experimental conditions must be maintained in order to obtain reproducible results. The coloured complex is not completely soluble in diisopropyl ether and there is deviation from the Beer - Lambert law. Absorbance readings are reproducible within 20% for up to 2 μg with recoveries of over 90% of antimony when 10 ml of whole blood are digested with concentrated H_2SO_4. Interference from several metals is reported.

Christopher and West[7] confirmed that in the Rhodamine B method it is difficult to obtain reproducible results and stated that the bromopyrogallol red method is simple, reproducible and has a high degree of selectivity. Straight-line graphs are obtained for 10–100 μg of Sb ($\varepsilon = 35\,000$). Certain metals are masked by EDTA, CN^- and F^- solutions and no solvent extraction step is involved. However, the method has not been adapted to the determination of antimony in any organic matrix.

The catechol violet method,[8] in which a ternary complex of the dye with antimony and cetyltrimethylammonium bromide is involved, has the same interferences as the bromopyrogallol red system, and linear graphs are obtained at 530 nm for 6.1–61 p.p.m. of Sb.

The methyl fluorone method has been the subject of several papers[9–11] and the 1:1 complex formed with antimony is measured at 495 nm giving linear graphs over the range 0.2–4.0 μg ml^{-1} of Sb. However, using manganese dioxide as a collector one determination of antimony in steel takes 1.5 h.[11] A spectrophotometric method,[12] involving the development of an ascorbic acid - potassium iodide coloured complex, has been used as a reference method for the determination of antimony in organoantimony compounds by atomic absorption spectrometry. The organic antimony compounds are decomposed by H_2SO_4 - H_2O_2 and a linear calibration graph is obtained for 4–20 μg ml^{-1} of Sb.

Decomposition of the matrix of organic or biological materials using strong acids (H_2SO_4, HNO_3 and $HClO_4$) is time consuming, tedious and may result in the loss of antimony by volatilisation when perchloric acid is present.[13]

Atomic absorption spectrometry with electrothermal atomisation (AAS-ETA) cannot be used directly on urine because of interference from the high inorganic solid content.[14] For this reason Smith and Griffiths developed a solvent extraction procedure using ammonium tetramethylenedithiocarbamate in isobutyl methyl ketone (IBMK) and determined the antimony in the organic solvent by AAS-ETA.[14] The extraction of excreted antimony is not complete until an acidity of 2 M is reached.

Antimony is an element that can be conveniently determined by candoluminescence, a technique in which the emission is measured from certain matrices that contain trace metals when exposed to the outer edge of a hydrogen flame burning in air.[17] The trace amounts of the metal ions serve as activators and the intensity of the emission produced depends on the activator concentrations if all the other parameters are kept constant. The most widely used matrix is CaO - $CaSO_4$ but other materials such as Al_2O_3 - $CaSO_4$, $CaWO_4$ and $Sr_3(PO_4)_2$ have been used.[17] It was thought that a suitable method, employing a solvent extraction procedure, could be developed using a porous CaO - $CaSO_4$ matrix, although solvent extraction has not previously been used in conjunction with candoluminescence spectrometry.

Experimental

Apparatus

The candoluminescence intensities were measured at 480 or 580 nm using a Unicam SP 900 flame spectrophotometer fitted with a Meker-type burner and a Pye Unicam AR 55 chart recorder. Full details of the burner assembly, measurement and control of hydrogen, air and nitrogen gas flow-rates have been given previously.[18] For the preparation of calibration graphs using antimony solutions, slit widths of 0.45 and 0.9 mm were used for the Allen screw and rod techniques, respectively. Slit widths of 0.45 mm were also used for the solvent extraction experiments with the rod technique.

Reagents and Urine Samples

Calcium oxide. Prepared by heating analytical-reagent grade calcium carbonate at 800 °C.

Plaster of Paris. Analytical-reagent grade.

Antimony(III) standard solutions. Prepared from stock solutions (500 μg ml^{-1}) that were made up by dissolving 1.371 g of analytical-reagent grade antimony potassium tartrate (≥99.9%) in 500 ml of distilled water. Dilute solutions (1–20 μg ml^{-1}) were prepared fresh daily by dilution with distilled water using graduated glassware.

Ammonium tetramethylenedithiocarbamate (ammonium pyrrolidinedithiocarbamate, APDC). AAS grade with low amounts of cobalt, copper, iron, lead, nickel and zinc (all below 0.5 p.p.m.).

Synthetic urine for preliminary studies. Prepared as suggested by Smith[19] by adding 0.42 g of $MgCl_2.2H_2O$, 0.31 g of $CaCO_3$, 2.86 g of KCl, 3.09 g of $NH_4H_2PO_4$, 5.08 g of NaCl, 0.67 ml of concentrated H_2SO_4, 8.7 ml of concentrated HCl to distilled water and diluting to 1000 ml. All chemicals used were of analytical reagent grade.

Real urine samples. Obtained from workers engaged in the processing of lead and antimony and their alloys and supplied by B. M. Smith of Morganite Electrical Carbon Ltd., Swansea.

Urine samples for calibration. Required for the preparation of calibration graphs using the solvent extraction method and supplied by one of us. It was assumed that these samples contained no antimony. They were spiked with known amounts of standard antimony solutions (500 p.p.m.) to produce a series of dilute standards, 25–150 ng ml^{-1}. The calibration graphs passed through the origin.

Preparation of the Matrices

The matrix material for the Allen screw and rod technique was prepared by mixing equal masses of the ignited calcium carbonate and plaster of Paris and carefully adding small volumes of water until a uniform paste was obtained. This material was then either (a) inlaid into the cavities of Allen screws as previously described[18] or (b) using an aluminium mould and a hand press, pressed into rods of 5 mm diameter and 1.5 cm length using a procedure similar to that described by Dhaler and Kassir.[15] Materials were stored in desiccators containing calcium chloride.

Optimisation of Burner Conditions

These were optimised as follows.

(*a*) To study the effect of changing the flow-rates of H_2, N_2 and air on the candoluminescence intensity at 480 nm, 7.5 μl of 5 p.p.m. Sb solutions were applied to the tip of the rods (slit width 1.05 mm) and to the Allen screws (slit width 0.9 mm).

(*b*) An optimisation of the candoluminescence intensity on varying the burner head to matrix distance and on varying the horizontal position of the matrix with respect to the flame position was also carried out using 7.5 μl of 5 p.p.m. Sb solutions.

(*c*) Variation of the intensity of candoluminescence with wavelength was carried out using the optimum conditions of gas flow-rates as found in (*a*) and the matrix burner distances (vertical and horizontal positions) that gave maximum intensities, as found in (*b*).

In all experiments, the maximum emission was attained 30–45 s after insertion into the flame. Re-introduction of the rod or screw into the flame produced the same intensity of emission. All candoluminescence intensities were corrected for background emission, *i.e.*, using rod or screw matrices with no antimony added.

Solvent Extraction Procedure

A 10.0-ml volume of a standard urine sample adjusted to pH 3–4 was treated with 2.0 ml of a freshly prepared 1% solution of APDC in IBMK and the mixture was shaken for 3 min and subsequently centrifuged. Exactly 0.2 ml of the organic solvent was transferred on to the tip of the matrix using a Hamilton syringe. The organic solvent was quickly adsorbed by the porous matrix. Candoluminescence intensities (expressed in mm on chart paper) were measured at 580 nm, blank readings being deducted from each.

Urine samples (10 ml) from workers engaged in the processing of antimony were treated likewise, and their candoluminescence intensities measured.

Results and Discussion

Good reproducibility in the candoluminescence technique is only possible if very precise control of a large number of variable parameters is exercised. A comparison of results between workers is difficult because a variety of spectrophotometers have been used, burner designs vary, gas flow-rates are sometimes expressed in arbitrary units and there are minor variations from worker to worker in the way in which the matrix is prepared.

In this study a direct comparison between the Allen screw and rod techniques is possible because all the other variables are kept constant. Using 7.5 μl of 5 p.p.m. Sb solutions applied to the matrices, it was found that maximum emission was obtained at a burner head to matrix distance of 11 mm for Allen screws and 12 mm for the rod technique. The flame width was 12 mm and the maximum emissions for the Allen screw and rod techniques were found at 1 and 2 mm, respectively, into the surface of the flame. Using the Allen screw technique another position of maximum candoluminescence is obtained if the screw reaches the other surface of the flame, *i.e.*, about 10 mm from the initial position of the maximum candoluminescence.

Results for the optimisation of gas flow-rates are shown in Fig. 1. Maximum emissions for the two techniques occur at similiar flow-rates of nitrogen and air but a greater hydrogen flow-rate is required to produce maximum candoluminescence for the rod technique. The composition of the matrix is

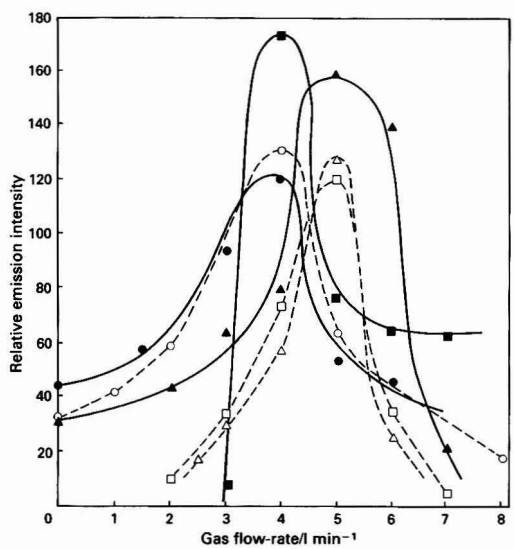

Fig. 1. Relative emission intensities with varying gas flow-rates for the Allen screw and rod techniques. Conditions: 7.5 μl of 5 p.p.m. of Sb for both techniques; emission wavelength, 480 nm; slit width, 0.9 mm for Allen screw technique and 1.05 mm for rod method. Graphs: ■, H_2; ●, air; ▲, N_2, Allen screw technique; and □, H_2; ○, air; and △, N_2, rod technique

Table 1. Results of the determination of antimony in urine samples

Sample No.	[Sb]/μg l^{-1} Candoluminescence* $n = 4$	AAS-ETA†
1	61 ± 3	69
2	28 ± 2	28
3	26 ± 2	36
4	17 ± 3	23
5	30 ± 3	33
6	64 ± 3	66
7	25 ± 3	24
8	60 ± 2	54
9	54 ± 2	59
10	12 ± 2	17

* Using the rod technique.

† Results supplied by Smith.[19] These results were from random samples taken from routine analyses. The standard deviation of a sample containing 43.8 μg l^{-1} of Sb was 2.6 (n = 11).

especially important in the rod technique as the material must be rigid and must not disintegrate in the flame. Emission intensities increase as the proportion of CaO increases but the material becomes weak if less than 40% of $CaSO_4$ is present and the best compromise appears to be 50% of $CaSO_4$. This composition was used for both procedures and has been found to be convenient by other users of the Allen screw technique.[15]

Background spectra are produced in the candoluminescence technique. An emission due to the OH band occurs at 306 nm (with a second-order band at 612 nm) and as the matrix materials inevitably contain sodium there is some emission at 589 nm.[16] The region between 320 and 600 nm is free of these interferences but the matrix materials of CaO and especially $CaSO_4$ contain some manganese, which gives a broad emission at 580 nm. Linear calibration graphs (over the range 0–50 ng of Sb) are obtained at both 480 and 580 nm using aqueous standard antimony solutions. Linear calibration graphs are also obtained over the range 0–150 ng of Sb using antimony transferred into the matrix by an ADPC - IBMK solvent extraction procedure.

As the same batch of CaO - $CaSO_4$ matrix is used for the preparation of rods for blanks, samples and calibrations, and as all other parameters are kept constant, the enhancement of the emission with the addition of an aqueous or organic solvent containing antimony can (after the deduction of the emission of the blank matrices) be regarded as a true measurement of the amount of the element present. In the solvent extraction procedure, in which the antimony in urine is complexed with APDC in IBMK, larger samples can be analysed by the rod procedure because the organic solvent is adsorbed rapidly into the matrix and volatilises quickly. On insertion into the flame, slight charring of the rod occurs and the ligand is destroyed, but this does not affect the emission intensity when the rod is subsequently inserted into the flame and a linear calibration graph is obtained. Heavy-metal concentrations in urine vary widely from person to person and although other metals are extracted from acidic solution using APDC[20] only lead and antimony give candoluminescence emissions and only antimony is quantitatively extracted at pH 3. Any emission from (partially extracted) lead ($\lambda_{max.}$ 390 nm) does not interfere. The results (Table 1) obtained by the APDC - IBMK solvent extraction candoluminescence technique are comparable to those obtained by AAS-ETA.[8]

It is possible that solvent extraction procedures may find other applications when used in conjunction with candoluminescence spectrometry. The agreement between the technique and AAS-ETA for the determination of antimony in urine is satisfactory and this suggests that interferences are not a major problem in this particular application.

The authors thank Morganite Electrical Carbon Ltd. for supplying real urine samples and for the AAS-ETA results in Table 1.

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A New Way of Organising Spectral Line Intensity Ratio Fluctuations

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A new method for organising spectral line intensity ratio fluctuations has been developed to determine which line pair of analysis is more or less stable for repeated measurements. A linear graph was obtained for iron lines when the spectral line intensity ratio fluctuations were plotted *versus* the photon energies of these lines. When these fluctuations were plotted *versus* the upper energies instead, a non-linear arrangement was observed. Therefore, non-linearity was obtained with the conventional intensity equation incorporating the Boltzmann exponent, but linearity was observed if $\exp(-h\nu_{mn}/kT)$ was used instead. The same result was also achieved for absolute intensities of iron lines using intensity tables (NBS) from d.c. arc measurements with photographic registration.

The light source used in this work was an inductively coupled plasma with a slot-type nebuliser system. The analyses were carried out using a computerised image dissector échelle spectrometer system.

Keywords: *Inductively coupled plasma; intensity equation; line intensity ratio fluctuations; échelle spectrometry*

The basis of modern analytical atomic spectrometry was introduced by Gerlach[1] in 1925 when an internal standard technique was used instead of a current external standard technique. The principle of the internal standard technique is based on relating the intensity ratio of selected spectral lines of the analytical and internal standard element to concentration ratios. In the five decades following Gerlach's innovation, quantitative analytical spectrometry has grown in sophistication and application and is today one of the most important methods available to the analyst. During the same period, analysts evolved a large number of "rules," which were intended to ensure the selection of the best possible analytical line pair in every situation. These "rules" have been summarised in the literature[2,3] and are divided into two groups—those concerned with the choice of the internal standard element and those concerned with the choice of the specific lines to be used.

During the last decade the application of the inductively coupled plasma (ICP) to analytical spectroscopy has provided a light source that is far more manageable for studying the excitation behaviour of an analytical line pair.

The principles that affect the choice of analysis and internal standard lines in analytical atomic spectrometry have been investigated theoretically by Barnett *et al.*,[4] using an ICP. They studied the effect of excitation energy, ionisation energy, partition functions and electron density on the analytical line pair intensity ratio.

Suckewer described the essential sources of spectroanalytical errors connected with the determination of the density ratios of atoms in plasmas.[5] He studied the effect of the distribution function of the atom density over the plasma radius and deviations from local thermal equilibrium (LTE) in the plasma using a powerful pulsed discharge system.

In this work a method of organising spectral line intensity ratio fluctuations has been developed,[6,7] *i.e.*, fluctuations of simultaneously measured line intensity ratio of an ICP have been organised into a linear graph in a new way so that it is possible to see which line pair of analysis is more or less stable for repeated measurements. The theoretical basis of these results has been developed[8] and newer theoretical results have recently been presented.[9]

Experimental

The experimental part of the work was carried out at the Swedish Geological Survey (SGU) in Uppsala, where a horizontally mounted ICP light source was used in combination with a computerised image dissector échelle spectrometer (IDES) system.[10]

A Plasma Therm (2.5 kW) ICP unit with an automatic tuning system was used. The experimental parameters of this ICP system in combination with a new slot-type nebuliser and a cyclone spray chamber[11] are given in Table 1. The sample used was a synthetic iron solution (1%). A similar experiment using a side-on ICP (Plasma Therm, 2.5 kW) with the same nebuliser system and also in combination with an IDES was performed at the Swedish Institute for Metals Research in Stockholm on the following five dissolved steel samples: NBS 361, 362, 363, 364 and 365 (1 g per 100 ml).

As a registration system the IDES system was used. This system, which works with photon counting, is very versatile and consists of an échelle spectrometer with high resolution, an image dissector tube and a minicomputer system (Table 1).

For the measurements and analyses using the IDES system, a computer program for peak measurements was used. In this program every spectral line is measured for 0.1 s at the top of each integration. By integrating 15 times, each spectral line is measured for 1.5 s, which is sufficient to obtain accurate results with this spectrometer system.

Theoretical

The usual method of analysing emission line spectra is based on studying spectral line pairs from a sample. However, no satisfactory explanation for some large discrepancies observed

Table 1. Instrumental conditions for the ICP light source and spectrometer

ICP light source—	
Experimental parameters	Slot-type nebuliser with cyclone spray chamber
Power	1.2 kW
Henry frequency	27.12 MHz
Argon cooling gas flow-rate	12 l min^{-1}
Spectrometer—	
Type	Échelle (IDES)
Wavelength region	200–800 nm
Linear dispersion	0.16 nm mm^{-1} at 200 nm 0.32 nm mm^{-1} at 400 nm
Entrance and exit slits	35 μm
Integration time	60 s
Integration time per line	1.5 s
Integration time per measured point	0.1 s

in this analysis method has been presented so far. There has been a tendency to exclude some poor line pairs from the analysis without giving any satisfactory explanation.

As all line pairs do not fit into the analysis scheme, it is reasonable to question whether the basic theory is correct. As yet it is not clear why certain line pair combinations are more or less stable for the repeated analyses of a sample. A detailed survey of the experimental and theoretical principles involved has been presented by Barnett *et al.*[4] In addition, Suckewer[5] has discussed the different parts of the partition functions. These workers and others assume that the line intensity from an atomic transition $E_m \rightarrow E_n$ is proportional to the Boltzmann factor, $\exp(-E_m/kT)$, where E_m is the energy of the upper energy level, E_n that of the lower energy level and $h\nu_{mn} = E_m - E_n$ the energy of the emitted photon. Thus the conventional intensity equation can be written as

$$I^a_{mn} = C^a_{mn}[\Sigma_i g_i \exp(-E_i{}^a/kT)]^{-1}\exp(-E^a_m/kT) \quad .. \quad (1)$$

where I^a_{mn} is the intensity of light with the photon energy $h\nu_{mn}$ from sample constituent a; C^a_{mn} is a combination of factors that are derived from the transition probability, electron and atomic density, quenching, absorption effects, sample geometry and apparatus constants; $\Sigma_i g_i \exp(-E_i/kT)$ is the partition sum over all energy levels of an atom; and g_i is the statistical weight of an energy level.

Forming the ratio between the intensities of two simultaneously measured lines from sample constituents a and b, respectively, and by using logarithmic differentiation of equation (1), the following equation is obtained:

$$\frac{d(I^a_{mn}/I^b_{m'n'})}{(I^a_{mn}/I^b_{m'n'})} = \frac{d(C^a_{mn}/C^b_{m'n'})}{(C^a_{mn}/C^b_{m'n'})} + \frac{1}{kT}\cdot\frac{dT}{T}(-\bar{E}^a + \bar{E}^b + E^a_m - E^b_{m'}) \quad (2)$$

where $\bar{E}^a$ is the Boltzmann mean value and is equal to

$$\Sigma_i g_i E_i \exp(-E_i/kT)/\Sigma_i g_i \exp(-E_i/kT)$$

and is derived in the following way:

$$d\log[\Sigma_i g_i \exp(-E_i/kT)]^{-1} = \frac{1}{kT}\cdot\frac{dT}{T}\left[-\frac{\Sigma_i E_i \exp(-E_i/kT)}{\Sigma_i \exp(-E_i/kT)}\right] = -\frac{1}{kT}\cdot\frac{dT}{T}\cdot\bar{E} \quad .. \quad .. \quad (3)$$

It is necessary to identify the absolute values of the logarithmic derivatives with the maximum relative deviations observed for the set of data obtained in a series of repeated simultaneous measurements of line intensities. This is carried out by forming the ratios of pairs of simultaneously measured intensities and determining the average value of each line pair ratio. The maximum relative deviation from the average value of a line pair ratio will hence be identified with the absolute value of the left-hand side of equation (2). The term R value is used to represent the maximum relative deviation and the R value of a quantity x is $R(x)$. On defining

$$D(E) = |-\bar{E}^a + \bar{E}^b + E^a_m - E^b_{m'}| \quad .. \quad .. \quad (4)$$

equation (2) can be written as

$$R(I^a_{mn}/I^b_{m'n'}) = D(E)(T)/kT + R(C^a_{mn}/C^b_{m'n'}) \quad .. \quad (5)$$

The choice of terms on the right-hand side of this equation maximises the R value of the intensity ratio. For this we have assumed that the R value of the C factor ratio, $R(C^a_{mn}/C^b_{m'n'})$, is independent of $R(T)$, the maximum relative temperature fluctuation. Using equation (5) it is possible to obtain a straight line with a direction coefficient of $(1/kT)(T)$ when plotting $R(I^a_{mn}/I^b_{m'n'})$ *versus* D(E) for all studied line pairs of the elements a and b because equation (5) is a linear differential equation. In this equation the R value of the C factor ratios is assumed to be constant. This assumption does not mean that the relative fluctuations of the factors in C are necessarily small. Large fluctuations arising from "bulk" effects such as changes in the instrument parameters or the density of the sample constituents will not significantly affect the relative deviation of the ratio of the C factors between two simultaneously measured spectral lines.

The material of observation can be treated as a sample from a normal population as there is only a very small probability that a statistical point will be situated far from the normal population.[12] The probability of obtaining values deviating by more than σ from the mean value is 33%, and for 2σ and 3σ it is 5 and 0.25%, respectively. Therefore, there is only a very small probability of selecting a measurement that deviates significantly from the mean. Such a value can be easily recognised and eliminated. The linearly connected differentials in equation (2) can be interpreted as measured deviations from mean values. To observe experimentally the linear relationship in equation (2), equation (5) must be used with the logarithmic differentials interpreted as maximum relative observed fluctuations. This allows the use of maximum relative deviations of intensity ratios in this investigation. Therefore, the probability of finding an extremely deviating intensity ratio is very small.

The R values of the part of the C factor ratios that come from the apparatus constants (IDES system) are, however, very small compared with the other C factor ratio fluctuations. This is due to the very accurate photon counting system and allows the contribution to the fluctuations resulting from the spectrometer system to be neglected in equation (5).

When plotting $R(I^a_{mn}/I^b_{m'n'})$ *versus* $D(E)$ and using data obtained by repeated measurements, it was found that the conventional intensity equation did not show any linear

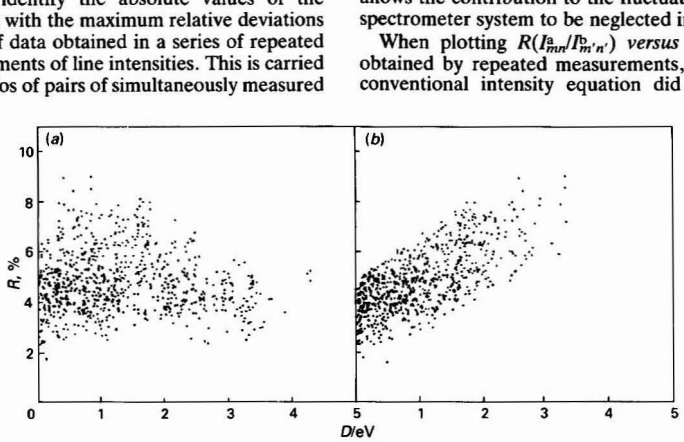

Fig. 1. Maximum relative deviation of spectral line pair (iron) intensity ratios. (*a*) Plotted *versus* $D(E) = |E_m - E_{m'}|$ as defined in the text ($r = -0.08$); and (*b*) plotted *versus* $D(E) = |h\nu_{mn} - h\nu_{m'n'}|$ ($r = 0.74$). The data in (*b*) were obtained from 30 repeated measurements of Fe I line intensity ratios with an ICP

relationship as could have been expected according to equation (5).

Results and Discussion

By using a very stable ICP light source for making repeated intensity measurements on different iron lines with the IDES system, no linear structure was obtained with $D(E) = |E_m - E_{m'}|$ in equation (5). An example of this lack of linearity is shown in Fig 1(*a*). These points form a cloud with no linear structure at all (negative correlation coefficient). It was then discovered that if the R values of the intensity ratios were plotted *versus* $D(E) = |h\nu_{mn} - h\nu_{m'n'}|$ the linear pattern in Fig. 1(*b*) was obtained with a much better correlation coefficient. Thus the correctness of the temperature- and energy-dependent exponential factor in the conventional intensity equation, $\exp(-E_m/kT)$, becomes questionable. According to Fig. 1(*b*) this exponent should be $\exp(-h\nu_{mn}/kT)$, where $h\nu_{mn} = E_m - E_n$ is the photon energy. The two exponents are obviously identical for emissions from transitions to the ground state (having zero energy value). From the graphs it must be considered how to obtain such results without changing the intensity equation. The width of the graph in Fig. 1(*b*) originates from the fluctuations in the C factors and the slope of the line from the temperature and temperature fluctuation in equation (5).

In the analyses, 39 iron lines in the wavelength range 240–750 nm were simultaneously and repeatedly measured 30 times using the IDES system. This means that it was possible to obtain $1/2(39 \times 38) = 741$ intensity ratio combinations between these iron lines. Each point in Fig. 1(*a*) and (*b*) constitutes the R value from 30 repeated intensity ratio measurements, and the calculations were carried out with a computer.

The spectral lines were chosen so that they originate from completely different upper level energies (3.2–7.5 eV). In this way the differences between the two exponents can be more easily seen. By using the same element (iron) in these two graphs, the influence of the partition sum of the intensity equation (1) is cancelled. In Fig. 1(*b*) both the ground-state and the upper-state transitions are organised together in a linear way, where the ground-state transition points have the same co-ordinates in Fig. 1(*a*) and (*b*). In Fig. 1(*a*) the ground-state transitions are mostly situated in the lower left-hand part of the graph, while the upper-state transitions are situated in the upper left-hand and the right-hand parts of the graph. To obtain the linearisation in Fig. 1(*b*) the upper-state transitions in Fig. 1(*a*) must move towards higher $D(E)$ values for those at the upper left-hand part and towards lower $D(E)$ values for those at the right-hand part of Fig. 1(*a*).

Similar graphs with the same result as in Fig. 1(*a*) and (*b*) have also been obtained using different types of demountable hollow-cathode lamps in combination with the IDES system. In these lamps, solid samples were used and both the same and different elements were used in the intensity ratios. These results will be published in subsequent papers. Similar studies to those for the maximum relative deviation from equation (5) have also been carried out for the relative standard deviation (r.s.d.) of the line intensity ratios. These studies have been carried out using ICP and hollow-cathode lamps and have produced similar results to those in Fig. 1(*a*) and (*b*).

It is well known that the maximum relative deviation (R) has a greater sampling variance than the r.s.d. The maximum relative deviation (R) is used here because equation (2) is a linear differential equation. This is important when determining which $D(E)$ value gives a linear arrangement. Identifying the logarithmic differentials with R values yields the linear relationship (5); using r.s.d. values instead does not yield linear relationships. With r.s.d. values it is easy to show that the graph is weakly parabolic in shape for small $D(E)$ values, tending asymptotically to a straight line for larger $D(E)$ values. Therefore, maximum relative deviations (R) are more convenient to use when evaluating which $D(E)$ expression gives a linear arrangement, although the r.s.d. can also be used to see the effect.

In the ICP the spectral lines are excited by different mechanisms in various parts of the plasma but the observed line intensities are averaged over the whole plasma column. The reason why equation (2) seems to hold for $D(E) = |h\nu_{mn} - h\nu_{m'n'}|$ could be that the slope of the graph, $(1/kT)R(T)$, is averaged over the whole plasma column. In this investigation an end-on mounting of the ICP was used. Studies have also been carried out on an ICP viewed side-on (the conventional type) with similar results and they show the same phenomenon but of course with another mean value of $(1/kT)R(T)$. This is because there are other parts of the plasma that are then exposed. With this conventional type of ICP, different salt concentrations have also been studied, all yielding similar results. Therefore, the higher the salt concentration (lower mean temperature) in the plasma, the steeper the slope of the line and the larger are the temperature fluctuations and, hence $(1/kT)R(T)$ will be higher. However a linear graph is always obtained with $D(E) = |h\nu_{mn} - h\nu_{m'n'}\nu|$ but not with $D(E) = |E_m - E_{m'}|$.

An example of such a side-on experiment can be seen in Fig. 2(*a*) and (*b*), which can be compared with Fig. 1 from the end-on experiment. In this side-on experiment five different dissolved steel samples (1 g per 100 ml) were repeatedly run ten times each and nine different iron lines were studied. Intensity ratio fluctuations were collected from five different steel samples and the mean value of $R(I^a/I^b)$ was calculated and plotted *versus* $D(E)$. This means that each point in the new graphs had collected intensity ratio fluctuations from 50 repeated measurements. From these data it is possible to compare the two exponents in the new graph. The linearisation effect in Fig. 2(*a*) is obvious in this side-on experiment also. Hence, greater linearisation is obtained for data points with the equation $D(E) = |h\nu_{mn} - h\nu_{m'n'}|$ however the ICP light source is turned.

An obvious question is whether or not these fluctuations come from the IDES system only. A simple way of illustrating that this is not the case is to run a very stable closed hollow-cathode lamp from the AAS technique in the IDES system. This has been carried out and these fluctuations are very small ($R \approx 0.5\%$) in comparison with Fig. 1(*a*) and (*b*) ($R \approx 2$–9%). From this is it obvious that the fluctuation studied in this paper come mostly from the light source.

Another observation that supports the results in Fig. 1(*a*) and (*b*) is a study carried out on iron lines concerning absolute intensities by using NBS data of an arc spectrum from a photographic registration.[13] From these data it is possible to plot the logarithm of the absolute intensities in equation (1) of a large number of spectral lines (*ca.* 500 lines) *versus* the upper

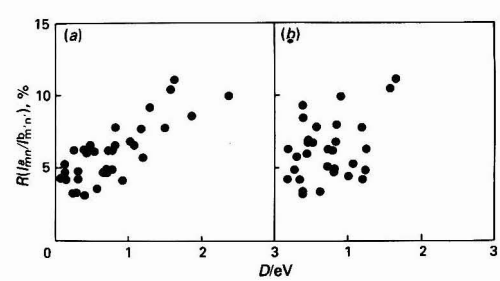

Fig. 2. Maximum relative deviations of spectral line pair (iron) intensity ratios. (*a*) Plotted *versus* $D(E) = |h\nu_{mn} - h\nu_{m'n'}$ ($r = 0.80$) as defined in the text (side-on experiment); and (*b*) plotted versus $D(E) = |E_m - E_{m'}|$ ($r = 0.43$). The data set in (*b*) were obtained from 50 repeated measurements of Fe I line intensity ratios with an ICP as defined in the text (side-on experiment)

energies as carried out previously when determining transition probabilities.[14]

Because lines with different intensities are used in this study, a broad linear relationship is obtained at lower upper energies. At higher upper energies (>6 eV) the broad linear arrangement becomes non-linear. On the other hand, when the same data are plotted *versus* the photon energies, a linear relationship is obtained over the whole wavelength region, as predicted mathematically. This exercise was repeated for the iron lines from the ICP experiment, with the same results.

The results in this paper are experimentally obvious and unequivocal, but they contradict the basic theory of optical emission spectrometry. Whatever the reason for this, the results are interesting and should be considered in greater detail to determine if the basic theory is correct.

From a more practical point of view, the results in Fig. 1(*b*) show that it seems to be possible to pick out which line ratios are more or less stable for analysis work, and this means that line ratios with small $D(E)$ values should be used. This can have practical importance in analysis work for workers choosing spectral lines for spectrometer systems. The fact that line pairs should be used with about the same wavelength to obtain accurate analytical results has been known in practice for a long time, but the line pairs have not been organised in a graph before, where these facts are easily seen.

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Spectrofluorimetric Determination of Hafnium and Zirconium with 3,7-Dihydroxyflavone

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The absorptive and fluorescent characteristics of the hafnium and zirconium complexes of 3-hydroxyflavone and its 12 hydroxy and methoxy derivatives have been studied. The fluorescence of the 1:1 hafnium - 3,7-dihydroxyflavone complex ($\lambda_{ex.}$ 397 nm, $\lambda_{em.}$ 465 nm) in 3 M hydrochloric acid has been used to determine 2–40 ng ml^{-1} of hafnium. The fluorescence of the 1 : 1 zirconium - 3,7-dihydroxyflavone complex ($\lambda_{ex.}$ 395 nm, $\lambda_{em.}$ 465 nm) at pH 2.0 in 0.02 M sulphate solution has been used to determine 2–40 ng ml^{-1} of zirconium. These methods are very sensitive and can be used for the simultaneous determination of hafnium and zirconium with an error of about 5%.

Keywords: *Hafnium determination; zirconium determination; spectrofluorimetry; 3,7-dihydroxyflavone; 3-hydroxyflavone derivatives*

It is well known that hafnium and zirconium react with derivatives of 3-hydroxyflavone in acidic solutions to form fluorescent complexes. Spectrofluorimetric methods for the determination of hafnium have been reported using the following derivatives of 3-hydroxyflavone: 3-hydroxyflavone,[1] morin,[2,3] myricetin,[4] myricetin-3′-glucoside,[5] quercetin,[6–9] quercetinsulphonic acid[10] and quercetin-7-glucoside.[11] In addition different derivatives of 3-hydroxyflavone have also been used for the spectrofluorimetric determination of zirconium.[12] The fluorescence intensities of hafnium and zirconium complexes are strongly influenced by substituents at different positions on 3-hydroxyflavone and moreover by different types and the concentrations of acids. We have previously reported the spectrofluorimetric determination of hafnium[13] and zirconium[14] with 3-hydroxychromone, of which 3-hydroxyflavene is a 2-phenyl derivative. In these investigations, we thoroughly examined the effects of different types and concentrations of acids on the fluorescence intensity. Further, we studied fluorescent reactions of antimony(III) and tin(IV) with 3-hydroxyflavone and its 12 hydroxy and methoxy derivatives in acidic media, and the spectrofluorimetric determination of antimony(III) in 1 M perchloric acid[15] and tin(IV) in 3 M phosphoric acid[16] with 3,7-dihydroxyflavone. This paper describes the fluorescent reactions of hafnium and zirconium with 3-hydroxyflavone and its 12 hydroxy and methoxy derivatives, and the spectrofluorimetric determination of hafnium in 3 M hydrochloric acid and zirconium at pH 2.0 in the presence of 0.02 M sulphate solution with 3,7-dihydroxyflavone. In addition, the simultaneous determination of hafnium and zirconium has been attempted.

Experimental

Reagents

Hafnium and zirconium solutions were prepared by dissolving hafnium chloride (Mituwa Chemicals) and dichlorooxozirconium (Merck) in 3 M hydrochloric acid, 3 M sulphuric acid or 3 M perchloric acid and standardising by back-titration with EDTA. Working solutions were prepared by dilution with 3 M hydrochloric acid, 3 M sulphuric acid or 3 M perchloric acid.

3,7-Dihydroxyflavone and the other 3-hydroxyflavone derivatives listed in Table 4 (except morin and quercetin) were synthesised as described earlier.[15] Morin and quercetin (Merck) were recrystallised from ethanol. These reagents were used as methanolic solutions. All other chemicals were of analytical-reagent grade.

Apparatus

Fluorescence spectra were recorded with a Hitachi Model 650-10S spectrofluorimeter fitted with a xenon lamp. Fluorescence spectra were not corrected. A Hitachi Model 203 spectrofluorimeter fitted with a medium-pressure mercury lamp was used for quantitative measurements. An aqueous solution (1 μg ml^{-1}) of sodium fluorescein was used to adjust the sensitivity of the spectrofluorimeter. Absorption spectra were recorded with a Hitachi Model 124 spectrophotometer and absorbance measurements were obtained with Hitachi Model 139 and 101 spectrophotometers. Quartz cells (10 × 10 × 45 mm) were used for all measurements. A Toadenpa Model HM-10B pH meter was used for pH measurements.

Procedures

Determination of hafnium

To a sample solution containing 0.05–1.0 μg of hafnium, add 1 ml of a methanolic solution (2×10^{-3} M) of 3,7-dihydroxyflavone, 11.5 ml of methanol (the final methanol content should be 50% *V/V*) and a sufficient amount of hydrochloric acid to adjust its concentration in the final solution to 3 M. Dilute the mixture to 25 ml with water. After about 1 h, irradiate the solution with the mercury lamp (405-nm line) and measure the total fluorescence intensity.

Determination of zirconium

To a sample solution containing 0.05–1.0 μg of zirconium, add 1 ml of a methanolic solution (2×10^{-3} M) of 3,7-dihydroxyflavone, 11.5 ml of methanol (the final methanol content should be 50% *V/V*), 2.5 ml of a 0.2 M solution of sodium sulphate and sufficient hydrochloric acid to adjust the pH to 2.0. Dilute the mixture to 25 ml with water. After about 1 h, irradiate the solution with the 405-nm mercury line and measure the total fluorescence intensity.

Results and Discussion

Spectrofluorimetric Determination of Hafnium with 3,7-Dihydroxyflavone

Hafnium reacts with 3,7-dihydroxyflavone in media of pH < 4 up to 6 M hydrochloric acid to form a water-soluble complex. The hafnium complex has an absorption maximum at 393 nm, which decreases with increasing concentration of hydrochloric acid. The complex shows an intense fluorescence in

2–4 M hydrochloric acid. The effect of concentrations of hydrochloric acid, sulphuric acid and perchloric acid on the fluorescence intensity is shown in Fig. 1. The maximum fluorescence intensity of the complex is shown in hydrochloric acid. Fluorescence is not observed using phosphoric acid. The fluorescence intensity of the reagent blank is minimal in hydrochloric acid and therefore 3 M hydrochloric acid is recommended for the determination of hafnium.

Fluorescence spectra

The excitation and emission spectra of the hafnium complex and the reagent in 3 M hydrochloric acid are shown in Fig. 2; the excitation and emission spectra have maxima at 397 and 465 nm, respectively. When excitation is carried out by a mercury lamp, the 405-nm mercury line is suitable.

Effect of reaction variables

The effect of the concentration of the reagent was examined. The maximum fluorescence intensity is obtained in 4.0×10^{-5} M reagent solution. Higher reagent concentrations cause a decrease in the fluorescence intensity, probably because of an inner filter effect. Taking into account the consumption of the reagent with coexisting ions, an 8.0×10^{-5} M reagent solution was used for the determination of hafnium. Water-soluble solvents were added to prevent the precipitation of the reagent. The effect of the content of methanol, ethanol, acetone and dioxane on the fluorescence intensity was also examined. In dioxane solution the complex is very unstable and the fluorescence intensity decreases with time. For the other three solvents, there is no difference with respect to the effect on the fluorescence intensity, although the fluorescence intensity increases gradually with increasing solvent content. A 50% *V/V* methanol content is therefore recommended. No difference in the fluorescence intensity is observed over the temperature range 15–40 °C and the fluorescence intensity remains constant for at least 2 h.

Calibration graph

Under the recommended conditions, the calibration graph is linear over the range 2–40 ng ml^{-1} of hafnium. The coefficient of variation obtained from five measurements of 0.5 μg of hafnium is 0.60%. The sensitivity of the proposed method is approximately the same as that of the quercetin-7-glucoside method,[11] which is generally referred to as the most sensitive method for the spectrofluorimetric determination of hafnium.

Effect of diverse ions

The effect of diverse ions on the determination of 1.00 μg of hafnium was examined. The tolerance limit was defined as the amount of diverse ion causing an error of not more than 5%.

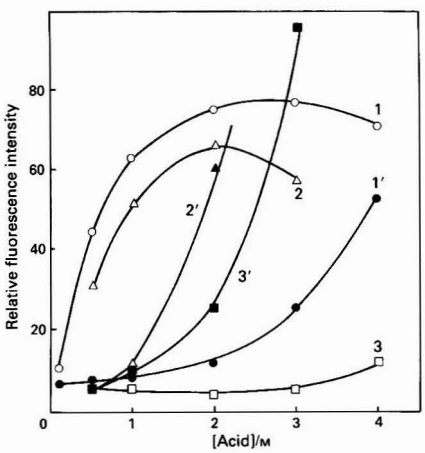

Fig. 1. Effect of acids on the relative fluorescence intensity of hafnium - 3,7-dihydroxyflavone complex: 1–3 are hafnium complexes (2.5×10^{-7} M Hf) in: 1, hydrochloric acid; 2, perchloric acid; and 3, sulphuric acid; 1′–3′ are corresponding reagent (8.0×10^{-5} M)

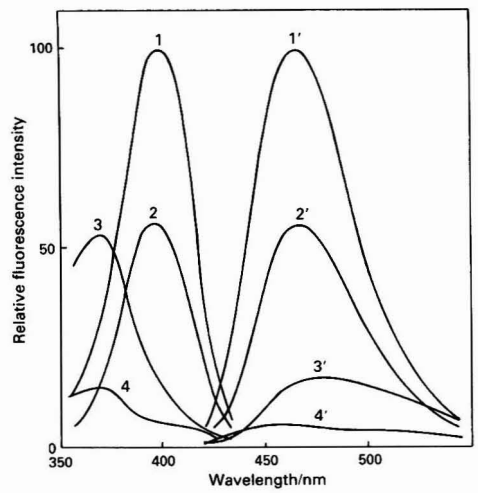

Fig. 2. Fluorescence spectra of hafnium and zirconium complexes of 3,7-dihydroxyflavone: 1–4 are excitation spectra of: 1, hafnium complex; 2, zirconium complex; 3 and 4, reagent in 1 and 3, 3 M hydrochloric acid; and 2 and 4, 0.02 M sulphate solution (pH 2.0); 1′–4′ are corresponding emission spectra

Table 1. Effect of diverse ions on the determination of 1.00 μg of hafnium

Ion	Amount added/μg	Hf found/μg	Ion	Amount added/μg	Hf found/μg
F^-	1	0.99	Sn(IV)	10	1.00
	10	0.89		100	0.82
EDTA	10	0.92	V(V)	10	0.99
	100	0.88		100	0.87
Zr	0.1	1.01	Mo(VI)	10	0.99
	1	1.21		100	0.85
Ge	1	1.12	W(VI)	10	0.96
Ga	10	1.04		100	0.87
	100	1.49	Fe(III)	10	0.98
Ti	10	0.97		100	0.78
	100	0.80			

The diverse ions that interfere below 100-fold amounts (by mass) are summarised in Table 1. EDTA and fluoride cause negative errors, whereas 100-fold amounts of oxalate, phosphate and citrate, 10000-fold amounts of tartrate and 100000-fold amounts of perchlorate, sulphate, nitrate and acetate have no effect.

The positive interferences of zirconium, germanium and gallium can be attributed to the fact that these elements also form fluorescent complexes. In the presence of more than 1 mg, scandium and aluminium also cause positive errors for the same reason. Various cations give negative errors probably by formation of non-fluorescing complexes. The maximum tolerable concentrations of other cations are as follows: copper(II), aluminium, scandium, lanthanum, cerium(IV) and bismuth(III), 0.1 mg; beryllium, calcium, mercury(II), yttrium(III), indium, thallium(III), lead, antimony(III), chromium(III), selenium(IV), tellurium(IV), manganese(II), cobalt(II), palladium, cerium(III), and nickel(II), 1 mg; and zinc, strontium, cadmium, barium and arsenic(III, V), 10 mg.

Spectrofluorimetric Determination of Zirconium with 3,7-Dihydroxyflavone

Zirconium reacts with 3,7-dihydroxyflavone in media of pH <4 up to 4 M hydrochloric acid to form a water-soluble complex. The effect of pH is shown in Fig. 3. Unlike the hafnium complex, the zirconium complex exhibits a faint fluorescence in dilute hydrochloric acid as shown in Fig. 3, whereas in the presence of a suitable amount of sulphate this complex exhibits a strong fluorescence in the pH range 1.0–2.0. Taking into account the simultaneous determination of zirconium and hafnium, a pH of 2.0 is recommended for the determination of zirconium.

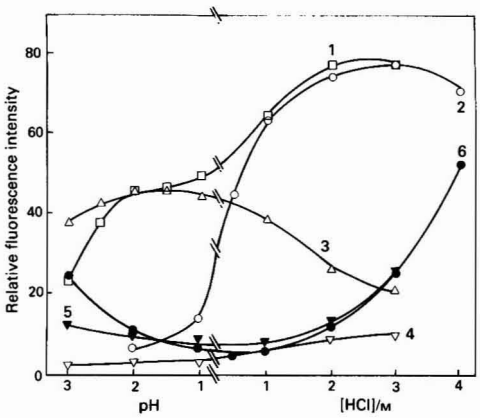

Fig. 3. Effect of hydrochloric acid concentration and pH on the relative fluorescence intensity of hafnium and zirconium complexes of 3,7-dihydroxyflavone. Graphs: 1 and 2, hafnium complex (2.5×10^{-7} M Hf); 3 and 4, zirconium complex (2.5×10^{-7} M Zr); 5 and 6, reagent (8.0×10^{-5} M) using sulphate concentrations of 1, 3 and 5, 0.02 M and 2, 4 and 6, 0 M

Fluorescence spectra

The excitation and emission spectra of the zirconium complex and the reagent at pH 2.0 are shown in Fig. 2. The excitation and emission spectra showed maxima at 395 and 465 nm, respectively. When excitation is carried out by a mercury lamp, the 405 nm mercury line is suitable.

Effect of reaction variables

The effect of the concentration of sulphate was examined and it was observed that the fluorescence intensity of the complex increases with an increase in the concentration of sulphate. The complex shows maximum fluorescence intensity in about 0.02 M sulphate solution. At higher concentrations of sulphate the fluorescence intensity of the complex gradually decreases on increasing the concentration of sulphate, probably because of the formation of a non-fluorescing sulphato complex. Therefore, 0.02 M sulphate solution is recommended for the determination of zirconium.

The effect of the concentration of the reagent was examined. The maximum fluorescence intensity was obtained in $6.0–8.0 \times 10^{-5}$ M reagent solution. An 8.0×10^{-5} M reagent solution was subsequently used.

The effect of the content of methanol, ethanol, acetone and dioxane on the fluorescence intensity is shown in Fig. 4. In the

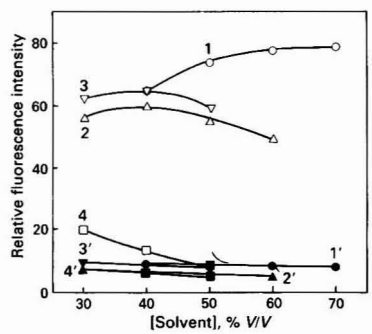

Fig. 4. Effect of the amount of solvent on the relative fluorescence intensity of zirconium - 3,7-dihydroxyflavone complex. Graphs 1–4, zirconium complex (4.0×10^{-7} M Zr) using 0.2 M sulphate solution (pH 2.0) and different solvents: 1, methanol; 2, ethanol; 3, acetone; and 4, dioxane. 1′–4′, Corresponding reagent (8.0×10^{-5} M)

Table 2. Effect of diverse ions on the determination of 1.00 μg of zirconium

Ion	Amount added/μg	Zr found/μg	Ion	Amount added/μg	Zr found/μg
EDTA	0.1	0.94	Al	1	1.04
	1	0.63		10	1.40
F^-	1	0.94	In	1	1.05
	10	0.66		10	1.49
Oxalate	1	0.93	V(V)	1	1.02
	10	0.55		10	0.84
Phosphate	100	0.92	Mo(VI)	1	0.97
Tartrate	100	0.92		10	0.84
Hf	0.1	1.04	Fe(III)	10	0.90
	1	1.51	Sc	10	1.02
Ga	1	1.85		100	1.31
Sn(IV)	1	1.65	Ti(IV)	10	0.97
Sb(III)	1	1.13		100	2.69

presence of a small amount of water-soluble solvents the reagent is precipitated, whereas sodium sulphate is deposited in the presence of a large amount of solvents. A 50% *V/V* methanol content is therefore recommended. No difference in the fluorescence intensity is observed over the temperature range 15–30 °C and the fluorescence intensity remains constant for at least 2 h.

Calibration graph

Under the recommended conditions, the calibration graph is linear over the range 2–40 ng ml^{-1} of zirconium. The coefficient of variation obtained from five measurements of 0.8 μg of zirconium is 0.50%.

Effect of diverse ions

The effect of diverse ions on the determination of 1.00 μg of zirconium was examined. The tolerance limit was defined as the amount of diverse ions causing an error of not more than 5%. Diverse ions that interfere below 100-fold amounts (by mass) are summarised in Table 2. EDTA, fluoride and oxalate cause serious negative errors. Metallic ions that cause positive errors are as follows: hafnium, gallium, tin(IV), antimony(III), aluminium, indium, scandium and titanium(IV). The maximum tolerable concentrations of other ions are as follows: citrate, silver, gold(III), germanium, arsenic(III, V), palladium, platinum(IV), 0.1 mg; lithium, copper(II), beryllium, magnesium, calcium, zinc, strontium, cadmium, barium, mercury(II), yttrium(III), lanthanum, cerium(III), thallium(I, III), lead, bismuth(III), chromium(III), selenium(IV, VI), tellurium(IV), tungsten(VI), manganese(II), cobalt(II) and nickel(II), 1 mg; and perchlorate, acetate and nitrate, 10 mg. Compared with the determination of hafnium, many ions interfere with the determination of zirconium, probably because of the weak acidity.

Simultaneous Determination of Hafnium and Zirconium

Hafnium reacts with 3,7-dihydroxyflavone to form a water-soluble complex, which shows an intense fluorescence in 2–4 M hydrochloric acid. Zirconium also reacts with this reagent to form a water-soluble complex, but the zirconium complex shows a faint fluorescence in dilute hydrochloric acid. The fluorescence intensity of the hafnium complex is about ten times that of the zirconium complex in 3 M hydrochloric acid. On the other hand, in the presence of 0.02 M sulphate, both zirconium and hafnium form an intensely fluorescent complex with 3,7-dihydroxyflavone at pH 1–2. At pH 2.0, the fluorescence intensity of the hafnium complex is the same as that of the zirconium complex at the same molar concentration, as shown in Fig. 3. Making use of the above-mentioned experimental results, the simultaneous determination of hafnium and zirconium was attempted. Thus, the fluorescence intensity of two sets of solutions containing hafnium and zirconium can be measured: the first is the fluorescence intensity in 3 M hydrochloric acid (F_1) and the second the fluorescence intensity at pH 2.0 in 0.02 M sulphate (F_2). The following equations can be written:

$$F_1 = \phi_1 c_1 + \phi_2 c_2$$
$$F_2 = \phi_3 (c_1 + c_2)$$

where c_1 and c_2 are the concentrations of hafnium and zirconium and ϕ_1 and ϕ_2 are the fluorescence intensities of hafnium and zirconium per mole in 3 M hydrochloric acid, respectively, and ϕ_3 is the fluorescence intensity of hafnium or zirconium per mole at pH 2.0 in 0.02 M sulphate solution. ϕ_1, ϕ_2 and ϕ_3 are preliminarily found by the measurement of solutions containing a known concentration of hafnium and zirconium. Hafnium and zirconium concentrations are obtained by solving two simultaneous equations. Some results of this simultaneous determination are summarised in Table 3. This method can be used to determine hafnium and zirconium concentrations with an error of about 5%.

Composition of the Complexes

The molar ratio of hafnium to ligand was found to be 1:1 in 3 M hydrochloric acid by spectrophotometric and spectrofluorimetric continuous variations methods. It was also found that the molar ratio of hafnium to ligand was 1:2 in 0.1 M hydrochloric acid by the spectrophotometric method. Similarly, it was found that the molar ratio of zirconium to ligand at pH 2.0 was 1:2 in the absence of sulphate and 1:1 in the presence of 0.02 M sulphate solution. On the basis of the above results, and with reference to the previous papers that described the composition of hafnium and zirconium complexes with 3-hydroxychromone,[13,14] it is likely that a 3,7-dihydroxyflavone of non-fluorescing 1:2 hafnium complex is replaced by sulphate ions or chloride ions to form a 1:1 complex, which is fluorescent. Similarly, it is thought that a reagent bound to non-fluorescing 1:2 zirconium complex is replaced by sulphate ions to form a 1:1 fluorescent complex.

Comparison of Substituent Effects

Hafnium reacts with 3-hydroxyflavone and the 12 derivatives in dilute hydrochloric acid to form water-soluble complexes. The wavelengths of maximum absorption, the molar absorption coefficients and the maximum excitation and emission wavelengths of the hafnium complexes in the optimum concentration of hydrochloric acid are summarised in Table 4, together with the relative fluorescence intensities measured at the maximum wavelengths of the relevant spectrum. In addition, the relative fluorescence intensities of zirconium complexes with these reagents under the optimum conditions of hafnium are also summarised in Table 4, in order to predict the degree of interference of zirconium on the determination of hafnium. The wavelengths of maximum absorption are shifted to longer wavelengths by the insertion of a hydroxy or

Table 3. Simultaneous spectrofluorimetric determination of hafnium and zirconium

Concentration/M × 10^{-7}		Fluorescence intensity		Concentration found/M × 10^{-7}	
Hafnium	Zirconium	3 M HCl	pH 2.0	Hafnium	Zirconium
0	3.0	9.6	55.5	0	3.00
0.5	2.5	22.1	55.3	0.45	2.54
1.0	2.0	37.1	55.4	0.99	2.00
1.5	1.5	52.9	55.4	1.56	1.43
2.2	0.8	72.3	54.7	2.26	0.70
3.0	0	93.0	55.6	3.00	0

Table 4. Absorptive and fluorescent characteristics of hafnium complexes of 3-hydroxyflavone and derivatives in hydrochloric acid

Flavone	HCl concentration/M	Absorption spectra		Fluorescence spectra		Fluorescence intensity*		Fluorescence intensity,* Zr complex
		$\lambda_{max.}$/nm	$\varepsilon \times 10^4$/l mol^{-1} cm^{-1}	$\lambda_{ex.}$/nm	$\lambda_{em.}$/nm	Complex	Reagent	
3-Hydroxy-	3	390	2.1	395	461	66	3	8
2',3-Dihydroxy-	1	388	2.7			0	0	0
2'-Methoxy-3-hydroxy-	1	374	1.3	380	470	20	1	7
3,3'-Dihydroxy-	2	395	2.2			0	0	0
3-Hydroxy-3'-methoxy-	2	394	2.3	398	467	10	1	6
3,4'-Dihydroxy-	2	409	3.0	413	475	65	9	14
3-Hydroxy-4'-methoxy-	2	407	2.5	410	475	100	10	24
3,5-Dihydroxy-	2	410	1.4			0	0	0
3,7-Dihydroxy-	3	393	2.2	397	465	74	12	6
3-Hydroxy-7-methoxy-	3	391	2.2	395	465	76	11	7
3,4',7-Trihydroxy-	2	409	3.1	410	480	74	33	8
Morin	4	418	2.5	421	508	23	2	4
Quercetin	4	423	2.4	428	500	7	0	3

* Relative to the 3-hydroxy-4'-methoxy derivative.

methoxy group at the 4'-position of 3-hydroxyflavone. The molar absorption coefficients of all the complexes are moderately large. The insertion of a hydroxy or methoxy group at the 2'-, 3'- or 5-position of 3-hydroxyflavone decreases the fluorescence intensity. The weak fluorescence of morin and quercetin complexes may be attributed to this effect. In contrast, the insertion of a hydroxy or methoxy group at the 4'- or 7-position increases the fluorescence intensity. The fluorescence intensity of the hafnium complex of 3,7-dihydroxyflavone is about three quarters (75%) that of the complex of 3-hydroxy-4'-methoxyflavone, which shows the greatest fluorescence intensity of the 13 reagents. Nevertheless, 3,7-dihydroxyflavone was selected for the detailed study as described above, taking into account the fluorescence intensity of the zirconium complex and the solubility of the reagent.

Zirconium reacts with these 13 reagents in the pH range 1–3 in the presence of 0.02 M sulphate solution to form water-soluble complexes. The absorptive and fluorescent characteristics of the zirconium complexes at pH 2.0 were also measured. The substituent effects of these characteristics of zirconium complexes were almost the same as those of hafnium. Of the 13 reagents, the greatest fluorescence intensity was shown by 3,4',7-trihydroxyflavone. The order of decreasing fluorescence intensity is 3-hydroxy-7-methoxyflavone > 3,7-dihydroxyflavone > 3-hydroxyflavone > 3,4'-dihydroxyflavone. Taking into account the fluorescence intensity and the solubility of the reagent, 3,7-dihydroxyflavone was selected. The substituent effects on the absorptive and fluorescent characteristics of hafnium and zirconium complexes are almost the same as those of the antimony(III) and tin(IV) complexes reported previously.[15,16]

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Leucoquinizarin as an Analytical Spectrophotometric and Fluorimetric Reagent: Application to the Determination of Magnesium in Pharmaceutical Preparations

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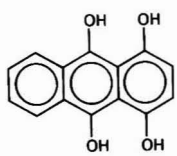
The physico-chemical characteristics and analytical applications of leucoquinizarin are described. The reaction between magnesium and the reagent was studied spectrophotometrically, and a method for the determination of the ion is proposed on the basis of the red complex formed. The spectrophotometric procedure is sensitive ($\varepsilon_{562} = 8.100$ l mol^{-1} cm^{-1}) and the colour is developed at pH 9.5 in a solution containing 58% *V/V* of ethanol. The method has been applied to the determination of magnesium in several pharmaceutical preparations.

Keywords: *Leucoquinizarin; magnesium determination; pharmaceuticals; spectrophotometry; fluorimetry*

The ketohydroxy derivatives of naphthalene and anthracene rings have been extensively studied and proposed as analytical reagents. Among these compounds the hydroxyanthraquinone type are the more classical reagents and, since 1915 when Atack proposed the use of alizarin, other hydroxyanthraquinones have been reported for the spectrophotometric and fluorimetric determinations of metal ions and anions, especially those with a donor-oxygen atom affinity. Attention is now being centred on hydroxyanthracene compounds, which have not been the subject of such analytical consideration, and in this work we focused on the possibilities of leucoquinizarin (1,4,9,10-tetrahydroxyanthracene), **I**, which has only previously been studied as a reagent for amines[1–4] and aromatic aldehydes.[5]



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Experimental

Reagents

All chemicals used were of analytical-reagent grade and distilled water was used throughout.

Leucoquinizarin solution in ethanol, 0.05% *m/V*. Prepared from the commercial product (EGA-Chemie).

Magnesium standard solution, 1.0011 g l^{-1}. Prepared from magnesium chloride hexahydrate and standardised by EDTA titration. Working solutions were prepared by suitable dilution.

Apparatus

Two spectrophotometers were used: a Perkin-Elmer Model Coleman 55 (digital instrument used for measuring absorbances at fixed wavelengths) and a Perkin-Elmer 554 recording spectrophotometer for absorbance scanning. Matched glass and quartz cells of 1.00 cm optical path length were used. A Beckman 70 pH meter with a combined saturated calomel - glass electrode was used for pH measurements. All fluorescence measurements were made with a Perkin-Elmer LS-5 spectrofluorimeter, equipped with Ultrathermostat Colora K5 and 1.00-cm cells.

Procedures

Spectrophotometric determination of magnesium

Into a 25-ml calibrated flask transfer a volume of solution (up to 2.5 ml) containing 12.5–50 μg of magnesium, add 12 ml of 0.05% *m/V* leucoquinizarin in ethanol, adjust the pH to 9.5 with 8 ml of ammonium chloride - ammonia buffer solution and dilute to the mark with ethanol. Heat the sample at 80 °C for 15 min, cool and measure the absorbance at 562 nm against a blank.

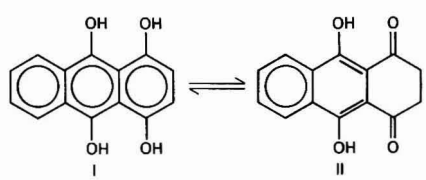
Preparation of sample solutions

The destruction of organic matter in the pharmaceutical preparations was carried out by the established dry ashing method based on the use of a crucible and furnace (controlled temperature).

Results and Discussion

Physico-chemical Properties of the Reagent

The spectra of the reagent solutions (10^{-3} M) in ethanol suffer a hyperchromic effect for 3 h, finally remaining stable in terms of absorbance for at least 14 d; this effect is possibly owing to the stabilisation of the keto - enol tautomeric form (**II**). This assumption is supported by Sterk's study,[6] which indicated that leucoquinizarin is in solution in form **II**, being stabilised by a hydrogen bond.



I II

In addition, the stability of more dilute reagent solutions (2.5×10^{-5} M) in several solvents (water, ethanol, methanol and dimethylformamide) was tested. Leucoquinizarin is more stable in water and ethanol (for at least 4–5 d), with lower stability in methanol, in which the absorbance is only constant for 24 h, and especially in dimethylformamide, which hydrolyses the reagent.

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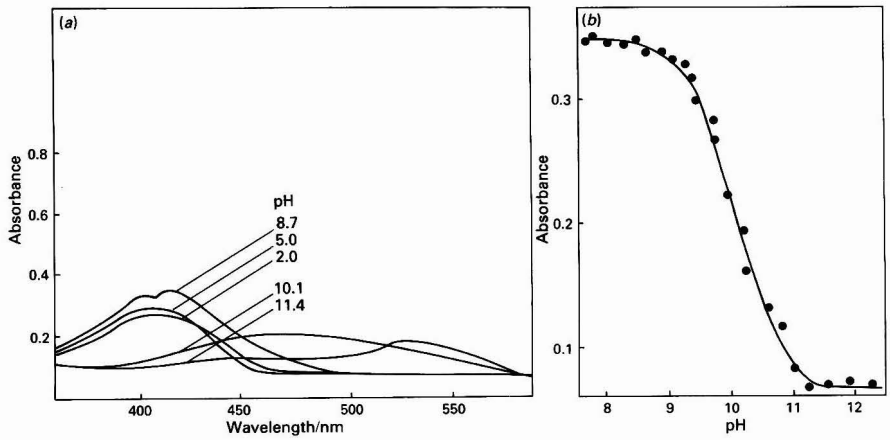


Fig. 1. Influence of pH on the absorption of the reagent. (*a*) Absorption spectra in 11.1% *V/V* ethanol at different pH values. (*b*) Graph of absorbance *versus* pH; $C_R = 5 \times 10^{-5}$ M; $\lambda = 415$ nm

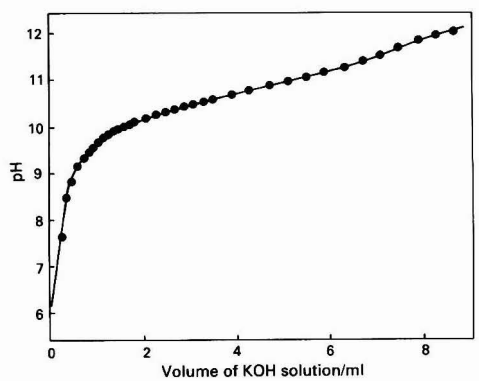


Fig. 2. Potentiometric titration curve for leucoquinizarin using a glass electrode

The influence of pH on the absorbance of aqueous solutions of the reagent (2.5×10^{-5} M) was studied. The absorbance remains constant in acidic media of pH below 6, but decreases at higher pH, especially in alkaline media (pH 11.4), which cause drastic hydrolysis of the reagent.

Reducing agents (such as ascorbic acid) do not affect the spectrum of the reagent; however, oxidising agents (such as potassium persulphate and hydrogen peroxide) decrease the absorbance rapidly.

The ultraviolet spectrum of leucoquinizarin was recorded in 10% *V/V* ethanol solution, $\lambda_{max.}$ being about 400 nm in acidic and neutral media and 535 nm in alkaline media [Fig. 1 (*a*)]. The dissociation constant of the reagent was determined by both spectrophotometric[7-9] and potentiometric[10] methods. Fig. 1(*b*) shows the absorbance *versus* pH graphs at 415 nm; only one of the two structurally possible pK values was determined, pK_1 = 10.0; this value is analogous to other dihydroxyanthraquinones,[11] being verified by Buděšínský's method[12] to represent the interchange of one proton in this equilibrium. The evaluation of the second dissociation step is not possible because it takes place in more alkaline media in which the reagent suffers hydrolysis. The evaluation of the dissociation equilibrium from the titration curve (Fig. 2) gives a pK_1 value in good accord with that from the spectrophotometric method.

The reagent exhibits fluorescence properties in ethanol solution, the excitation and emission wavelengths being 397 and 460 nm, respectively; neither the excitation nor the emission wavelengths are affected by the pH of the medium in the range 1–9, but fluorescence is not observed at higher pH.

Reaction with Metal Ions

The chromogenic properties of the reagent on reaction with metal ions at pH 4.5 and 9.0 were tested and the absorption spectra of these systems recorded. Table 1 summarises the sensitivity of the reactions given as molar absorptivity (ε, $1\,mol^{-1}\,cm^{-1}$). Leucoquinizarin exhibits a reactivity analogous to those of other structurally similar reagents such as quinizarin-2-sulphonic acid,[13] anthragallol and anthrapurpurin,[14] but its selectivity is greater, especially for the Mg(II) chelate at pH 9.0 with an absorption maximum at 562 nm.

The fluorescence spectra of the chelates (pH 4.5 and 9.0) were studied, and a summary of the main spectral characteristics of the fluorogenic reactions of leucoquinizarin with metallic ions is given in Table 2. The relative fluorescence intensities of the chelates with Be(II) and Al(III) are extremely high.

Study of the Magnesium - Leucoquinizarin System

In an excess of leucoquinizarin the magnesium solutions give a red colour whose absorption spectrum shows maxima at 530 and 562 nm.

Stability of the Absorbance Intensity

The stability of the absorbance value of the system at pH 9.5 was carefully studied. The colour developed slowly and the absorbance increased for about 2 h at room temperature. For a more rapid preparation of the samples, they were heated at 80 °C for 15 min and then cooled to room temperature.

Absorbance *versus* pH Graph

The influence of pH on chelate formation and on the absorbance value was tested. The optimum pH range corresponded to an alkaline medium (pH between 9 and 10) (Fig. 3), the absorbance decreasing at acidic and strongly basic pH values.

Effect of the Amount of Reagent

It has been verified that the absorbance of the leucoquinizarin - magnesium system increases appreciably with increasing

Table 1. Analytical characteristics of the main colour reactions of leucoquinizarin

Ion	pH 4.5 $\lambda_{max.}$/nm	pH 4.5 $\varepsilon_{max.} \times 10^{-3}$/l mol^{-1}cm^{-1}	pH 9.0 $\lambda_{max.}$/nm	pH 9.0 $\varepsilon_{max.} \times 10^{-3}$/l mol^{-1}cm^{-1}
Be(II)	437	1.89	439	3.42
Mg(II)	—	—	562	8.10
Al(III)	438	3.35	442	5.39
Co(II)	—	—	442	13.26
Zn(II)	—	—	424	2.35
Ni(II)	—	—	450	11.15
Cu(II)	446	5.08	436	1.90
Fe(III)	448	7.82	438	10.61
Zr(IV)	446	2.55	400	6.15
Ca(II)	—	—	423	1.00
V(V)	—	—	398	4.58
Th(IV)	422	3.62	422	16.24
U(VI)	421	4.28	399	11.90
Ti(IV)	436	3.00	392	5.26
Ga(III)	—	—	423	1.74
In(III)	—	—	440	2.00

Table 2. Analytical characteristics of the main fluorogenic reactions of leucoquinizarin (pH 4.5)

Ion	$\lambda_{exc.}$/nm	$\lambda_{em.}$/nm	Relative fluorescence intensity*
Be(II)	450	476	8.80
Mg(II)	300	461	0.10
Al(III)	442	479	8.91
Co(II)	431	457	0.12
Zn(II)	430	459	0.13
Ca(II)	300	461	0.33
U(VI)	303	460	0.25
Ga(III)	352	466	0.35
In(III)	298	458	0.20

* The relative fluorescence intensity of 0.1 p.p.m. quinine sulphate is 0.8.

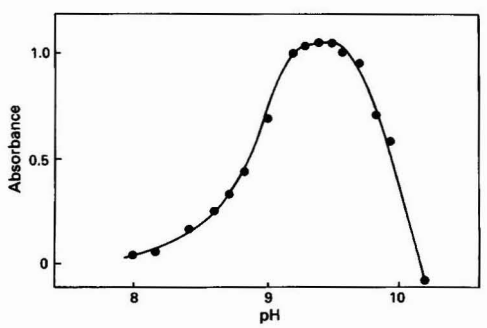


Fig. 3. Absorbance *versus* pH graph for the magnesium - leucoquinizarin chelate at 562 nm; C_{Mg} = 4 p.p.m.; C_R = 5.0 ml of 0.05% leucoquinizarin in ethanol

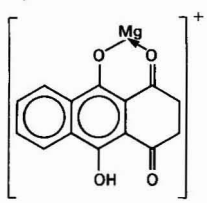
concentration ratio of reagent to magnesium. This is probably due to the formation of a dissociated chelate, and a high concentration of reagent is necessary to guarantee constant absorbance of the samples. In all experiments a 10^{-3} M concentration of leucoquinizarin was used, giving a ratio of magnesium to reagent of 1 : 20; larger amounts of reagent had no additional effect.

Effect of the Order of Addition of Reagents and the Salt Concentration

From experiments in which magnesium, leucoquinizarin, buffer solution and ethanol were added in all possible orders, it was concluded that the absorbance obtained is independent of the order of addition. In addition, the influence of salt concentration (using potassium chloride) was found to be immaterial.

Stoicheiometry and Nature of the Chelate

Because magnesium forms a dissociated chelate with leucoquinizarin, classical methods such as those of Yoe and Jones[15] and Job[16] do not yield reliable results for the determination of the stoicheiometric ratio. For this reason, the Asmus[17] and the modified Holme and Langmyhr[18] methods, which are more suitable for this type of chelate, were tried. Fig. 4 shows the results obtained, which indicate a 1 : 1 molar ratio. In addition, negative tests of solvent extraction of the aqueous magnesium - leucoquinizarin chelate solution in non-polar solvents such as chloroform and carbon tetrachloride, and fixation of the chelate as the counter ion in a cation-exchange resin (Dowex 50-X8), indicated the cationic nature of the complex, and the following structure may be proposed:



Spectrophotometric Determination of Magnesium with Leucoquinizarin

Applying the procedure described earlier it was found that, for the absorbance of the chelate at 562 nm, Beer's law is obeyed over the concentration range 0.4–2.0 μg ml^{-1}. The straight line passed through the origin. The molar absorptivity is 8.100 l mol^{-1} cm^{-1} and the sensitivity of the reaction as defined by Sandell is 0.003 μg cm^{-2} of Mg. The optimum concentration range as evaluated by a Ringbom plot is 0.5–2.0 μg ml^{-1}. The relative error (ρ = 0.05) of the method is 1.61%.

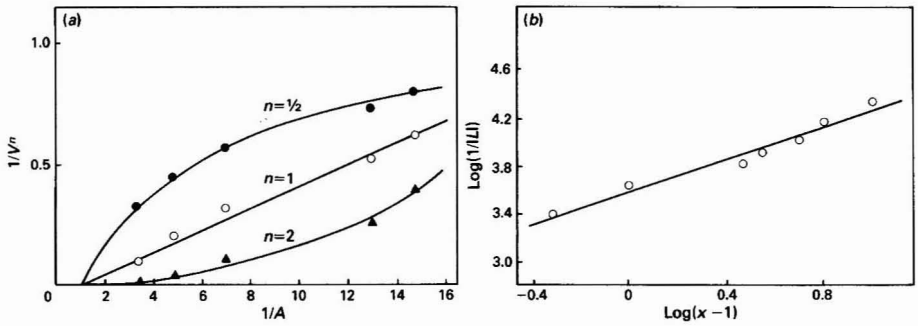


Fig. 4. Determination of the composition of the magnesium - leucoquinizarin chelate. (*a*) Asmus method; and (*b*) modified Holme and Langmyhr method. Conditions: pH 9.5 and λ = 562 nm. A = absorbance; L = free ligand concentration; V = volume of reagent stock solution taken; $X = A_{max}/A$; n = ratio of ligand to metal ion in the complex

Table 3. Tolerance to foreign ions in the determination of 1.2 p.p.m. of magnesium

Amount tolerated, p.p.m.	Ion or species
600	Alkali metals, ClO_4^-
360	Tartrate
300	SO_4^{2-}, I^-
240	SCN^-, Br^-
180	ClO_3^-
120	CH_3COO^-
60	NO_3^-, phthalate, AsO_2^-
30	CO_3^{2-}, SO_3^{2-}, NO_2^-, BrO_3^-
18	F^-
12	Zn(II), Cd(II), Ga(III), Tl(I), IO_4^-, $S_2O_8^{2-}$
1.2	Alkaline earth metals, Ti(IV), Zr(IV), Cr(III), Mn(II), Fe(III), Fe(II), Co(II), (Ni(II), Pd(II), Cu(II), Hg(I), Hg(II), Al(III), Sn(II), Pb(II), Sb(III), Bi(III), Mo(VI), W(VI), EDTA, $S_2O_3^{2-}$

Table 4. Spectrophotometric determination of magnesium in pharmaceutical preparations

	Mg content, % *m/m*		
		Mg found	
Sample	Label specification	Leucoquinizarin*	AAS†
Protergan	2.12	2.08 ± 0.02	2.09
		2.07 ± 0.00	2.09
		2.09 ± 0.01	2.09
Sulmetin	0.23	0.25 ± 0.00	0.22
		0.24 ± 0.00	0.22
		0.23 ± 0.00	0.21
Sulmetin Papaverina . .	2.09	2.41 ± 0.00	2.39
		2.33 ± 0.01	2.30
		2.22 ± 0.01	2.21
Aspartono	3.49	4.47 ± 0.05	4.27
		4.85 ± 0.03	4.11
		5.06 ± 0.03	4.55

* Mean and standard deviation of triplicate determinations.
† Single determinations by atomic absorption spectrometry.

Interferences

In the determination of 1.2 μg ml^{-1} of magnesium by the recommended procedure, foreign ions can be tolerated at the levels given in Table 3. The criterion for the interference was an absorbance value varying by more than ±3% from the expected value for magnesium alone. The maximum concentration tested was 600 p.p.m. for cations and anions.

Numerous interferences restrict the applicability of the procedure. However, some cations, such as Zn(II), Cd(II), Ga(III) and Tl(I), can be tolerated in a molar ratio of 10 : 1; also there is a high tolerance to numerous anions, SO_4^{2-}, OAc^-, SCN^-, I^-, AsO_2^-, phthalate and tartrate in a 20 : 1 molar ratio and CO_3^{2-}, F^-, SO_3^{2-}, IO_4^- and $S_2O_8^{2-}$ in a 10 : 1 molar ratio.

Applications

The method has been applied to the determination of magnesium in the following pharmaceutical preparations: Aspartono, for stress treatment; Sulmetin Papaverina, an antispasmodic - analgesic and sedative product; Sulmetin, an antiemetic preparation; and Protergan, an antianorexic product. These products contained magnesium as the α-ketoglutarate (Protergan), gluconate (Sulmetin and Sulmetin Papaverina) or L-aspartate (Aspartono), some other salts also being present, such as potassium L-aspartate (in Aspartono), plus organic matter to complete the total percentage. The recommended procedure for magnesium was carried out after destruction of the organic matter. As can be seen in Table 4, the results were in good agreement with the label specifications of the pharmaceutical preparations and with those obtained by atomic absorption spectrometry.

Comparison with Other Reagents for Magnesium

Other reagents have been proposed for the spectrophotometric determination of magnesium, and Table 5 summarises some of the more important, comparing them with leucoquinizarin. Some of them exhibit greater sensitivity than leucoquinizarin, but all lack adequate selectivity or require liquid - liquid extraction or the use of stabilisers. The leucoquinizarin method is sensitive and does not require special apparatus or toxic chemicals; also, as leucoquinizarin is a commercial product, the procedure can be performed with the chemicals and equipment to be found in most laboratories, and would therefore be useful for increasing the analytical possibilities for the determination of magnesium.

Table 5. Characteristics of other reagents for the spectrophotometric determination of magnesium

Compound	$\lambda_{max.}$/nm	Optimum pH	Solvent	Molar absorptivity/ l mol^{-1} cm^{-1}	Range of adherence to Beer's law, p.p.m.	Interferents	Ref.
Eriochrome Black T . .	530	10.0	Methanol - water	24×10^3	0–0.4	Al(III), Ca(II), Co(II), Fe(III), Mn(II), Ni(II), Zn(II)	19
Quinolin-8-ol	380	10.5–11.5	Chloroform	5.2×10^3	0–0.4	Ca(II), Ti(IV), V(V), U(VI)	19
Xilidyl Blue	510	9–11	Ethanol - water	42×103	0–0.28	As(III), Be(II), Ce(IV), Cr(III), Au(III), Mo(VI), Pd(II), Tl(I)	19
Titan Yellow	450–500	~10	Water	—	0.2–1.6	Ca(II), PO_4^{3-}, Al(III), Ni(II), Zn(II), Fe(III), Cu(II), Mn(II)	20
Chlorphosphonazo III	669	7.3	Chloroform	4.8×10^3	0.1–10	Transition elements	21
Magon sulphonate . .	548	—	Dimethyl sulphoxide - water	—	0–75	Cu(II), Fe(III)	22
1,2,7-Trihydroxyanthraquinone . .	530	~11	Ethanol - water	—	1–6	Ca(II), Sr(II), Ba(II), Cu(II), Mn(II), Zn(II), Al(III), Sb(III), Ge(IV), Ce(IV), U(VI), CN^-, Be(II), PO_4^{3-}, citrate, Cd(II), Ni(II), Fe(III)	23
Calcichrome	630	11.4	—	—	Up to 0.7	Ca(II), Co(II), Cr(III), Mn(II), Ni(II), Ti(IV), Zr(IV)	24

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Solvent Extraction Separation of Gallium, Indium and Thallium with High Relative Molecular Mass Amine from Citrate Solutions

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A novel method is proposed for the solvent extraction separation of aluminium, gallium, indium, thallium(I) and thallium(III). Gallium was quantitatively extracted from 0.01 M citric acid between pH 2.0 and 3.5 with 0.1 M Amberlite LA-2 in xylene, stripped with 0.5 M hydrochloric acid and determined spectrophotometrically at 505 nm as its complex with 4-(2-pyridylazo)resorcinol (PAR). Indium was quantitatively extracted from 0.01 M citric acid between pH 2.5 and 3.6 with 0.1 M Aliquat 336S in xylene, stripped with 0.5 M hydrochloric acid and determined spectrophotometrically at 510 nm with PAR. Thallium was quantitatively extracted with 0.1 M Aliquat 336S in xylene from 0.01 M citric acid between pH 2.0 and 6.0, stripped with 0.5 M perchloric acid and determined spectrophotometrically at 610 nm as its complex with crystal violet. The separation of aluminium, gallium, indium, thallium(I) and thallium(III) was effected after extraction by stripping aluminium or thallium(I) with water followed by stripping of gallium with 2.5 M hydrobromic acid, indium with 0.5 M hydrochloric acid and finally thallium(III) with 0.5 M perchloric acid. These elements were also separated from several multi-component mixtures. The method was extended to the analysis of real samples.

Keywords: *Gallium, indium and thallium separation; solvent extraction; spectrophotometry*

Gallium, indium and thallium have been separated from each other by solvent extraction[1] with high relative molecular mass amines as the complexing reagent from hydrochloric, sulphuric and nitric acids.[2] Gallium and indium have been extracted from 2–6 M hydrobromic acid with Amberlite LA-1 in xylene.[3] Similarly, gallium has been extracted from hydroiodic acid with Amberlite LA-1.[4] The separations of gallium from indium and other elements in thiocyanate media with either trioctylamine[5] or tridodecylamine[6] have been also carried out.

Thallium has been separated from interfering elements such as cadmium, zinc and mercury in acetate solution.[7] The separations of gallium, indium and thallium have been carried out from oxalic[8] and succinic acids.[9] The mechanism of extraction of the tartrate complex of gallium and indium has been studied.[10,11] The separation of indium from gallium in 0.01 M tartaric acid at pH 2.0 with trioctylamine[10,11] has been carried out.

The solvent extraction separation of gallium, indium and thallium from citrate media with Amberlite LA-1 or LA-2 and Aliquat 336S with xylene as the diluent is presented in this paper. Methods are suggested for the separation of these three elements not only from one another but also from associated elements and real samples.

Experimental

Apparatus

An Orion Research Microprocessor Ion Analyser-901 (Orion, USA), with glass and calomel electrodes and a GS866C spectrophotometer (ECIL, India), with matched 10 mm Corex glass cuvettes were used. A wrist-action flask shaker (Toshniwal, India) and a centrifuge (Remi, India) with a speed of 6000 rev. min^{-1} were employed.

Reagents and Materials

Gallium stock solution. Prepared by dissolving 0.5 g of pure metal (Johnson Matthey, UK) in 20 ml of concentrated hydrochloric acid. The solution was evaporated to dryness and the residue was dissolved in distilled water and diluted to 250 ml, maintaining the concentration of hydrochloric acid at 1%. The solution was standardised complexometrically[12] and contained 2.07 mg ml^{-1} of gallium. A dilute solution containing 10 μg ml^{-1} of gallium was prepared by appropriate dilution.

Indium stock solution. Prepared by dissolving 0.5 g of pure metal (Indium Corporation of America, USA) in 5 ml of concentrated hydrochloric acid and diluting to 100 ml. The

Table 1. Extraction (%) as a function of pH

Amine	Element	pH 1	2	3	4	5	6
Amberlite LA-1 ..	Ga	6.6	70.6	99.4	92.6	77.9	70.6
	In	0	14.0	96.0	81.1	66.0	49.5
	Tl(III)	13.3	30.9	51.1	93.3	99.8	97.8
Amberlite LA-2 ..	Ga	13.2	99.4	99.4	98.5	95.6	72.0
	In	0	15.0	98.5	62.5	59.5	35.0
	Tl(III)	45.1	75.6	95.5	99.8	99.0	82.4
Aliquat 336S	Ga	10.3	51.5	85.3	99.4	94.1	76.5
	In	37.5	60.1	99.9	92.7	34.0	14.7
	Tl(III)	29.5	99.8	99.8	99.8	99.8	99.8
TOA	Ga	20.6	44.1	64.7	71.9	71.9	52.9
	In	0	50.0	70.1	70.1	48.5	29.8
	Tl(III)	0	23.1	25.3	61.2	99.8	90.5

solution was standardised complexometrically[12] and contained 5.0 mg ml^{-1} of indium. A dilute solution containing 40 μg ml^{-1} of indium was prepared by appropriate dilution.

Thallium stock solution. Prepared by dissolving 0.65 g of thallium oxide (BDH Chemicals, UK, AnalaR grade) in 5 ml of concentrated nitric acid and diluting to 100 ml. The solution was standardised complexometrically[12] and contained 0.57 mg ml^{-1} of thallium. A dilute solution containing 75 μg ml^{-1} of thallium was prepared by appropriate dilution.

Liquid anion exchangers. Amberlite LA-1 [*N*-dodecyl(trialkylmethyl)amine]. Amberlite LA-2 [*N*-lauryl(trialkylmethyl)amine] (Rohm & Hass), Aliquat 336S (tricaprylmethylammonium chloride) (General Mills Ltd.) and trioctylamine (TOA) (Riedel de Häen) were used without further purification. The exchangers were converted into the citrate form as described earlier.[13]

Colour reagents. 4-(2-Pyridylazo)resorcinol (PAR) (BDH Chemicals, AnalaR grade) was used as a 0.1% aqueous solution and crystal violet (E. Merck) as a 0.001% aqueous solution.

General Procedure

An aliquot of solution containing either 10 μg of gallium, 40 μg of indium or 75 μg of thallium was taken, 0.01 M citric acid was added, the pH was adjusted to an appropriate value with 0.01 M sodium hydroxide or citric acid solution and the volume was made up to 10 ml. The solution was transferred into a separating funnel and shaken with 10 ml of either 0.1 M Amberlite LA-2 or Aliquat 336S solution in xylene for about 5 min on a wrist-action flask shaker. After allowing the phases to settle and separate, the organic phase was shaken with 10 ml of 0.5 M hydrochloric or perchloric acid to strip out the element concerned. From the aqueous phase, gallium and indium were determined spectrophotometrically with PAR at 505 and 510 nm and thallium as its crystal violet complex at 610 nm.[14]

Results and Discussion

Effect of pH

The optimum pH for the quantitative extraction of all three elements was ascertained by extracting them with a 0.1 M solution of the liquid anion exchanger in xylene over the pH range 2–8 (Table 1). The phase volume ratio was maintained at 1:1. Gallium was quantitatively extracted with Amberlite LA-1 (pH 3.0–3.5), Amberlite LA-2 (pH 2.0–3.5) and Aliquat 336S (pH 3.5–4.0). Indium was extracted with Amberlite LA-1 (pH 3.0–3.5), Amberlite LA-2 (pH 2.5–3.0) and with Aliquat 336S (pH 2.5–3.6). TOA was a poor extractant for both elements. Thallium was extracted with Amberlite LA-1 from pH 4.5 to 5.5, with Amberlite LA-2 from pH 3.5 to 5.0 and with Aliquat 336S from pH 2.0 to 6.0. Amberlite LA-2 was the best extractant for gallium and Aliquat 336S was the best extractant for indium and thallium.

Effect of Varying Concentration of Liquid Anion Exchanger

The concentration of Amberlite LA-2 required for quantitative extraction of gallium and that of Aliquat 336S for the quantitative extraction of indium and thallium were investigated at appropriate pH values with various concentrations of liquid anion exchangers from 1×10^{-2} to 1×10^{-1} M (Table 2). Extraction was quantitative at liquid anion exchanger concentrations of 6.25×10^{-2} M for gallium, 7.5×10^{-2} M for indium and at 8.0×10^{-2} M for thallium.

Effect of Citric Acid Concentration

The optimum concentration of citric acid required for the formation of anionic complexes of the elements was ascertained by varying the concentration of citric acid at an appropriate pH. The extraction was quantitative above 7×10^{-4} M citric acid. When an attempt was made to extract the elements from 1×10^{-1} M citric acid, the extent of extraction decreased owing to the occurrence of competitive equilibria between the anion of the ligand and the anionic complex of the metal with the ligand (Fig. 1). The optimum concentration of citric acid was 1×10^{-2} M.

Nature of Extracted Species

An attempt was made to evaluate the composition of the extracted species by plotting log D against log[citric acid] (D = distribution ratio) at a fixed liquid anion exchanger concentra-

Table 2. Effect of amine concentration on extraction

Amine concentration/ M × 10^{-2}	Extraction, % pH 3.0, Ga, Amberlite LA-2	pH 3.0, In, Aliquat 336S	pH 4.6, Tl(III), Aliquat 336S
1.25	77.2	30.8	28.0
2.50	80.0	49.5	37.5
3.75	90.8	59.0	72.9
5.0	96.1	89.5	85.9
6.25	99.4	96.5	92.0
7.50	99.4	99.8	96.6
8.75	99.4	99.9	99.8
10.0	99.4	99.9	99.8

Table 3. Effect of diluents on extraction

Diluent	Dielectric constant	Extraction, % Ga	In	Tl(III)
Benzene	2.28	95.5	96.0	99.2
Nitrobenzene	35.5	38.0	Nil	Nil
Toluene	2.38	99.4	90.0	99.0
Xylene	2.30	99.4	99.9	99.8
Hexane	1.89	99.2	—	72.1
Cyclohexane	2.05	82.2	—	68.1
Carbon tetrachloride	2.24	52.8	81.0	32.4
Chloroform	4.8	73.2	85.0	99.0

Table 4. Efficiency of stripping agents

Stripping agent	Concentration/M	Stripping, % Ga	In	Tl(III)
HCl	0.1	77.6	29.5	—
	0.5	99.4	99.9	—
	1.0	88.7	28.0	—
	2.0	24.3	16.0	—
	4.0	8.8	5.0	—
HBr	0.1	69.0	48.5	—
	0.5	99.1	99.9	—
	1.0	99.4	16.0	—
	2.0	99.4	0.0	—
	4.0	77.6	0.0	—
HNO_3	0.1	93.8	59.0	—
	0.5	99.4	85.0	—
	1.0	98.9	85.0	—
	2.0	99.5	43.5	—
	4.0	98.9	15.4	—
H_2SO_4	0.1	88.7	16.0	—
	0.5	44.4	40.0	—
	1.0	44.4	62.0	—
	2.0	35.5	95.1	—
	4.0	44.0	100.1	—
$HClO_4$	0.1	—	—	90.4
	0.5	—	—	99.8
	1.0	—	—	99.8
	2.0	—	—	99.8

Table 5. Separation of gallium, indium and thallium from multi-component mixtures

Mixture No.	Element	Taken/ μg	Found/ μg	Recovery, %	Stripping agent	Reagent for spectrophotometric analysis ($\lambda_{max.}$/nm)
1	Cu	270	268	99.8	H_2O	Diethyldithiocarbamate (580)
	Ge	31.2	31.1	99.7	5 M HCl	Phenylfluorone (530)
	Ga	15.0	14.8	98.6	0.5 M HCl	PAR (505)
2	Al	310.0	309.0	99.6	H_2O	Alizarin Red S (490)
	Ga	15.0	14.9	99.4	0.5 M HCl	PAR (505)
	Bi	43.5	43.0	98.8	0.5 M citric acid	Iodide (460)
3	Sc	62.3	62.1	99.6	0.05 M H_2SO_4	Alizarin Red S (525)
	Zr	47.8	47.2	98.7	5 M HCl	Arsenazo III (665)
	In	50.1	50.0	99.8	0.5 M HCl	PAR (510)
4	Tl	268.0	267.8	99.9	H_2O	Crystal violet (610)
	In	50.0	50.0	100.0	0.5 M HCl	PAR (510)
	Tl(III)	35.4	35.3	99.7	0.5 M $HClO_4$	Crystal violet (610)
5	Pb	245.0	245.0	100.0	H_2O	PAR (520)
	Ga	72.0	71.6	99.4	2.5 M HBr	PAR (505)
	In	50.0	49.5	99.0	0.5 M	PAR (510)
6	Al/Cd	474.0	473.0	99.9	H_2O	Arsenazo III (600)
	Bi	373.0	372.9	99.9	0.5 M H_2SO_4	Iodide (460)
	Tl(III)	105.0	104.9	99.9	0.5 M $HClO_4$	Crystal violet (610)
7	Cu/Pb/Cd	584.0	583.8	99.9	H_2O	PAR (520)
	Ga/Sb	120.0	119.5	99.6	0.5 M HCl	Iodide (555)
	Tl(III)	105.0	104.9	99.9	0.5 M $HClO_4$	Crystal violet (610)

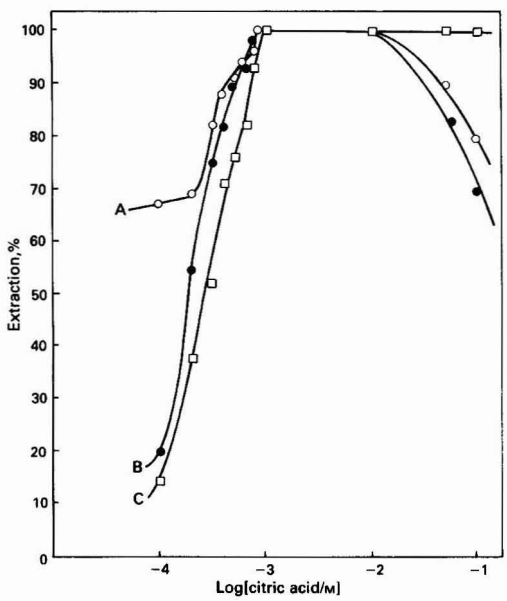

Fig. 1. Effect of citric acid concentration on the extraction of (A) gallium, (B) indium and (C) thallium

tion and also log D against log[liquid anion exchanger] at a constant citric acid concentration, both at constant pH. The slopes were, respectively, 1.80 and 2.70 for gallium, 1.96 and 3.27 for indium and 1.84 and 3.17 for thallium. The general mechanism of extraction can be written as

$$(R_4N)_3^+ L^{3-} + M(L)_2^{3-} \rightarrow [(R_4N)_3^+ M(L)_2^{3-}] + L^{3-}$$

or

$$(R_2NH_2)_3^+ L^{3-} + M(L)_2^{3-} \rightarrow [(R_2NH_2)_3^+ M(L)_2^{3-}] + L^{3-}$$

where M = gallium or indium or thallium and L = citric acid.

Similar behaviour has been observed in oxalate media.[8]

Table 6. Separation of gallium, indium, thallium and aluminium from each other

Element	Amount taken/ g	Amount found/ g	Recovery, %	Stripping agent
Al	310.0	308.0	99.0	H_2O
Ga	15.0	14.9	99.4	2.5 M HBr
In	42.0	41.9	99.7	0.5 M HCl
Tl(III)	51.2	50.8	99.4	0.5 M $HClO_4$
Al	322.0	321.7	99.9	H_2O
Ga	72.0	71.9	99.8	2.5 M HBr
In	50.0	49.7	99.7	0.5 M HCl
Tl(III)	70.8	70.6	99.7	0.5 M $HClO_4$
Al	322.0	321.0	99.6	H_2O
Ga	72.0	71.7	99.6	2.5 M HBr
In	50.0	49.4	98.7	0.5 M HCl
Tl	35.4	35.3	99.7	0.5 M $HClO_4$
Tl(I) - Al	467.8	467.7	99.9	H_2O
Ga	120.0	119.8	99.8	2.5 M HBr
In	130.0	129.9	99.9	0.5 M HCl
Tl(III)	105.0	104.9	99.6	0.5 M $HClO_4$

Effect of Various Diluents

Keeping all other factors constant, benzene, toluene, xylene, nitrobenzene, chloroform, carbon tetrachloride, hexane and cyclohexane were tested as the diluent for the extraction of gallium with Amberlite LA-2 and indium and thallium with Aliquat 336S (Table 3). The phase volume ratio was kept at 1:1 to eliminate the problem of emulsification. Toluene, xylene and hexane were suitable diluents for gallium, xylene for indium and benzene, toluene and xylene for thallium. Thus xylene was found to be a common suitable diluent for all three elements.

Effect of Stripping Agents

After extraction, the elements were stripped with different mineral acids (Table 4) and alkalis. Gallium and indium were stripped with 0.5 M hydrochloric or hydrobromic acid and thallium with 0.5–4.0 M perchloric acid. It was noteworthy that thallium could be stripped only with perchloric acid. Alkalis proved to be poor stripping agents. Therefore, 0.5 M hydrochloric acid was selected for stripping gallium and indium and 0.5 M perchloric acid for stripping thallium.

The optimum period of extraction was evaluated by extracting these elements for various periods of time ranging from 1 to 5 min. A 5-min period of equilibrium was adequate for the quantitative extraction of all three elements.

Separation of Gallium, Indium and Thallium from Other Elements in Binary Mixtures

A systematic investigation of the extraction of various elements in citrate media was carried out with 0.1 M liquid anion exchanger solutions in xylene. It was interesting that alkali and alkaline earth elements, thallium(I) and silver were not extracted as they could not form a citrato complex. Manganese, nickel, copper, zinc, lead, cadmium, yttrium, lanthanum and aluminium formed weak citrato complexes (D = 10). Therefore, such complexes, although extracted, were washed with water. Vanadium, thorium, uranium, antimony, gold, platinum, iron, germanium and tin formed citrato complexes (D = 200). Hence they were co-extracted with gallium, indium and thallium. They were later stripped with mineral acids that could not form an anionic complex with any of these elements. Finally, mercury, scandium, zirconium, hafnium, tungsten and bismuth formed relatively strong complexes and were therefore extracted. They were later stripped with a higher concentration of mineral acid. The alkali and alkaline earth elements were separated as they were not extracted and remained in the aqueous phase. The elements having D = 10 were first stripped with distilled water. The distribution ratios of gallium, indium and thallium ranged from 200 to 1000. Therefore, the gallium and indium were subsequently stripped with 0.5 M hydrochloric acid and thallium was removed with 0.5 M perchloric acid.

Separation of Gallium, Indium and Thallium from Tertiary Mixtures (Table 5)

Copper, germanium and gallium were separated, after extraction, by stripping copper with distilled water, germanium with 5 M hydrochloric acid and gallium with 0.5 M hydrochloric acid.

The separation of aluminium, gallium and bismuth was effected by washing aluminium with distilled water, gallium with 0.5 M hydrochloric acid and bismuth with 0.5 M citric acid.

Scandium, zirconium and indium were separated by stripping scandium with 0.05 M sulphuric acid, zirconium with 5 M hydrochloric acid and indium with 0.5 M hydrochloric acid. Similarly, thallium(I), indium and thallium(III) were separated by washing thallium(I) with water, indium with 0.5 M hydrochloric acid and thallium with 0.5 M perchloric acid. The separation of lead, gallium and indium was achieved by stripping lead with water, gallium with 2.5 M hydrobromic acid and indium with 0.5 M hydrochloric acid.

The mixture of aluminium or cadmium, bismuth and thallium was separated by stripping the aluminium or cadmium with water, bismuth with 0.5 M sulphuric acid and thallium with 0.5 M perchloric acid. An interesting separation of either copper, lead or cadmium from gallium, antimony and thallium was effected by stripping copper, lead or cadmium with water, gallium or antimony with 0.5 M hydrochloric acid and finally thallium with 0.5 M perchloric acid.

In all these separations the elements, after back-stripping, were determined quantitatively by spectrophotometric methods utilising appropriate chromogenic ligands (Table 5). It was further noted that there was no cross-contamination and that the analytical methods used for the determinations were selective.

Separation of Gallium, Indium, Thallium and Aluminium from One Another (Table 6)

When a mixture of aluminium, gallium, indium and thallium was extracted with Amberlite LA-1 or Aliquat 336S, aluminium was stripped with distilled water as it formed a weak complex and the other elements were retained by the organic phase. Then gallium was stripped with 2.5 M hydrobromic acid, indium and thallium being re-extracted into the organic phase as an anionic bromo complex. Subsequently, indium was stripped with 0.5 M hydrochloric acid, thallium(III) being retained in the organic phase and later stripped with 0.5 M perchloric acid. It was possible to separate these elements in different proportions. This procedure provided a rapid and selective method for the extraction of all the four elements belonging to the same group of the periodic classification.

Determination of Gallium in Bauxite and Thallium in Sediments

The method was extended to the determination of gallium in bauxite. A 0.5-g amount of bauxite was dissolved in mineral acid, extracted with distilled water and made up to a known volume. An aliquot of the solution containing gallium was taken and was extracted according to the general procedure. Aluminium and iron(II) were first washed with distilled water and gallium was stripped with 0.5 M hydrochloric acid and determined spectrophotometrically. The amount of gallium found was 140 p.p.m. compared with the reported value of 141 p.p.m. found by atomic absorption spectrometry.

The method was also extended to the determination of thallium in sediments. A 4-g amount of sample was dissolved in nitric and perchloric acid and evaporated to dryness. The residue was dissolved in hydrochloric acid and the solution made up to 100 ml. An aliquot of solution containing thallium was extracted according to the general procedure. The extracted copper, zinc, aluminium and cobalt were washed with distilled water, while titanium and iron were stripped with 0.5 M sulphuric acid and thallium with 0.5 M perchloric acid. The amount of thallium found was 5.8 p.p.m. compared with the standard value of 6.0 p.p.m., which was verified by atomic absorption spectrometry.

Conclusion

The proposed method is simple, rapid and selective. It provides a rapid method not only for the separation of gallium, indium and thallium from each other but also from associated elements normally encountered in real samples. The amounts of gallium, indium and thallium after separation were quantitatively determined by spectrophotometric methods as described earlier. From the results of the analysis of eight synthetic samples of these elements the relative standard deviation was found to be 1.25%, which indicates that the method is reproducible.

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Extraction - Spectrophotometric Determination of Gallium with 1,2,4,6-Tetraphenylpyridinium Perchlorate

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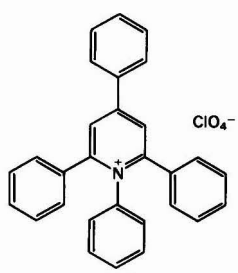
The characteristics and applications of 1,2,4,6-tetraphenylpyridinium perchlorate (TPPP) as a reagent for the spectrophotometric determination of gallium are described. This reagent forms a 1 : 1 complex with $GaCl_4^-$ ($\lambda_{max.}$ 310 nm, molar absorptivity 2.91×10^4 l mol^{-1} cm^{-1}) that is slightly soluble in water and can be extracted with isopentyl acetate with an extraction efficiency of 97.4%. The TPP^+ $GaCl_4^-$ complex obtained is useful for the spectrophotometric determination of gallium in the concentration range 0.1–2.2 μg ml^{-1} in the organic phase, and the method is applicable to the determination of gallium in aluminium.

Keywords. *Gallium determination; 1,2,4,6-tetraphenylpyridinium perchlorate; ion-association complex; spectrophotometry*

The recently introduced spectrophotometric reagent 1,2,4,6-tetraphenylpyridinium perchlorate (TPPP) forms ion-association complexes with a small number of halogenometal ions. These complexes can be used for the spectrophotometric determination of the metal after extraction with an appropriate solvent.[1,3] This paper discusses the formation and extraction of the ion-association complex of gallium(III) with TPPP in a strong hydrochloric acid medium and a spectrophotometric method for the determination of gallium(III) is proposed.



TPPP

Several extraction - spectrophotometric methods for the determination of gallium involving the formation of ion-association complexes have been reported.[4–7] The procedure reported here has an extraction efficiency and a sensitivity higher than those of most similar methods, and also good selectivity, precision and accuracy.

Experimental

Apparatus

A Pye Unicam SP8-200 spectrophotometer with 1-cm silica cells was used for recording spectra and absorbance measurements in the ultraviolet region.

A Perkin-Elmer 177 grating infrared spectrophotometer, a Hewlett-Packard 5980A mass spectrometer and a Varian FT-80A nuclear magnetic resonance spectrometer were used for the identification of the TPPP reagent.

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Reagents

All solutions were prepared using analytical-reagent grade chemicals and doubly distilled water.

Synthesis of TPPP. TPPP was synthesised from 2,4,6-triphenylpyrylium perchlorate and aniline, as described previously.[1]

1,2,4,6-*Tetraphenylpyridinium perchlorate solution*, 10^{-3} M. Prepared by dissolving 0.1209 g of the reagent in 250 ml of ethanol. The solution is stable for several months if protected from light.

Gallium(III) stock standard solution, 0.01 M. Prepared by dissolving gallium(III) nitrate in 0.5 M hydrochloric acid and standardised by titration with EDTA.[8] Working standards were prepared from this solution as required.

Procedure for the Determination of Gallium(III)

To a volume of sample solution in a separating funnel containing up to 11 μg of gallium(III), add 25 ml of 12 M hydrochloric acid and 2 ml of 10^{-3} M TPPP solution, dilute to 50 ml with doubly distilled water and extract the mixture with 5 ml of isopentyl acetate by shaking vigorously for 2 min. Allow the phases to separate for 10 min, then transfer the organic layer into a centrifuge tube and centrifuge it to give an organic layer free from water. Measure the absorbance of the organic layer against a reagent blank prepared in a similar manner but without gallium.

Prepare a calibration graph using different volumes of the standard solution of gallium(III) treated in the same manner. Beer's law is obeyed for 0.1–2.2 μg ml^{-1} of gallium in the organic phase.

Determination of Gallium in Aluminium

The samples were dissolved in 6 M hydrochloric acid and boiled. The solutions were transferred into 100-ml calibrated flasks and diluted to volume with doubly distilled water.

To avoid the interference of iron(III), 15% titanium(III) chloride solution was added to the separating funnel until a concentration of 0.03 M was reached, then the mixture was allowed to stand for 5 min. Under these conditions most of the iron(III) is reduced. The last trace amounts of iron(III) are difficult to eliminate, and after the reduction with titanium(III) a simultaneous determination of gallium(III) and iron(III) was followed by measuring the absorbance at 310 and 363 nm.

Results and Discussion

Absorption Spectra

TPPP reacts with gallium(III) in a strong hydrochloric acid medium to form an ion-association complex that can be extracted with isopentyl acetate. Fig. 1 shows the absorption spectra of a 2×10^{-5} M solution of TPPP in ethanol (A) and of the isopentyl acetate extract (B) of the ion-association complex obtained by mixing gallium(III) solution (2.8×10^{-6} M) and TPPP solution (4×10^{-5} M) in 6 M hydrochloric acid. Only a small shift of the 311-nm absorption maximum of the reagent was observed for the complex in isopentyl acetate. Line C corresponds to the spectrum of TPPP in isopentyl acetate, showing the low extraction of the reagent by this solvent.

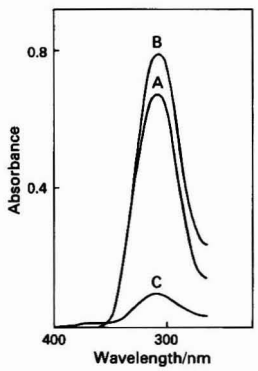

Fig. 1. Absorption spectra of: A, 2.0×10^{-5} M TPPP in ethanol; B, $TPP^+ GaCl_4^-$ complex extracted with 5 ml of isopentyl acetate obtained in 50 ml of aqueous solution with a Ga(III) concentration of 2.8×10^{-6} M, a TPPP concentration of 4×10^{-5} M and 6 M hydrochloric acid; and C, TPPP in isopentyl acetate

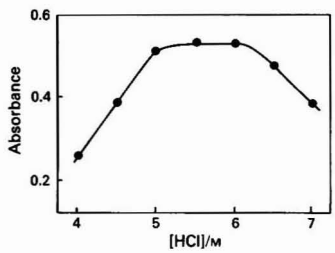

Fig. 2. Effect of hydrochloric acid concentration on absorbance of the complex at 310 nm, with 0.13 μg ml^{-1} of gallium(III). Reference, reagent blank

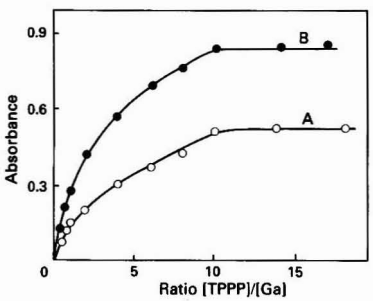

Fig. 3. Effect of reagent concentration on the absorbance of the complex. Gallium(III) concentration: A, 0.13; and B, 0.21 μg ml^{-1}

Effect of Hydrochloric Acid Concentration

The effect of the concentration of hydrochloric acid on the formation of the $TPP^+ GaCl_4^-$ complex and its extraction into isopentyl acetate was studied using fixed concentrations of gallium(III) (0.13 μg ml^{-1}) and TPPP (2.7×10^{-5} M) and varying the hydrochloric acid concentration from 4 to 7 M. Results are shown in Fig. 2. Maximum absorbance values were obtained in isopentyl acetate extracts for hydrochloric acid concentrations in the range 5.5–6 M. All subsequent investigations were carried out in 6 M hydrochloric acid medium.

Effect of Reagent Concentration

The effect of the reagent concentration was studied by extracting 10.5 and 6.5 μg of gallium(III) from 6 M hydrochloric acid with isopentyl acetate in the presence of different amounts of TPPP. As can be seen from Fig. 3, a molar ratio of TPPP to Ga(III) higher than 10 is sufficient for the complete formation and extraction of the complex.

Effect of Shaking Time

Shaking times ranging from 1 to 4 min did not produce any change in absorbance, so a 2-min shaking time was selected.

Extraction Efficiency

The selected ratio of aqueous phase to isopentyl acetate was 10 + 1 *V/V*. The extraction efficiency was calculated by a procedure similar to that used by Hasegawa *et al.*,[5] by extracting the aqueous phase with two consecutive volumes of organic solvent. For different volumes of aqueous and organic phases, V_a and V_o, the equation used by Hasegawa *et al.* for the distribution ratio is converted into $D = V_a(A_1 - A_2)/V_oA_2$, where A_1 and A_2 represent the absorbances of the first and second extracts, respectively. An extraction efficiency of 97.4% was found.

Stability

The absorbance of the ion-association complex extracted into isopentyl acetate remains constant for at least 72 h if the solution is kept away from sunlight and ultraviolet radiation, which affect the tetraphenylpyridinium cation.[9]

Composition of the Complex

To establish the composition of the complex, the continuous variations[10,11] and molar ratio[12] methods were applied. The molar ratio of gallium to TPPP was found to be 1 : 1 by these two methods (Figs. 3 and 4).

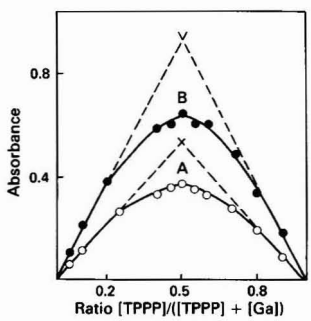

Fig. 4. Stoicheiometry of gallium - TPPP complex determined by the continuous variations method. Concentration of gallium(III) plus TPPP: A, 7×10^{-6} M; and B, 1×10^{-5} M

Table 1. Interference of other ions in the determination of gallium. Concentration of gallium(III), 0.13 μg ml^{-1}

Ion added	Molar ratio [ion added]/[Ga(III)]	Ion added	Molar ratio [ion added]/[Ga(III)]
Al(III)	2000	Mn(II)	3000
Sb(V)	0.05	Hg(II)	100
As(V)	1	Pt(IV)	20
Bi(III)	100	Ag(I)	500
Cd(II)	5	Se(IV)	1
Cr(III)	100	Tl(III)	0.02
Cu(II)	500	Sn(IV)	10
Au(III)	0.02	Zn(II)	500
In(III)	1	Nitrate	1000
Fe(III)	0.03	Perchlorate	5
Pb(II)	50	Sulphate	50000

Table 2. Determination of trace amounts of gallium in aluminium

Gallium added/ mg g^{-1}	Gallium obtained*/ mg g^{-1}	Recovery, %
0	0.123	—
0.063	0.184	96.8
0.133	0.256	100.0

* Average of three determinations.

Calibration Graphs, Sensitivity and Precision

Under the recommended conditions, the calibration graph was linear over the range 0.1–2.2 μg ml^{-1} of gallium(III) in the organic phase. Sandell's sensitivity is 2.4×10^{-3} μg cm^{-2} and the molar absorptivity of the complex is 2.91×10^4 l mol^{-1} cm^{-1} at 310 nm. The coefficient of variation for 0.16 μg ml^{-1} of gallium(III) (ten determinations) is 1.7%.

Effect of Other Ions

In the determination of 0.13 μg ml^{-1} of gallium(III), extraneous ions can be tolerated at the levels given in Table 1. The limiting value of the concentration for each ion was taken as that value which caused an error of not more than 2.5% in the absorbance values. Cations were added as chlorides, nitrates or sulphates, and anions in the form of sodium or potassium salts.

Gold(III) and thallium(III) cause the largest interferences. These interferences can be avoided by reducing the ions to gold metal and thallium(I), respectively. The best results were obtained with the use of hydroxylamine as a reducing agent for thallium in 0.05 M sulphuric acid, and sodium nitrite for reduction of gold(III); the excess of nitrite must be eliminated with urea.

The interference of iron(III) can be eliminated by reducing it to iron(II) with titanium(III) chloride. The last traces of iron(III) (*ca.* 0.3%) are difficult to reduce and if necessary a simultaneous determination of gallium(III) and iron(III) can be followed. The $TPP^+FeCl_4^-$ extracted in isopentyl acetate has two maxima, at 363 and 310 nm. As absorbances are additive, the relationships for gallium(III) and iron(III) are

$$A_{310} = [\mathrm{Ga}]_0\, \varepsilon_{310}^{\mathrm{Ga}} + [\mathrm{Fe}]_0\, \varepsilon_{310}^{\mathrm{Fe}} \quad \ldots \quad (1)$$

$$A_{363} = [\mathrm{Fe}]_0\, \varepsilon_{363}^{\mathrm{Fe}} \quad \ldots \quad (2)$$

where $\varepsilon_{310}^{\mathrm{Ga}} = 2.91 \times 10^4$, $\varepsilon_{310}^{\mathrm{Fe}} = 3.10 \times 10^4$ and $\varepsilon_{363}^{\mathrm{Fe}} = 7.2 \times 10^3$ l mol^{-1} cm^{-1}. Under the selected conditions, the extraction efficiencies, E_{Ga} and E_{Fe}, for gallium(III) and iron(III), calculated as above, were 97.4 and 80.0%, respectively.

To illustrate the correction for iron(III), a sample containing known concentrations of Ga(III) (1.5×10^{-6} M) and Fe(III) (1.5×10^{-6} M) in the aqueous phase gave absorbances in the organic phase after extraction of $A_{310} = 0.798$ and $A_{363} = 0.086$. The concentration of gallium in the organic phase, correcting the interference of iron, can be calculated from equations (1) and (2) as follows:

$$[\mathrm{Ga}]_0 = \frac{1}{\varepsilon_{310}^{\mathrm{Ga}}}\left(A_{310} - \frac{A_{363}\,\varepsilon_{310}^{\mathrm{Fe}}}{\varepsilon_{363}^{\mathrm{Fe}}}\right) = 1.47 \times 10^{-5}\ \mathrm{M}$$

The total concentration of gallium initially in the aqueous phase is

$$[\mathrm{Ga}]_{\mathrm{t,a}} = \frac{100V_0}{E_{\mathrm{Ga}}V_{\mathrm{a}}}\,[\mathrm{Ga}]_0 = 1.51 \times 10^{-6}\ \mathrm{M}$$

in agreement with the known initial concentration of gallium of 1.5×10^{-6} M.

Applications

The method has been applied satisfactorily to the determination of gallium in aluminium. In the absence of a standard sample, the method was tested by the standard additions technique. An aluminium sample was analysed with and without the addition of 0.063 and 0.133 mg of gallium per gram of aluminium following the recommended procedure. The results are shown in Table 2.

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Determination of Copper in Biological Tissue by Means of Photochemically Generated (*Z*)-2-Thiophenaldehyde 2-Pyridylhydrazone

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An improved and sensitive photometric determination of copper, based on the formation of a coloured chelate with photochemically generated (*Z*)-2-thiophenaldehyde 2-pyridylhydrazone [(*Z*)-TAPH], is described. The calibration graph is linear over the range 0.017–1.2 μg ml^{-1} of Cu and the method has a detection limit of 5 ng ml^{-1} and a relative standard deviation of 0.70%. Colour development is instantaneous and remains stable for at least 4 h; the stoicheiometry of the chelate is 3 : 1 (L : M). The influence of reaction variables and the effect of foreign ions are also discussed. The method is sensitive for the determination of copper(II) and has been satisfactorily applied to the determination of copper in pig liver tissue.

Keywords: *Copper determination; spectrophotometry; (Z)-2-thiophenaldehyde 2-pyridylhydrazone; pig liver tissue analysis*

Copper is an essential element for the normal metabolism of many living organisms; it is a constituent of numerous proteins and enzymes. The tissues most rich in copper are those of the liver, nervous system, pancreas and bone. Copper is excreted in the bile and urine but in Wilson's disease, a state of altered metabolism, it accumulates in the liver and tissues.[1–5]

Copper is a strong inhibitor of numerous cellular enzymes, particularly those of the glucolytic pathways, and is toxic when in excess of normal levels. It also suppresses ATPhosphatase activity and may produce intravascular haemolysis. Toxic levels may produce liver, neurological and neuropsychic disorders and pigmentation phenomena. The determination of hepatic copper concentrations in tissue obtained by liver biopsy is one of the important criteria in the diagnosis of Wilson's disease.

Copper is an essential element for many living organisms but often the margin between normal and toxic levels is small. Toxic, copper-based, marine anti-fouling paints are used to prevent algal and molluscal growth on ship bottoms. However, in Crustaceae a copper - protein complex, haemocyanin, is a respiratory pigment and functions similarly to the haemoglobin iron - protein complex in higher animals.

Copper levels in blood, urine and other tissues sometimes have great significance in medical and biochemical research; photometric determination methods are less expensive than atomic absorption or atomic emission spectrometry.

This paper describes the photometric determination of copper in pig liver tissue by photochemically generated (*Z*)-2-thiophenaldehyde 2-pyridylhydrazone [(*Z*)-TAPH]. Usually, *N*-heterocyclic hydrazones are used as colour-forming reagents with metal ions[7–9]; however, the several problems associated with the photochemical transformations of this type of ligand have received little attention in spite of the fact that they may cause an inaccurate analysis because of their photolability.

The photometric determination of copper with (*Z*)-TAPH has not previously been reported although the *E* - *Z* photoisomerisation behaviour of (*Z*)-TAPH has been studied[10] in addition to photosensitised reactions that are observed with several metallic ions. The photometric determination of nickel has also been reported.

The proposed method is sensitive (limit of detection 5 ng ml^{-1}) and permits a rapid and simple determination of trace amounts of copper. The effects of experimental variables and those resulting from chelate stereochemical changes have also been investigated.

Experimental

Reagents

All reagents were of analytical-reagent grade and all solutions were prepared with distilled, de-ionised water, unless stated otherwise.

Copper(II) standard solution, 0.1 M. Prepared by dissolving $CuSO_4.5H_2O$ in de-ionised water and standardising complexometrically.

(E)-*TAPH*. Synthesised as described previously.[10] The (*Z*)-isomer was prepared from the (*E*)-isomer by the direct irradiation of a 1 × 10^{-3} M solution in ethanol for 6 h at 365 nm. Characterisation was performed by UV, IR and NMR spectroscopy.

Buffer solution, pH 8.75. Prepared from 0.1 M boric acid in 0.1 M potassium chloride and 0.1 M sodium hydroxide solutions.

Sodium hydroxide solution, 2 M. Prepared freshly.

Citric acid trisodium salt dihydrate solution, 5 g l^{-1}.

Apparatus

Spectrophotometric measurements were carried out using Shimadzu UV-240 Graphicord and Beckman DU-2 spectrophotometers, with matched 1.00-cm quartz cells.

The pH measurements were carried out with a Crison Digit 501 pH meter. The irradiation light source was an Atom-70 vapour lamp (200–250 V, 8 W), whose strongest emission was at 365 nm. The equipment consisted of a black box equipped with a mercury lamp; the reaction cells were standard flasks of Pyrex glass (lower limit of transparency 300 nm); and the irradiation distance was 10 cm.

Procedures

Spectrophotometric determination of copper

Into a 25-ml calibrated flask, transfer a volume of sample solution containing 0.42–30 μg of copper, add 6 ml of 1 × 10^{-3} M irradiated (*E*)-TAPH ethanolic solution, 6.5 ml of ethanol, 5 ml of pH 8.75 buffer solution and dilute to the mark with de-ionised water. Measure the absorbance at 435 nm against the reagent blank. Use a calibration graph, or empirical equation, to convert absorbances into concentrations. If the samples contain other metal ions, add a suitable amount of masking agent prior to addition of the reagent.

Determination of copper in pig liver tissue

Cut fresh pig liver (10–15 g) into small portions, taking care to exclude major blood vessels and connective tissue. Dry the liver at 120 °C until loss of mass ceases and treat 3–4 g with 10 ml of concentrated nitric acid and 5 ml of concentrated sulphuric acid in a Kjeldahl flask; next heat strongly until the evolution of nitrous vapours ceases. After cooling to room temperature, add 3 ml of concentrated perchloric acid and again heat strongly until nearly dry. Make up to 50 ml with de-ionised water to give a clear solution. Analyse aliquots of this solution by the standard photometric procedure, adding sodium citrate (100 μg ml^{-1}) to mask the iron and, when necessary, re-adjusting the pH to 10.25 with NaOH solution.

Optimisation of pH and ethanol concentration

Into a 250-ml calibrated flask, transfer appropriate aliquots of irradiated (*E*)-TAPH and copper solutions to give 8×10^{-5} M and 2×10^{-5} M solutions, respectively. Add sufficient ethanol to give concentrations of 50% *V/V* when diluted to the mark. Dilute to 250 ml with de-ionised water and measure the absorbance at 435 nm against the reagent blank at several values of pH covering the range 6–12. Adjust the pH by the addition of discrete amounts of dilute HCl or NaOH solution.

Under the same conditions, but in 50-ml calibrated flasks, vary the concentration of ethanol between 10 and 70% *V/V*. Adjust the pH in each instance to 10.25 by adding dilute HCl or NaOH solution. Measure the absorbance at 435 nm against the reagent blank.

Optimisation of irradiation time and heating time (route A)

In 25-ml calibrated flasks, place aliquots of (*E*)-TAPH and copper solutions to give 8×10^{-5} and 2×10^{-5} M solutions, respectively. Add sufficient ethanol to give concentrations of 50% *V/V* when all the reagents are present, and 5 ml of pH 8.75 borate buffer. After diluting to the mark, irradiate the samples for different times between 15 and 45 min. Maintain one sample in darkness.

To optimise the heating times, prepare several samples as above, irradiate for 30 min and maintain in a water-bath at 35 °C for different times between 15 and 45 min, keeping one sample at room temperature.

In both optimisation experiments, following appropriate treatment store the samples in darkness and measure their absorbances several times between 3 and 90 min at 427 nm to check the stability.

Results and Discussion

Study of the Copper - TAPH Complex

The reaction of 2-thiophenaldehyde with 2-pyridylhydrazine produces (*E*)-2-thiophenaldehyde 2-pyridylhydrazone [(*E*)-TAPH]. Sunlight or UV irradiation promotes *E* - *Z* photo-isomerisation.

(*E*)-TAPH acts as a potentially tridentate ligand because of the linear arrangement of the thiophenic sulphur, pyridine nitrogen and azomethine nitrogen to produce two five-membered chelate rings. In the (*Z*)-position the torsion of the C=N bond stereochemically impedes the donor participation of the sulphur atom, and thus creates a five-membered chelate ring between the azomethine and pyridine nitrogens.[10]

Copper(II) and (*E*)-TAPH formed a yellow complex that was unstable even in darkness. The absorption of the complex increased with time, with a $\lambda_{max.}$ at 427 nm. On the other hand, (*E*)-TAPH irradiated solutions reacted with copper(II) to form yellow complexes with $\lambda_{max.}$ at 435 nm; the molar absorptivities remained constant for several hours at room temperature.

These data indicate the optimum conditions for complex formation by two alternative routes: route A, by irradiation of the (*E*)-TAPH - copper mixture and route B, by the addition of the metal ion to previously irradiated (*E*)-TAPH.

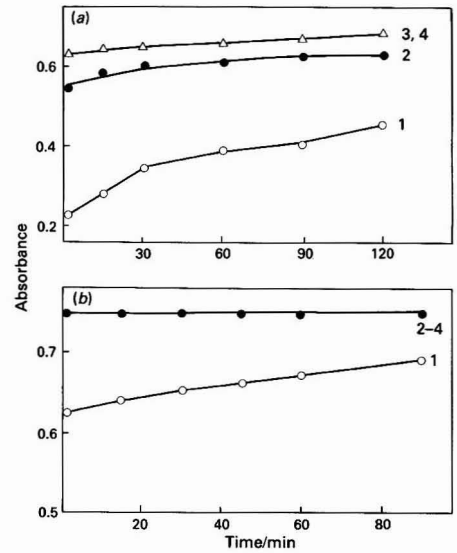

Fig. 1.(*a*) Influence of irradiation times and stability of the samples after irradiation. [Cu] = 2×10^{-5} M; [(*E*)-TAPH] = 8×10^{-5} M; λ = 427 nm; pH = 10.25. Irradiation time: 1, 0; 2, 15; 3, 30; and 4, 45 min. (*b*) Influence of heating times and stability of the samples after heating. Irradiation time, 30 min; λ = 427 nm. Heating time: 1, 0; 2, 15; 3, 30; and 4, 45 min

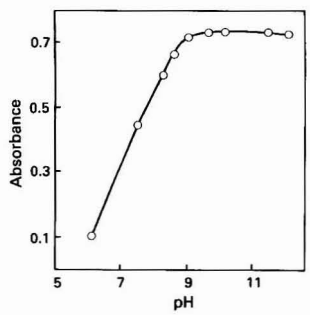

Fig. 2. Absorbance *versus* pH dependence for the (*Z*)-TAPH - copper complex. [Cu] = 2×10^{-5} M; [(*Z*)-TAPH] = 8×10^{-5} M; λ = 435 nm; and pH = 10.25

Fig. 1(*a*) shows the results for ligand and copper ion mixtures at pH 10.25 in water - ethanol media (50 + 50 *V/V*) and for different irradiation times. Reproducible data were obtained when the irradiation times were greater than 30 min; absorbance measurements slowly increased with time. Fig. 1(*b*) gives the results after irradiation for 30 min and heating several times to 35 °C, as described under Experimental. Fig. 1(*b*) reveals that there is complete (*Z*)-chelate formation after 15 min of heating as the absorbance measurements are then constant.

Assuming that the ligand is in a large excess, this behaviour suggests that three reactions occur in solution:

$$(E)\text{-TAPH} + \text{Cu(II)} \rightleftharpoons (E)\text{-TAPH - Cu(II)} \quad .. \quad (1)$$

$$(E)\text{-TAPH} + h\nu \rightleftharpoons (Z)\text{-TAPH} \quad .. \quad (2)$$

$$(Z)\text{-TAPH} + \text{Cu(II)} \rightleftharpoons (Z)\text{-TAPH - Cu(II)} \quad .. \quad (3)$$

Reactions (1) and (3) compete for copper ion in a displacement equilibrium.

Probably this competitive reaction is slow and accelerates with increase in temperature. Some support for this hypothesis is given by the results of the route B experiment, *i.e.*, irradiation of the (*E*)-TAPH and subsequent mixture with copper ions. The maximum absorbance readings were obtained following irradiation and heating of the (*E*)-TAPH - copper ion mixture.

These results suggest that irradiation of the ligand is preferred to irradiation of the mixture, because the reaction proceeds rapidly and heating is not necessary. Thus prior photogeneration of the (*Z*)-isomer with the subsequent addition of copper ion is recommended.

Under these conditions, for 50% ethanolic solutions, chelate formation was complete at pH 9.0 and the absorbance was constant up to pH 12.0 (Fig. 2). In the suggested procedure, the pH can be adjusted to 10.25 by adding 5 ml of pH 8.75 borate buffer solution.

Similar experiments, carried out at pH 8.75 to determine the optimum percentage of ethanol, showed the need for a high ethanol content. A 50% *V/V* ethanol solution was finally chosen because of the insolubility of the chelate in water. Complex formation was rapid and gave a constant absorbance for at least 4 h. For complete complexation, a 10 M excess was adequate. Complex formation was not affected by the sequence in which the reagents were added.

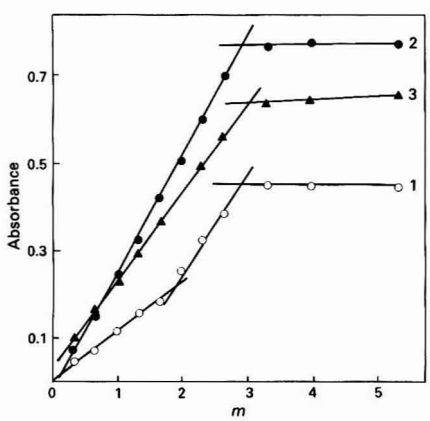

Fig. 3. Molar ratio method to study the variation of the stoicheiometry of the (*E*)-TAPH - copper complex with time. [Cu] = 3×10^{-5} M; λ = 427 nm; pH = 10.25; and reaction carried out in darkness. Formation time: 1, 0; 2, 3; and 3, 24 h

Table 1. Spectral data for the (*E*)- and (*Z*)-TAPH - copper mixtures

Sample	λ_{max}/nm	ε/l mol^{-1} cm^{-1}
A	427	13.5×10^3
B	430	21.5×10^3
C	435	38.5×10^3

Stoicheiometry

Both isomers react with copper(II) ions to form complexes with absorption maxima at 427 nm [(*E*)-complex] and 435 nm [(*Z*)-complex]. We would expect that the linkage process with the (*E*)-isomer would increase the molecular rigidity by formation of two five-membered chelate rings to impede the torsion of the C=N bond.

The expected complexation reaction does take place, but surprisingly, the (*E*)-chelate is unstable, probably because of the great delocalisation of the unshared heterocyclic electron pair donated by the sulphur atom in the tridentate chelate.[10] The (*E*)-TAPH - Cu complex is gradually converted into the (*Z*)-complex, even in darkness. This process was studied by following the variation in the stoicheiometry of the complexes formed between (*E*)-TAPH and Cu(II) in an appropriate medium (Fig. 3).

Initially, two complexes were observed, with stoicheiometries of 2:1 and 3:1. However, even in darkness, the mixture converts spontaneously into one stable chelate with a stoicheiometry of 3:1 (ligand:metal). These observations support the theory that the initial equilibrium between the two complexes is displaced towards the more stable (*Z*)-form.

The electronic spectra of the copper and (*Z*)-TAPH mixtures were recorded immediately after preparation and after 3 h in darkness (samples A and B). They were compared with another sample, C, which was similar except that instead of irradiated TAPH, an equal volume of previously irradiated TAPH was used.

The spectral data are summarised in Table 1 and, in comparison with sample A, bathochromic displacement of the absorption maxima in samples B and C is observed together with progressive increases of the molar absorptivity (ε) values. The linkage process produces torsion of the C=N double bond, and then the molecule undergoes spontaneous gradual decay to a lower energy state, that is, to the most stable chelate. Paradoxically, the stability of the resulting bidentate chelate, with only one five-membered ring, is greater than that of those chelates with two rings (terdentate chelates).[11]

Analytical Parameters

Straight-line calibration graphs were obtained using the recommended procedure. The linear equation obtained following treatment by the least-squares method was

$$A = 0.589c + 0.012, r = 0.998$$

The molar absorptivity (ε) at $\lambda_{max.}$ = 435 nm was 3.85×10^4 l mol^{-1} cm^{-1} and the Sandell sensitivity was 0.0016 μg cm^{-2}. A Ringbom plot showed that the optimum range for accurate determination was 0.3–1.2 μg ml^{-1} of Cu.

The calculated detection limit (k = 3) and limit of quantitation (k = 10) for the method, together with other details about precision and sensitivity, the standard deviation of the blank measurements (S_B) and the analytical signal (S_S) are given in Table 2.

To compare the sensitivity of the method, in a coherent units system, we used analytical sensitivity, $S_A = S_S/m$, where S_S is the standard deviation of the analytical signal at a particular concentration and m is the slope of the calibration graph. These values indicate the ability to distinguish a

Table 2. Characteristics of the method

Analytical sensitivity ($S_A = S_S/m$)/ng ml^{-1}	S_B, A	S_S, A	C_L (k = 3)/ ng ml^{-1}	C_Q (k = 10)/ ng ml^{-1}	LDR*/ ng ml^{-1}	Amount taken/ μg ml^{-1}	Amount found/ μg ml^{-1}	n	RSD,† %	RE,‡ %
7	0.001	0.004	5	17	17–1200	1.0	0.997	10	0.70	0.50

* LDR = linear dynamic range.
† RSD = relative standard deviation.
‡ RE = relative error (= $100ts/\bar{x}n^{\frac{1}{2}}$).

Table 3. Tolerance limits for various ions in the determination of 1 μg ml^{-1} of copper

Ion added	Tolerance ratio, ion to Cu^{2+} (*m/m*) Without masking	With masking	Masking agent	Amount added/ μg ml^{-1}
I^-, OAc^-	20 000			
NH_3, urea	5 000			
F^-, PO_4^{3-}, TEA*	1 000			
H_2O_2, $S_2O_3^{2-}$, SCN^-, thiourea	200			
Citrate, oxalate, tartrate, SO_3^{2-}	100			
Ascorbic acid, dimethylglyoxime, Cd^{2+}	10			
Mn^{2+}, Zn^{2+}, Pb^{2+}, Mo^{6+}	5			
Al^{3+}	5	50	OAc^-	20 000
V^{5+}, S^{2-}	2			
EDTA	1			
Ag^+	1	20	NH_3	5 000
Hg^{2+}	1	5	TEA	1 000
CN^-, Ni^{2+}	0.1			
Pd^{2+}	0.1	20	TEA	1 000
Fe^{3+}	0.1	10	Citrate	100
Cr^{3+}	0.05	5	Citrate	100
Co^{2+}	0.05	1	H_2O_2	200
			NH_3	5 000

* TEA = triethanolamine.

Table 4. Determination of copper in samples of pig liver tissue

Sample	Copper present/ μg ml^{-1}	Copper found/ μg ml^{-1}	Copper content/ μg Cu per g dried liver
1	0.43†	0.410 ± 0.015	70.4
2	0.40	0.387 ± 0.020	66.5
3	0.21	0.203 ± 0.012	68.8

* Mean ± standard deviation of triplicate determinations.
† By standard additions method.

concentration difference at a determined confidence level. In this instance, S_A can be given in ng ml^{-1}, irrespective of the analytical signal.

Interference from Foreign Ions

Under the same optimum conditions used above and with 1 μg ml^{-1} of Cu, a systematic study of the effect of foreign ions was undertaken. The results are summarised in Table 3. The tolerance limit of an ion was fixed as the maximum amount causing an error of not greater than 2% in the absorbance. The most important interferences were from ions that formed coloured complexes with the reagent, such as Co(II), Ni(II) and Pd(II). The effects of these principal interferences were suppressed by using several common masking agents; only Ni(II) could not be masked. The interferences of Cr(III), Cd(II) and Fe(III) were effectively masked by citrate and those of Al(III), Hg(II) and Ag(I) by acetate, triethanolamine and NH_3, respectively. The most troublesome interference was that caused by cobalt ions; this was removed by adding one drop of dilute hydrogen peroxide to the sample in ammoniacal medium, which was adjusted to pH 6–8; reagent and buffer were then added as recommended. Absorbance readings remained constant for 15 min after this treatment.

Application to Clinical Samples

The recommended procedure for the determination of copper was applied to pig liver tissue to evaluate its effectiveness. The results obtained are summarised in Table 4.

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Spectrophotometric Determination of Palladium in Catalysts and Carbenicillin with 1-(2-Pyridylmethylidene)-5-(salicylidene)-thiocarbohydrazone

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A selective and sensitive method is described for the spectrophotometric determination of palladium using 1-(2-pyridylmethylidene)-5-(salicylidene)thiocarbohydrazone reagent. The orange complex is formed in pH 8.3 EDTA - bicine - borate buffer in a medium containing 32% *V/V* dimethylformamide, and the absorbance is measured at 505 nm. The molar absorptivity is 16 500 l mol^{-1} cm^{-1}, and in the determination of 0.75 μg ml^{-1} of palladium the relative standard deviation is about 2%. The effect of interferences was studied and the selectivity of the reaction was enhanced by using appropriate masking agents. The method was applied to the determination of palladium in synthetic mixtures, hydrogenation catalysts and carbenicillin (semi-synthetic penicillin).

Keywords: *1-(2-Pyridylmethylidene)-5-(salicylidene)thiocarbohydrazone; palladium determination; spectrophotometry; catalysts; carbenicillin*

This paper, which forms part of an investigation into the use of thiocarbohydrazones as analytical reagents,[1,2] describes a study of the optimum conditions for a selective and sensitive spectrophotometric method for the determination of palladium using 1-(2-pyridylmethylidene)-5-(salicylidene)thiocarbohydrazone (PST) in EDTA - bicine [*N,N*-bis(2-hydroxyethyl)glycine] - borate buffered medium (pH 8.3). By using the proposed method, the palladium content in five synthetic mixtures, seven catalysts and two pharmaceutical preparations, containing the semi-synthetic penicillin carbenicillin, was accurately determined.

Experimental

Reagents

All chemicals were of analytical-reagent grade or better. Glass-distilled and de-ionised water was used throughout. Stock standard palladium(II) solution (1.0033 g l^{-1}) was prepared by dissolving pure $PdCl_2$ (Merck) in 10 ml of concentrated hydrochloric acid and diluting to 1 l with distilled water. Working solutions were prepared by appropriate dilutions. PST was used at 0.1% *m/V* in dimethylformamide (DMF). This solution was stable for 3 months. The synthesis and analytical properties of this reagent have been reported previously.[2] A masking buffer of pH 8.3 was prepared by dissolving 186 g of $Na_2EDTA.2H_2O$, 31 g of boric acid and 32 g of sodium hydroxide in 500 ml of water. When a clear solution was obtained, 32.7 g of bicine were added and the solution was diluted to 1 l.

Apparatus

Perkin-Elmer Model 554 and Coleman Model 55 spectrophotometers and a Beckman Model 70 pH meter with a combined SCE - glass electrode were used.

Recommended Procedure

In a 25-ml calibrated flask place a suitable volume (up to 15 ml) of sample solution containing up to 125 μg of palladium and 2–10 ml of the pH 8.3 buffer described under Reagents. Allow the solution to stand for about 1 min and add 3 ml of 0.1% PST solution and 5 ml of DMF. Dilute to the mark with distilled water and mix well. Measure the absorbance at 505 nm using a 1.0 cm cell against distilled water. Calculate the palladium content by using a calibration graph or empirical equation. If the sample contains large amounts of interfering ions, add a suitable amount of masking agent prior to addition of the reagent (see under Interferences).

Determination of Palladium in Hydrogenation Catalysts

The following catalysts are listed in Table 4.

Palladium on $CaCO_3$, $BaCO_3$ or $CaCO_3$ poisoned with lead

About 0.3 g of catalyst was treated with 5 ml of 2 M HNO_3. When the evolution of gas had diminished, 20 ml of aqua regia, or concentrated HNO_3 if the catalyst contained lead, were added and the solution was evaporated nearly to dryness on a sand-bath. The residue was dissolved in 5 ml of 2 M HNO_3 and diluted to 100 ml with water. Aliquots of 0.5 ml were used in the spectrophotometric determination of palladium by the recommended procedure.

Palladium on $BaSO_4$ or alumina

A 0.3–2.5 g aliquot of catalyst was treated with 20 ml of aqua regia and evaporated nearly to dryness on a sand-bath. It was then treated with 20 ml of concentrated HNO_3 and evaporated to about 5 ml. On cooling, the solution was diluted with 20 ml of water and filtered into a 100-ml calibrated flask, washing the residue with water. Aliquots of 0.5 ml were analysed by the recommended procedure.

Palladium on activated charcoal

About 0.3 g of sample was treated twice with 20 ml of aqua regia and the solution evaporated to about 5 ml. On cooling, the solution was diluted with 20 ml of water and filtered into a 250-ml calibrated flask, washing the residue first with 20 ml of 2 M HNO_3 and then with water. Finally, the sample was diluted to the mark with water. The palladium content was

determined in 0.5-ml aliquots by the recommended procedure.

Determination of Palladium in Carbenicillin

About 5 g of sample in a silica crucible were placed in a muffle furnace, heated at a rate of 100 °C h^{-1} and ashed at 800 °C for 3 h. After slow cooling, the residue was treated with 10 ml of HNO_3 - HCl (1 + 1) and evaporated nearly to dryness on a sand-bath. It was then treated with 5 ml of concentrated HNO_3 and evaporated again to about 1 ml. On cooling, the solution was diluted with 5 ml of water and filtered into a 10-ml calibrated flask, washing the residue with water. Finally, the sample was diluted to the mark with water. To aliquots of 3 ml were added 2 ml of buffer and the pH was adjusted with sodium hydroxide to 8.3 ± 0.1 (pH meter). The solutions were quantitatively transferred into 10-ml calibrated flasks and 1.2 ml of PST solution and 2 ml of DMF were added. They were then diluted to the mark with water, mixed and the absorbance was measured at 505 nm against distilled water. The palladium content was calculated from a calibration graph.

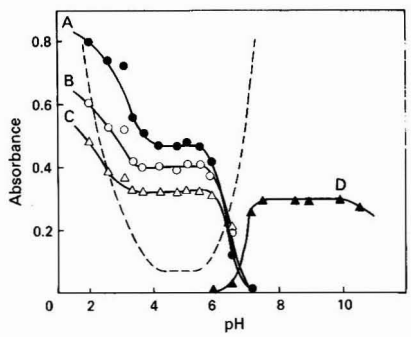

Fig. 1. Absorbance *versus* pH graph for the palladium(II) - PST complexes. Absorbances at A, 400 nm; B, 420 nm; C, 470 nm; and D, 500 nm. Dashed line, absorbance of the reagent blank at 400 nm measured against water. C_{Pd} = 1.8 μg ml^{-1}; C_{PST} = 3 ml of 0.1% solution per 25 ml; medium containing 40% DMF

Results and Discussion

Absorption Spectra and pH Effect

When a solution of PST in DMF is added to a palladium(II) solution, a yellow complex with a $\lambda_{max.}$ at 400 nm is formed instantaneously at an acidic pH. The yellow colour of the complex changes to orange when the pH is increased ($\lambda_{max.}$ at 505 nm); this system is reversible. The absorbance *versus* pH graph for the palladium complexes at several wavelengths are shown in Fig. 1. A constant absorbance was obtained at pH 4–5.5 (yellow complex) and at pH 7.5–10 (orange complex). The molar ratio and continuous variation methods were applied at pH 4.5 and 9.0. Both methods showed a 1 : 2 (M : L) stoicheiometry for both complexes (Fig. 2). The over-all conditional formation constants were evaluated from the molar ratio data in a medium containing 40% *V/V* DMF. The constants found for the yellow complex at pH 4.5 were $\log \beta'_1 = 5.57$, $\log \beta'_2 = 12.97$ and those for the orange complex at pH 9.0 were $\log \beta'_1 = 10.69$, $\log \beta'_2 = 16.85$.[2] Both complexes exhibited conditional formation constants higher than the Pd(II) - EDTA complex at these values of pH,[3] and it was confirmed experimentally that they can be formed in the presence of large amounts of EDTA. The structure of the yellow complex is probably similar to that described previously for the Hg(II) - PST complex,[4] but we failed to find a scheme for the orange complex structure.

We cannot explain the pH dependence of the spectral characteristics of the palladium(II) - PST system, firstly because there is no change in the stoicheiometry of the complexes, and secondly because both complexes are uncharged (they are both extracted into chloroform, though the orange complex is very unstable in this solvent), which implies that two sulphur atoms of two PST molecules are binding to the palladium(II) ion. However, this does not affect the use of PST for the spectrophotometric determination of palladium.

Evaluation of the Optimum Conditions for the Spectrophotometric Determination of Palladium

The yellow complex was unsuitable for the analytical procedure because of the blank absorbance at the wavelength of maximum absorption of the complex and because PST generally forms yellow complexes with other metal ions.[2,4] Therefore, the method was carried out in a basic medium, measuring the absorbance at the wavelength of maximum absorption of the

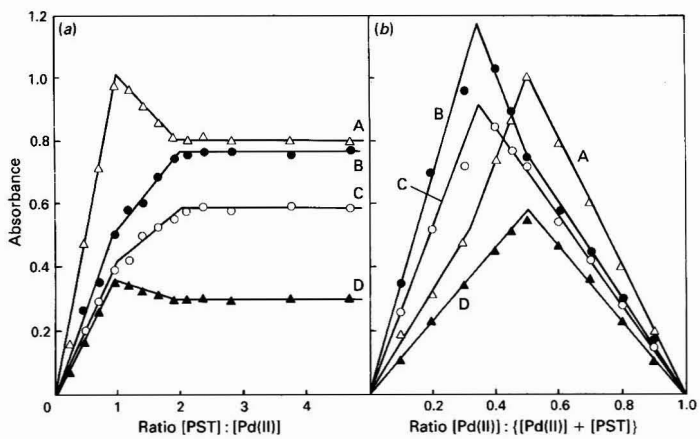

Fig. 2. Stoicheiometry of the palladium(II) - PST complex in 40% DMF. (*a*) Composition determined by the molar ratio method: A, at pH 9.0 (C_{Pd} = 5.0 μg ml^{-1}); B, C and D, at pH 4.5 (C_{Pd} = 2.9 μg ml^{-1}). (*b*) Composition determined by the continuous variations method: $C_{(Pd + PST)} = 1.09 \times 10^{-4}$ M. A, at pH 9.0; B, C and D, at pH 4.5. Absorbances at A, 500 nm; B, 400 nm; C, 420 nm; and D, 470 nm

Table 1. Tolerance of foreign ions in the determination of 1.00 μg ml^{-1} of palladium

Tolerance/ μg ml^{-1}	Ion or species	Tolerance/ μg ml^{-1}	Ion or species
>1000	Alkali and alkaline earth metals, Al(III), Ga(III), In(III), Tl(I), Pb(II), Y(III), La(III), Ce(IV), Th(IV), UO_2(II), V(V),† Mo(VI), W(VI), Mn(II), Zn(II), Cd(II), NH_4^+	>50 000	EDTA*
		>10 000	$S_2O_3^{2-}$, F^-, Cl^-, Br^-, I^-, tartrate
		5 000	SCN^-, TeO_3^{2-}, citrate
		1 000	NO_3^-, PO_4^{3-}, $P_2O_7^{4-}$, AsO_4^{3-}, AsO_3^{3-}, SO_4^{2-}, SO_3^{2-}, SeO_3^{2-}, ClO_3^-, ClO_4^-, BrO_3^-, IO_3^-, CO_3^{2-}, $C_2O_4^{2-}$, ascorbate
500	Bi(III),‡ Ni(II)	500	NO_2^-
100	Sb(III), Sn(II)‡, V(V), Pt(IV), Re(VIII)	100	IO_4^-
		1	S^{2-}
50	Zr(IV),‡ Fe(II), Fe(III), Cr(III)§		
20	Ti(IV)‡		
10	Co(II), Os(VIII), Ir(IV), Rh(III)		
5	Ag(I), Au(III), Ru(III),§ Cr(III)		
2	Cu(II), Hg(II), Ru(III)		

* The EDTA contained in the buffer is not included in the amount shown.
† Measured before 5 min.
‡ Filtered.
§ Measured against a blank containing the metal ion tested and the buffer.

Table 2. Elimination of interferences by the addition of masking agents. Palladium concentration, 1.00 μg ml^{-1}

Foreign ion	Amount tolerated/μg ml^{-1} Without masking agent	With masking agent	Masking agent added	Amount of masking agent/ μg ml^{-1}
Zr(IV)	50	100	Tartrate	10 000
Ti(IV)	20	100	Tartrate	10 000
Sn(II)	100	1000	Tartrate	10 000
Sb(III)	100	1000	Tartrate	10 000
Ag(I)	5	100	Thiosulphate	10 000
Hg(II)	2	20	Thiosulphate	10 000
Au(III)	5	100	Thiosulphate	10 000*
Cu(II)	2	10	EDTA	50 000
Co(II)	10	50	EDTA	50 000

* Before the addition of PST, neutralise, add thiosulphate and then the buffer; in any other order of addition elemental sulphur or gold appears.

Table 3. Determination of palladium in synthetic mixtures

Synthetic mixture/μg ml^{-1}	Palladium added/ μg ml^{-1}	Palladium found*/ μg ml^{-1}	Masking agent	Amount of masking agent/ μg ml^{-1}
80 Al(III) + 50 Ti(IV) + 50 Sn(II) + 50 V(V) + 50 Zr(IV) + 40 Mo(VI) + 20 Mn(II) + 10 Fe(III) + 1 Cu(II)	0.75	0.79 ± 0.03	Tartrate	10 000
	1.00	1.02 ± 0.03	Tartrate	10 000
	2.00	2.02 ± 0.04	Tartrate	10 000
100 Ni(II) + 10 V(V) + 2 Pt(IV)	0.75	0.76 ± 0.01	—	
	1.00	0.99 ± 0.02	—	
	2.00	2.02 ± 0.02	—	
30 Ag(I) + 30 Au(III) + 30 Zn(II) + 5 Cu(II) + 2 Pt(IV)	0.75	0.78 ± 0.03	Thiosulphate	10 000†
	1.00	0.99 ± 0.02	Thiosulphate	10 000†
	2.00	1.99 ± 0.04	Thiosulphate	10 000†
7 Ir(IV) + 2 Rh(III) + 2 Pt(IV) + 1 Fe(III) + 1 Cu(II)	0.75	0.79 ± 0.03	—	
	1.00	1.05 ± 0.02	—	
	2.00	2.04 ± 0.03	—	
100 Ag(I) + 15 Pt(IV) + 10 Au(III) + 2 Ru(III) + 2 Rh(III) + 2 Ir(IV)	1.00	1.08 ± 0.03	Thiosulphate	10 000†
	2.00	2.04 ± 0.04	Thiosulphate	10 000†

* Mean of three determinations.
† Using 5 ml of buffer. See footnote in Table 2.

orange complex (505 nm). As the complex can be formed in the presence of EDTA or bicine, a borate pH 8.3 buffer that was 0.5 M in EDTA and 0.2 M in bicin was selected for the analytical procedure. The volume of this buffer (1–10 ml in a final volume of 25 ml) had no effect. Increasing the ionic strength produced no significant changes in the absorption of the complex.

A constant absorbance was obtained when the ratio of reagent to palladium was greater than 2, and therefore 3 ml of 0.1% PST solution in a final volume of 25 ml or 1.2 ml in a final volume of 10 ml was chosen as the most suitable. Under these conditions, the reaction went to completion immediately after mixing the reagents and the absorbance of the reagent blank at

Table 4. Determination of palladium in hydrogenation catalysts

Catalyst	Palladium claimed, %	Mass of sample*/g	Palladium found,†/%
Pd - $CaCO_3$	5	0.3122	5.16 ± 0.04
		0.3004	4.99 ± 0.03
		0.3008	5.05 ± 0.08
Pd - $CaCO_3$ poisoned with lead	5	0.3034	5.11 ± 0.06
		0.3022	5.04 ± 0.05
		0.3215	5.12 ± 0.04
Pd - $BaCO_3$	5	0.3104	5.21 ± 0.05
		0.2998	5.18 ± 0.06
		0.3300	5.25 ± 0.04
Pd - $BaSO_4$	5	0.2993	5.27 ± 0.05
		0.3208	5.10 ± 0.03
		0.3023	5.10 ± 0.06
Pd - Al_2O_3	1	0.8819	1.01 ± 0.01
		0.7842	1.07 ± 0.02
		1.3217	1.04 ± 0.01
Pd - 1/8-in Al_2O_3 pellets	0.5	1.9017	0.486 ± 0.007
		2.4002	0.488 ± 0.006
		2.2313	0.493 ± 0.004
Pd - activated charcoal	10	0.3056	9.31 ± 0.18
		0.3296	9.41 ± 0.16
		0.3165	9.27 ± 0.15

* Samples dissolved as described under Procedure and made up to 100 ml, except for Pd - charcoal, which was made up to 250 ml.
† Mean of three determinations.

Table 5. Determination of palladium in carbenicillin

Preparation	Mass of sample*/g	Palladium found†/μg g^{-1}
Beecham carbenicillin‡	6.1249	8.69 ± 0.32
	5.4641	8.51 ± 0.48
Pyopen	4.6200	4.70 ± 0.53
	5.9941	4.37 ± 0.24

* The samples were ashed and the residue made up to 10 ml.
† Mean of three determinations.
‡ The palladium content in this preparation, determined by atomic absorption spectrometry, was *ca.* 8 μg g^{-1}.

505 nm was negligible. The order in which the reagents were added was found to be immaterial.

The influence of various water-soluble solvents was studied because the complex precipitated in aqueous media. In general, mixtures such as water - DMF were the most suitable. The percentage of DMF affected the absorbance of the complex but it was constant for 24–40% *V/V* of this solvent. A medium containing 32% DMF was selected. Variations of ±5% produced changes lower than 2% in the absorbance of the complex. In this medium the complex was stable for 3 h. The same stability was observed by using a pH 8.3 borate buffer containing neither EDTA nor bicin. The apparent pH in this medium was 8.7 ± 0.1.

Calibration and Precision

Beer's law was obeyed over the range 0–5.0 μg ml^{-1} of palladium. The straight line corresponded to the equation $A = 0.1555$ [μg ml^{-1} Pd(II)] + 0.003, with a correlation coefficient of 0.9999. The molar absorptivity, calculated from the slope of the graph, was 16500 l mol^{-1} cm^{-1} at 505 nm. The optimum concentration range, as evaluated by a Ringbom plot, was 0.7–5.0 μg ml^{-1} of palladium, and according to Sandell's expression, the sensitivity of the reaction was 6.43 ng cm^{-2}. The precision, expressed as a relative standard deviation ($P = 0.05$), was 1.35% for 1.00 μg ml^{-1} of palladium (11 samples). For the study of between-day variation, single determinations were made for each sample size on 11 different days, and the relative standard deviations were 2.03, 1.56 and 0.81% for 0.75, 1.00 and 2.00 μg ml^{-1} of palladium, respectively.

Interferences

In the determination of 1.00 μg ml^{-1} of palladium by the recommended procedure using 2 ml of buffer, foreign ions can be tolerated at the levels given in Table 1.

Results for the elimination of interferences by the addition of masking agents are given in Table 2. Good results were obtained on masking silver, gold and mercury with thiosulphate, and tin, titanium, zirconium and antimony with tartrate. Palladium was also accurately determined in some synthetic mixtures of foreign ions corresponding to concentrates[5] and jewellery alloys[6] (Table 3).

Applications

One of the most important industrial uses of palladium today is as a catalyst for hydrogenation reactions. The proposed method has been applied to the determination of palladium in a variety of such catalysts. The results obtained are presented in Table 4. Palladium is also used as a catalytic agent in the production of semi-synthetic penicillins. Because palladium can combine with some of the compounds during the reaction and persist in the pharmaceutical product, its levels, which should be less than 20 μg g^{-1}, must be controlled.[7] According to the previously described procedure, the palladium content in two pharmaceutical preparations containing carbenicillin was determined. As in a preliminary study we noticed that palladium cannot be directly determined because carbenicillin acts as a strong sequestering agent, the organic matter was previously destroyed in a similar way to that recommended in the British Pharmacopoeia.[8] The results obtained are shown in Table 5.

Conclusions

Many chromogenic reagents have been used for the spectrophotometric determination of palladium (Table 6), but most of them require a time-consuming extraction step or a heating period, or suffer from a large number of interferences, especially from the other noble metals. Many of them also

Table 6. Comparison with some other reagents for palladium

Reagent	Optimum pH	$\lambda_{max.}$/ nm	$\varepsilon \times 10^{-3}$/ l mol^{-1} cm^{-1}	Remarks	Reference
Dimethyl glyoxime	1.5–2.5	380	1.6	Extraction into $CHCl_3$	10
Phenylazobenzaldehyde oxime	1.0–5.5	550	3.6	*ca.* 90% ethanol	11
Ethyl 3-phenyl-5-isoxazolone-4-carboxylate	0.1–4.0 M $HClO_4$	370	3.9	Extraction into 4-methylpentan-2-ol	12
	1.0–6.5	370	3.7	Aqueous	12
4-(2-Pyridylazo)resorcinol	4.5–5.0	610	3.9	Extraction into mesityl oxide	13
Propericiazine	1.1–4.1	474	4.1	Ag^+, Au^{3+}, V^{5+} interfere	14
Biacetyl monoxime 2-pyridylhydrazone	1.2–1.8	560	7.5	Extraction into $CHCl_3$	15
Solochrome Red B	4.0–6.5	488	12.1	Pt^{4+} interferes	16
PST	7.5–10	505	16.5	32% DMF	This work
α-Furyl dioxime	0.1–1.0 M HCl	380	23.8	Extraction into $CHCl_3$	10
Dithizone + $SnCl_2$	1 M H_2SO_4	635	35.5	Extraction into CCl_4	17
3-(2′-Thiazolylazo)-2,6-diaminotoluene	1–2.5 M $HClO_4$	590	53.5	I^-, SCN^- interfere	18
p-Nitrosodimethylaniline	4.4–5.2	525	65.0	Au^{3+}, Pt^{4+}, Ni^{2+} interfere	10
p-Nitrosodiphenylamine	1.8–2.3	525	70.0	Extraction into diethyl oxalate	9

have the drawback of a low sensitivity [Pd(II) complex molar absorptivities are less than 10^4 l mol^{-1} cm^{-1}], and some of them are too unstable to be accepted as good spectrophotometric reagents. The PST method is rapid, precise and easy to use, and can be applied to the determination of palladium in materials containing as little as 5 μg g^{-1} by taking a 5-g sample. Further, because tellurite does not interfere in the determination, the procedure should be applicable, with little modification, to sulphides, meteorites and subsilicic rocks containing more than 0.1 μg g^{-1} of palladium, by coprecipitation of palladium with tellurium in the presence of tin(II) chloride, which provides an effective pre-concentration of palladium and separation from iron, copper, nickel and cobalt.[9] A further advantage of the PST method over other spectrophotometric reagents is that, by using thiosulphate as a masking agent, palladium can be directly determined in the presence of other noble metals.

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Spectrophotometric Determination of Boron in Nuclear-grade Uranium Oxides with Curcumin after Extraction with 2-Ethylhexane-1,3-diol

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Trace amounts of boron are determined in uranium oxides spectrophotometrically with curcumin after prior extraction with 2-ethylhexane-1,3-diol. The concentration range of the method is 0.1–5 μg of B per gram of U, with a standard deviation of 0.03 μg of B per gram of U. A low bias of 15% is observed in U_3O_8 reference standards.

Keywords: *Boron determination; 2-ethylhexane-1,3-diol; curcumin; spectrophotometry; uranium oxides*

The determination of microgram or sub-microgram amounts of boron in nuclear-grade uranium oxides is necessary to meet the specification limit of 0.3 μg of B per gram of U for natural sinterable UO_2 powder. The detection limit obtained with spectrographic[1,2] or emission spectroscopic[3] determinations is approximately 0.1 μg of B per gram of U at the 95% confidence level, which is barely adequate for the above determination.

Boron in uranium compounds can be determined spectrophotometrically with curcumin after separation by distillation as methyl borate.[4] However, the distillation procedure is slow and tedious, requires special apparatus and can be subject to boron losses and memory effects. Over the past several years, considerable work has been performed on the use of alkane-1,3- or 2,4-diols as selective extractants for boron. Agazzi[5] used 5% 2-ethylhexane-1,3-diol (EHD) in chloroform to extract acidic boron solutions prior to boron determination by flame photometry. Mair and Day[6] and Grotheer[7] used 10% EHD in chloroform for the extraction of boron, followed by a spectrophotometric determination with curcumin. The chloroform was evaporated prior to colour formation for samples containing low levels of boron. Donaldson[8] applied EHD - chloroform extraction and colour development with curcumin to the determination of boron in steel. Peterson and Zoromski[9] and Troll and Sauerer[10] back-extracted the EHD solution with NaOH prior to colour development with carmine. Mezger *et al.*[11] used NaOH back-extraction prior to boron determination by ICP spectrometry. Aznarez and co-workers[12,13] preferred 2-methylpentane-2,4-diol as the extractant. Wikner and Uppstrom[14] concluded that 2,2-dimethylhexane-1,3-diol was superior to several other diols, including both EHD and 2-methylpentane-2,4-diol, for the extraction of boron. However, 2,2-dimethylhexane-1,3-diol is less readily available than either EHD or 2-methylpentane-2,4-diol.

In this work, the extraction procedure of Donaldson[8] was followed throughout, using 10% EHD in chloroform as the extractant. To obtain the required sensitivity, the colour development procedure of Wikner and Uppstrom[14] was utilised. Colour development takes place in a homogeneous solution containing diol extract, curcumin, acetic acid, sulphuric acid and 4-methylpentan-2-one (isobutyl methyl ketone, IBMK). When colour development is complete, water is added. The solution separates into two phases with the upper layer (IBMK and chloroform) containing the rosocyanin complex. In preliminary experiments it was discovered that the volume of diol extract could be increased from 1 to 3 ml with this procedure to improve sensitivity without the prior evaporation of chloroform found necessary by other workers.[6,7,12]

Experimental

Apparatus

Absorbance measurements were performed on a Cary 219 spectrophotometer. Sample dissolution was performed in 125-ml PVC bottles (Nalgene). Air condensers were made by press-fitting a 150 mm length of thick-walled polyethylene or PTFE tubing of 2–3 mm i.d. in the cap of the PVC bottle. The bottom of the tubing was placed about 40 mm below the cap.

Plastic laboratory ware was used where possible to avoid boron contamination from glass. All laboratory ware was cleaned by soaking in a commercial cleaning solution, then rinsed consecutively with tap water, distilled water, methanol, and acetone and then dried.

Reagents

Unless stated otherwise, all chemicals were of analytical-reagent grade and were used as received.

2-Ethylhexane-1,3-diol (EHD) solution, 10%. Dilute 50 ml of 2-ethylhexane-1,3-diol (BDH Chemicals) to 500 ml with chloroform. Store in a polyethylene bottle.

Curcumin reagent. Using a 250-ml PTFE beaker, dissolve 0.50 g of curcumin (Aldrich Chemicals) in approximately 150 ml of IBMK (Aldrich Gold Label grade). Filter through a Whatman No. 41 filter-paper into a 250-ml polyethylene flask. Dilute to the mark with IBMK.

Sulphuric acid of low boron content. Select a bottle of sulphuric acid (Baker Instra-Analyzed grade) certified to contain less than 0.01 μg g^{-1} of boron.

Boron standard solutions. Prepared by serial dilution of 1000 mg l^{-1} standard boron solution (BDH Chemicals) and stored in plastic bottles.

Procedure

Sample dissolution

Convert U metal or UO_2 samples into U_3O_8 by ignition at 850 °C for 0.5 h. Transfer a sample containing up to 2.5 g of U (3.0 g of UO_3 or 2.9 g of U_3O_8) into a 125-ml PVC bottle. Add 10 ml of distilled, de-ionised water. If the sample contains U_3O_8 or if U_3O_8 samples are being analysed at the same time, also add 3 ml of 30% H_2O_2 to all samples and standards. Add 8 ml of dilute sulphuric acid (1 + 1) and fit the cap containing the air condenser. Heat on a water-bath at 65–70 °C until dissolution is complete. Cool, remove the cap and rinse the inside of the air condenser and the inside of the cap into the bottle with a small amount of water. Quantitatively transfer the contents into a 125-ml polypropylene separating funnel. Dilute to 75 ml.

Extraction procedure

Extract a 75-ml volume of sample containing 0–2.5 μg of B with 2 × 10 ml and 1 × 3 ml of 10% EHD. Filter the extracts through cotton-wool into a 25-ml polypropylene calibrated flask. Wash the cotton-wool with a further 2 ml of 10% EHD, combining with the previous extracts. Dilute to volume with 10% EHD. For each series of measurements also extract four calibration standards containing 8 ml of dilute sulphuric acid and 0.5, 1.0, 1.75 and 2.5 μg of B, and a reagent blank.

Colour development

Using a glass pipette, transfer 3.0 ml of EHD extract into a 125-ml polypropylene separating funnel. Add 10 ml of sulphuric acid - acetic acid (1 + 1). Using a glass pipette, add 10.0 ml of 2 g l^{-1} curcumin reagent. Stopper the separating funnel and mix by inversion. Allow to stand for 10 min. Add 40 ml of water and shake for 15 s. The solution will separate into two phases with the IBMK and chloroform in the top layer. Drain and discard the bottom layer. Transfer the top layer into a 12-ml screw-top glass centrifuge tube and centrifuge for 5 min. Transfer the solution into a 10 mm quartz cell and measure the absorbance at 550 nm *versus* IBMK. Determine the boron content from a graph of absorbance *versus* mass of B in the final solutions.

Results and Discussion

Minimisation of the Blank Absorption

A blank absorption of approximately 0.6 A was originally obtained with this determination. Experiments with different aliquots of diol extract showed little variation in the blank, indicating that it is mainly due to the colour development rather than the extraction step. The use of low-boron sulphuric acid (0.004 μg g^{-1} of boron) for sample dissolution and colour development allowed the reduction of the blank absorption only from 0.6 to 0.5 A.

The use of ultra-pure acetic acid did not change the blank absorption, nor did the substitution of different batches of curcumin. A reduction in absorbance from 0.5 to 0.3 A was achieved when spectrophotometric-grade IBMK was substituted for the reagent-grade IBMK used in the preliminary experiments. Spectral scans suggest that most of the absorbance of the blank is due to residual boron, possibly in the IBMK. However, the blank remains constant with each batch of samples and is not sufficiently high to cause significant photometric error over the range of standards employed. The spectrophotometric measurements are referenced to IBMK to allow the blank level to be monitored and to provide an additional degree of freedom for the calibration.

Tests with Uranium Compounds

Standard additions to two UO_3 samples and a U_3O_8 sample in the range 0–5 μg (0–2 μg g^{-1} of B in U) gave slopes for the final solutions of 1.09 ± 0.05 (95% confidence level), 1.04 ± 0.11 and 1.16 ± 0.12 A μg^{-1}, respectively. These are not significantly different at the 95% confidence level from the slope of 1.13 ± 0.14 A μg^{-1} observed for aqueous standards. The reason for the minor slope variations is not known, but may be due in part to different batches of curcumin reagent.

The uranium content of the diol extracts of two UO_3 samples and a U_3O_8 sample was determined fluorimetrically. The measured uranium concentrations were in the range 3–20 mg l^{-1}, indicating that 0.02% or less of the uranium was extracted by the diol.

The apparent molar absorptivity of the rosocyanin complex calculated from the slope of the calibration was typically 1.6 × 10^5, based on a nominal final volume of 13 ml (10 ml of IBMK +3 ml of EHD - chloroform). This value is slightly lower than the value of 2.0 × 10^5 reported by Wikner and Uppstrom[14] in the absence of diol and chloroform, but falls in the middle of the typical range[8] 1.3 × 10^5–2.0 × 10^5.

Analysis of Uranium Oxides

Results of the analyses of New Brunswick Laboratories (NBL) 98 series U_3O_8 standards are given in Table 1. A graph of mass of B found *versus* mass of B certified for these data gave a slope of 0.85 ± 0.08 (95% confidence level), an intercept of 0.10 ± 0.16 and a correlation coefficient of 0.987. These data indicate a low bias of approximately 15% in the analysis of U_3O_8.

The results of boron determinations in several UO_3 samples are given in Table 2. These samples were included in an internal company inter-laboratory exchange and were also analysed by carrier distillation[1] and by d.c. plasma spectrometry after extraction of the uranium with triethylhexylphosphate.[15] Results are given as μg of B per gram of sample, as the uranium content was not always determined, and they are generally the results of single determinations.

The spectrophotometric data are in good agreement with the d.c. plasma results except for the initial d.c. plasma result for sample A1153, which may have a high bias owing to contamination. However, the carrier distillation results for the exchange samples appear to be significantly lower than either the spectrophotometric or d.c. plasma results, although no bias between the three methods is evident in the replicate determinations on the control material.

The results of a precision study on a UO_3 control material are also given in Table 2. Over a three month period, the standard deviation of the spectrophotometric procedure was 0.024 μg of B per gram of sample at the level of 0.4 μg of B per gram of sample. This corresponds to expected 95% confidence limits of 0.05 μg of B per gram of sample, or 0.06 μg of B per gram of U for a sample containing 2.5 g of U. The estimated limit of detection is 0.1 μg of B per gram of U.

The results of a sample exchange organised by an external laboratory are given in Table 3. All results shown are based on single determinations except for the replicate spectrophotometric analysis of samples 1 and 4. The U metal samples were ignited to form U_3O_8 at each laboratory prior to the determination of boron.

A graph of the spectrophotometric data *versus* the assigned value from Laboratory 1 (not including the samples for which the Laboratory 1 assigned value in given as "less than 0.1"), gave a slope of 0.85 ± 0.08 (95% confidence level), an intercept of 0.07 ± 0.08 and a correlation coefficient of 0.991.

Table 1. Analysis of NBL 98 Series U_3O_8 standards

Standard	B/μg per g of U Certified	Found	Sample size/ g of U
98–1	5.5 ± 0.2	4.9*	0.50
		5.2	0.43
98–2	2.3 ± 0.1	2.1*	1.00
		2.3	0.42
98–3	1.2 ± 0.2	1.08	2.50
		1.11	2.50
		1.06	2.50
98–4	0.8 ± 0.2	0.63	2.50
		0.64	2.50
98–5	0.4 ± 0.1	0.26	2.50
		0.37	2.50
98–6	$0.2 \pm 0.0_3$	0.27	2.50
		0.28	2.50
98–7	$0.01 \pm 0.0_4$	0.08	2.50
		0.18	2.50

* Mean of two colour developments.

Table 2. Determinations of boron in UO_3 samples

Sample No.	B/μg per g of sample: Carrier distillation*	D.c. plasma spectrometry	Spectrophotometry	Comments
A1129	0.3	0.4	0.26	By calibration graph; spike recovery 93%
			0.24	By standard additions
B068	<0.1	0.1	0.18	By calibration graph
A1153	<0.1	0.4	0.19	By calibration graph
		0.1		
A1179	<0.1	0.2	0.20	By calibration graph
B125	0.1	0.1	0.12	By calibration graph
B132	0.1	0.3	0.36	By calibration graph
B141	0.1	0.2	0.20	By calibration graph
Control†	0.46 ± 0.04 (n = 4)	0.42 ± 0.03 (n = 20)	0.41 ± 0.02 (n = 13)	By calibration graph over 3 month period

* Ga_2O_3 - SrF_2 carrier.
† Error limits are 1 s.

Table 3. Comparative analyses for boron

Sample No.	Matrix	Laboratory 1 No.*	B/μg per g U: Laboratory 1 value†	Laboratory 2 value‡	Spectrophotometric value
2	U	9–5425	<0.10	0.2	0.02
5	U	9–5425	<0.10	0.2	0.06
15	U_3O_8	9–5425	<0.10	0.2	0.13
6	U	9–5426	<0.10	0.2	0.08
16	U_3O_8	9–5426	<0.10	0.1	0.11
3	U	5–2285	0.22	0.3	0.24
8	U	5–2285	0.22	0.4	0.31
9	U_3O_8	5–2285	0.22	0.3	0.22
10	U_3O_8	5–2285	0.22	0.4	0.23
7	U	5–2507	0.81	0.8	0.78
13	U_3O_8	5–2507	0.81	0.7	0.7
1	U	5–3424	1.1	1.0	1.08, 1.0
11	U_3O_8	5–3424	1.1	1.0	1.0
14	U_3O_8	5–3424	1.1	1.0	1.1
4	U	5–2498	1.6	1.6	1.4, 1.3
12	U_3O_8	5–2498	1.6	1.3	1.5

* Identification concealed by Laboratory 1 until comparisons completed.
† Carrier distillation, AgCl - LiF carrier.
‡ Carrier distillation, Ga_2O_3 - SrF_2 carrier.

These data again suggest a low bias of approximately 15% in the spectrophotometric analysis of U_3O_8 samples. In contrast, no firm evidence for a systematic bias can be concluded from an inter-comparison of the spectrophotometric data and the carrier distillation results from Laboratory 2.

Conclusions

Extraction with EHD and colour development with curcumin allow the determination of boron to be made in nuclear-grade uranium oxides over the range 0.1–5 μg of B per gram of U with a standard deviation of 0.03 μg of B per gram of U. An unexplained low bias of 15% was observed for U_3O_8 reference materials but was not clearly confirmed in other comparisons.

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Experimental Determinations and Computer Predictions of Trace Metal Ion Concentrations in Dilute Complex Solutions

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A comparison has been made of experimentally determined and computed metal ion concentrations in the following aqueous solutions of ionic strength <0.015 M containing: (i) up to four trace metals; (ii) ligands selected from glycine or citric, nitrilotriacetic or salicylic acids; (iii) calcium or potassium nitrates as a background electrolyte; and (iv) different pH values. Metal ion concentrations in these solutions were measured using an ion-exchange equilibrium technique and, where appropriate, Cu or Cd electrodes. The metal speciation of the same solutions was also calculated from stability constant data by computer simulation using the programs GEOCHEM and ECCLES. For the well defined solutions studied, the calculated values agreed closely with those determined experimentally, and the two programs gave identical results provided that they used identical stability constant data.

Keywords: *Ion-selective electrodes; ion-exchange equilibrium; metal speciation; stability constants; computational methods*

Sea and river waters, soil solutions and biological fluids contain trace metals such as copper, zinc and cadmium, whose concentrations may be increased by pollution. These metals exist as different chemical species, *e.g.*, the free hydrated cation, inorganic complexes and complexes with organic macromolecules. The relative concentrations of the different species of any one metal depend on the solution temperature, pH, ionic strength and concentrations of potential inorganic and organic ligands and of major cations such as Ca^{2+} and Mg^{2+}, which form complexes with some of the available ligands. It is desirable to determine concentrations of individual metal species as well as concentrations of total metals in these solutions because the rate of uptake of the metals by plants, aquatic organisms or tissues may depend on the concentration not only of the total metal but also of individual metal species.[1–4]

Experimental methods used for the determination of metal species (speciation) include electroanalytical techniques, ion-exchange equilibration and chromatographic methods; these have been reviewed[5,6] and also form part of the subject matter of several recent publications.[7–9] Many computer programs[10] have also been written in which complete descriptions of chemical species present within a system are calculated from the total concentrations of all the metals and ligands, the stability constants for the formation of metal complexes and mass balance equations. The predictions made by a number of these programs have been compared,[11] but apart from our own preliminary studies,[12] we are unaware of any comparisons of results obtained using both experimental methods and computational techniques on the same solutions. This paper compares results from two independent experimental methods: ion-selective electrode (ISE) measurements and ion-exchange equilibration (IEE), and two computer programs, GEOCHEM and ECCLES, which are described fully later.

The solutions used were of well defined composition containing up to four trace metals and organic complexing agents. We considered it necessary to compare experimental and computing methods on these solutions before attempting comparisons on soil solutions or natural waters that contain macromolecular organic complexing agents of ill-defined composition.

Experimental

Solutions

Stock solutions (10^{-1} M) of trace metal salts, $Ca(NO_3)_2$, KNO_3 and $NaClO_4$ and the organic complexing agents nitrilotriacetic

Table 1. Compositions of solutions (all concentrations as molarities)

Solution No.	pH range	Ligand	Concentration	KNO_3	$Ca(NO_3)_2$	$CdCl_2$	$ZnSO_4$	$CuSO_4$	$MnSO_4$	$MgSO_4$
1	3.5–5.0	NTA	4×10^{-5}	10^{-2}	—	10^{-5}	—	—	—	—
2	3.5–5.0	NTA	4×10^{-5}	—	4×10^{-3}	10^{-5}	—	—	—	—
3	3.5–5.0	NTA	4×10^{-5}	—	4×10^{-3}	5×10^{-6}	5×10^{-6}	—	—	—
4	3.5–5.0	NTA	10^{-4}	—	4×10^{-3}	—	5×10^{-6}	5×10^{-6}	5×10^{-6}	5×10^{-6}
5	4.5–6.0	SAL	2×10^{-3}	10^{-2}	—	—	—	10^{-5}	—	—
6	4.5–6.0	SAL	2×10^{-3}	—	4×10^{-3}	—	—	10^{-5}	—	—
7	4.5–6.0	GLY	4×10^{-4}	10^{-2}	—	—	—	10^{-5}	—	—
8	4.5–6.0	GLY	2×10^{-4}	—	4×10^{-3}	—	—	10^{-5}	—	—
9	4.5–6.0	GLY	2×10^{-4}	—	10^{-2}	—	—	2×10^{-5}	—	—
10	4.5–6.0	GLY	2×10^{-4}	—	4×10^{-3}	—	5×10^{-6}	5×10^{-6}	5×10^{-6}	5×10^{-6}
11	4.0–5.5	CIT	10^{-4}	10^{-2}	—	—	—	10^{-5}	—	—
12	4.0–5.5	CIT	10^{-4}	—	4×10^{-3}	—	—	10^{-5}	—	—
13	4.0–5.5	CIT	10^{-4}	—	10^{-2}	—	—	2×10^{-5}	—	—
14	4.0–5.5	CIT	10^{-4}	—	4×10^{-3}	—	5×10^{-6}	5×10^{-6}	5×10^{-6}	5×10^{-6}
15	4.0–5.5	GLY	2×10^{-4}	—	4×10^{-3}	—	5×10^{-6}	5×10^{-6}	5×10^{-6}	5×10^{-6}
		CIT	10^{-4}							

acid (NTA), salicylic acid (SAL), glycine (GLY) and citric acid (CIT) were made up from analytical-reagent grade chemicals and de-ionised water. They were diluted and mixed to give the experimental solutions listed in Table 1. Dilute $HClO_4$ or NaOH was added to give eight samples of each solution with a range of about 1.5 pH unit. The maximum pH value used was 6; higher pH values were avoided because of the possibility of formation of hydroxy or carbonate complexes. Reference solutions were also prepared; these contained the same metal concentrations but no organic ligands, and contained sufficient $NaClO_4$ to match the Na concentrations in experimental solutions. It was calculated that the ionic strengths of experimental solutions and their corresponding reference solutions were the same to within 0.001 M.

Ion-selective Electrode Studies

Copper or cadmium ISEs (Orion 94.29 and 94.48) were used in conjunction with a reference electrode (Orion 90.02) to determine copper(II) or cadmium ions in experimental and reference solutions. The electrodes were washed in the relevant solution and then immersed in 50-ml portions of the solution at 20 °C for 10 min before a reading was taken. After examination of each solution the electrodes were immersed in a standard solution and their reading taken again after 10 min; this compensated for any drift of the readings over the period of the experiments (3–4 h).

Ion-exchange Equilibrium Studies

These were carried out on solutions containing $Ca(NO_3)_2$ as a background electrolyte. Calcium-form ion-exchange resin was prepared by exchanging Amberlite CG120 Type 1 sodium-form resin with $CaCl_2$ solution in a column and drying at 40 °C. Duplicate 25-ml portions of experimental and reference solutions were shaken overnight at 20 °C with 100-mg portions of the calcium-form resin. Aliquots were taken from these equilibrium solutions and from the corresponding original solutions before resin treatment. The trace metals were determined using either flame atomic absorption spectrometry using a Shandon Southern A3400 spectrometer or inductively coupled plasma optical emission spectrometry using an ARL 34000 instrument.

Treatment of Experimental Data

General

Metal ion concentrations were used instead of activities in the following calculations because both experimental methods depended on comparisons of activities in solutions of similar ionic strengths.

All the solutions contained small concentrations of nitrate complexes of the trace metals (MNO_3^+) because of the use of calcium or potassium nitrate as background electrolytes. A value, f, was calculated for the ratio of ionic (M^{2+}) concentration to the total ($M^{2+} + MNO_3^+$) metal concentration for each trace metal M in each reference solution, using published stability constants and the extended Debye - Hückel equation; f was always >0.98.

Ion-selective Electrode Data

If $[M^{2+}]_{I1}$ and $[M^{2+}]_{I2}$ are the metal ion concentrations in an experimental solution I_1 and a reference solution I_2 (M = Cu or Cd) and if E_1 and E_2 are the measured total potentials of an ion-selective electrode - reference electrode pair in these solutions, then

$$E_1 = E_0 + c \log[M^{2+}]_{I1} \quad \ldots \quad (1)$$

and

$$E_2 = E_0 + c \log[M^{2+}]_{I2} \quad \ldots \quad (2)$$

E_0 is the constant portion of the potential due to reference and internal solutions and c is a constant whose value was found by calibrating the electrodes in pure metal nitrate solutions of constant ionic strength and was taken as 29.0 for Cu and 28.0 for Cd if E_1 and E_2 were expressed in mV.

From equations (1) and (2)

$$(E_1 - E_2)/c = \log([M^{2+}]_{I1}/[M^{2+}]_{I2}) \quad \ldots \quad (3)$$

$$[M^{2+}]_{I1}/[M^{2+}]_{I2} = \text{antilog}[(E_1 - E_2)/c] = A \ldots \quad (4)$$

If $[M_T]$ is the total concentration of M in both solutions,

$$\frac{[M^{2+}]_{I1}}{[M_T]} = \frac{[M^{2+}]_{I2}}{[M_T]} \cdot A = fA \quad \ldots \quad (5)$$

Ion-exchange Equilibrium Data

If an amount of calcium-saturated strongly acidic cation-exchange resin is equilibrated with a solution containing a large concentration of Ca^{2+} ions and a small concentration of any divalent M^{2+} ion, then calcium ions on the resin phase exchange with M^{2+} ions in solution, and at equilibrium

$$[M^{2+}]_S/[M^{2+}]_R = A_M[Ca^{2+}]_S/[Ca^{2+}]_R \quad \ldots \quad (6)$$

where [ ]$_S$ and [ ]$_R$ are concentrations in mol l^{-1} and mol kg^{-1} in solution and resin phases, respectively, and A_M is a constant. Because calcium ions are present in excess, nearly all the ions in the resin phase are still calcium ions at equilibrium so that $[Ca^{2+}]_R$ is effectively constant, and hence

$$[M^{2+}]_S/[M^{2+}]_R = B_M [Ca^{2+}]_S \quad \ldots \quad (7)$$

where B_M is another constant, in kg mol^{-1}.

The concentration of resin-phase ions $[M^{2+}]_R$ may be replaced by the concentration of metal in the initial solution $[M_T]_I$ minus the concentration of metal in the equilibrium solution $[M_T]_S$, divided by a factor depending on the ratio of mass of resin to volume of solution. This gives

$$[M^{2+}]_S/([M_T]_I - [M_T]_S) = K_M[Ca^{2+}]_S \quad \ldots \quad (8)$$

where K_M is a constant in l mol^{-1}. Further, if calcium is present in a very large excess compared with any of the trace metals, each metal can be considered to exchange independently with calcium, with its own characteristic value of K_M.

If an initial experimental solution I_1 and an initial reference solution I_2 contain equal calcium ion concentrations, then

$$\frac{[M^{2+}]_{S1}}{[M_T]_{I1} - [M_T]_{S1}} = \frac{[M^{2+}]_{S2}}{[M_T]_{I2} - [M_T]_{S2}} \quad \ldots \quad (9)$$

where S_1 and S_2 are the equilibrium solutions corresponding to I_1 and I_2, and $[M_T]$ and $[M^{2+}]$ are the total and ionic concentrations of M in any solution.

Rearranging equation (9) and dividing by $[M]_{S1}$ gives

$$\frac{[M^{2+}]_{S1}}{[M]_{S1}} = \frac{[M_T]_{I1} - [M_T]_{S1}}{[M_T]_{I2} - [M_T]_{S2}} \cdot \frac{[M^{2+}]_{S2}}{[M_T]_{S1}} \quad \ldots \quad (10)$$

and as

$$[M^{2+}]_{S2} = [M_T]_{S2} \cdot f \quad \ldots \quad (11)$$

then

$$\frac{[M^{2+}]_{S1}}{[M_T]_{S1}} = \frac{[M_T]_{I1} - [M_T]_{S1}}{[M_T]_{I2} - [M_T]_{S2}} \cdot \frac{[M_T]_{S2}}{[M_T]_{S1}} \cdot f$$

It was assumed that $[M^{2+}]_{S1}/[M_T]_{S1}$, the proportion of M occurring as the free ion in the equilibrium solution, was the same as that occurring in the initial solution. This is justified if no complexed metal species or free ligands are adsorbed by the resin phase, and this was shown to be so.

Computer Programs

Stability Constants

The individual metal complex species that were taken into account were those included in the comprehensive GEOCHEM databank. Stability constants for the formation of complexes at 20 °C between the cations H^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+} and Cd^{2+} and the anions OH^-, NO_3^-, Cl^-, SO_4^{2-}, NTA^{3-}, GLY^-, SAL^{2-} and CIT^{3-} were compiled from published sources.[13–16] The values so obtained did not always correspond to those in the GEOCHEM databank (see below). The stability constants were corrected to ionic strength 0.0 using the Davies equation[17]

$$\text{Log } \gamma = AZ^2 [\sqrt{I}/(1 + \sqrt{I})] - 0.3I \quad .. \quad (12)$$

to determine the activity coefficients of the ions and the equation[18]

$$\text{Log } \gamma = 0.1I \quad .. \quad .. \quad .. \quad (13)$$

to determine the activity coefficients of the uncharged species. In these equations γ is the activity coefficient, I the ionic strength, Z the ionic charge and $A = -0.504$ at 20 °C. The values of the stability constants [log $K_{(20)}$values] so calculated were rounded to the nearest 0.1 unit and used for both programs.

Where values of stability constants at 20 °C could not be found in the literature, values at 25 °C and $I = 0$ were calculated from experimental values using equation (12) with $A = -0.509$. These were converted into values at 20 °C and $I = 0$ using the equation[19]

$$\text{Log } K_{(20)} = \text{log } K_{(25)} - 0.0029\Delta H \quad .. \quad (14)$$

where ΔH is the enthalpy of the complexation reaction in kJ mol^{-1}.

It was found that log $K_{(20)}$ and log $K_{(25)}$ for any individual complexation reaction never differed by >0.1 unit. Consequently, when neither log $K_{(20)}$ nor ΔH values were found in the literature, log $K_{(25)}$ values were used in the databank.

GEOCHEM

GEOCHEM[20] was developed at the University of California, Riverside, CA, USA, from earlier programs and extended for use with soil solutions; details of this program and comparisons with similar programs have been published.[11] The total metal and ligand concentrations in our experimental solutions, together with the appropriate pH, were input to the program at intervals of 0.5 pH unit. The program calculated the ionic strengths of the solutions and corrected the stability constants in the databank to this ionic strength using equations (12) and (13) above; the program would use other equations to deal with more concentrated solutions. It then used a Newton - Raphson approach to solve mass balance equations, which determine the equilibrium concentrations of constituent species, and printed the results as percentages of each metal complexed with each anion (and *vice versa*).

The GEOCHEM databank contains stability constants for individual metal - ligand pairs at 25 °C and ionic strength 0. The values at 20 °C and ionic strength 0 which we used in our study did not differ appreciably from those at 25 °C except for copper, zinc, manganese and cadmium complexes of salicylic and citric acids where there were large discrepancies of log K values of >1 unit. Individual corrections to the original GEOCHEM databank at 25 °C were made in these instances.

ECCLES

The computer program ECCLES[21] was developed at the University of Cape Town, South Africa, and at UWIST, Cardiff, UK, for medical applications. In this study stability constants at 20 °C and $I = 0$ were manually corrected to the ionic strength of the solution using equations (12) and (13) before being input to the program together with the pH values, pOH values and metal and ligand concentrations. The program is based on a three-tier successive-approximation procedure to arrive at the concentrations of species in equilibrium systems. This program also printed results as percentages of each metal complexed with each anion.

Results and Discussion

Figs. 1 and 2 show the variation of metal ion concentrations with pH in two representative solutions, as determined by ISE

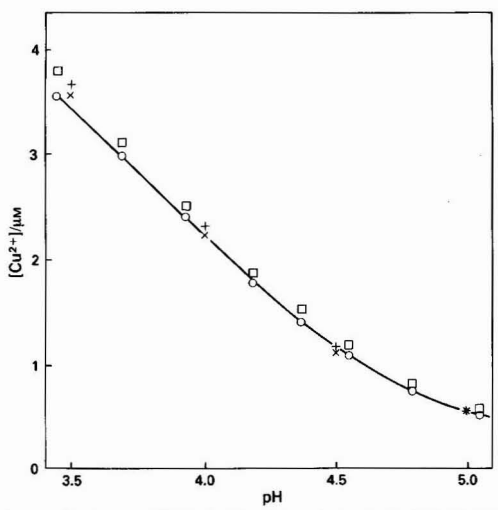

Fig. 1. Variation of $[Cd^{2+}]$ with pH in solution 3. ○, ISE; □, IEE; +, GEOCHEM; and ×, ECCLES; for clarity only the line between ISE points is shown

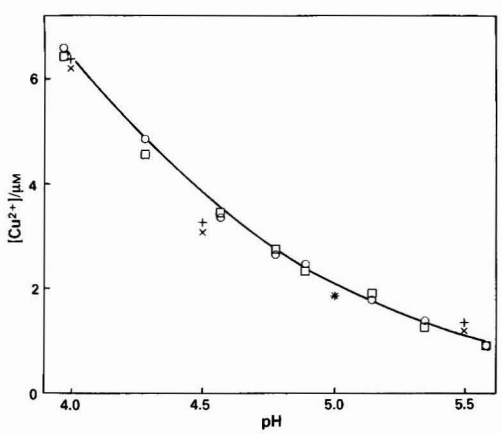

Fig. 2. Variation of $[Cu^{2+}]$ with pH in solution 12. ○, ISE; □, IEE; +, GEOCHEM; and ×, ECCLES; for clarity, only the line between ISE points is shown

Table 2. M^{2+} concentrations (μM) in solution 15

M	pH	ISE	IEE	GEOCHEM	ECCLES
Cu	4.0	2.60	2.40	3.05	2.95
	4.5	1.50	1.40	1.55	1.45
	5.0	0.90	0.90	0.85	0.80
	5.5	0.50	0.55	0.55	0.55
Zn	4.0		4.40	4.55	4.50
	4.5		4.00	3.85	3.80
	5.0		3.60	3.20	3.20
	5.5		3.10	2.95	2.90
Mn	4.0		4.90	4.90	4.90
	4.5		4.85	4.80	4.75
	5.0		4.75	4.65	4.60
	5.5		4.70	4.55	4.55
Mg	All			>4.90	>4.90

Table 3. Equations relating metal ion concentrations (μM) determined or predicted by four methods, with numbers of sample pairs (n) and correlation coefficient (r). IS, IE, GE, EC, EX, CO = concentrations given by ISE, IEE, GEOCHEM, ECCLES, experimental methods (average of ISE and IEE) and computing (average of programs)

Metal ion	Equation	n	r*
Cd^{2+}	$IS = 0.924 \times IE + 0.072$	8	0.998
Cd^{2+}	$GE = 1.029 \times EX + 0.028$	12	1.000
Cd^{2+}	$EX = 0.923 \times CO + 0.057$	12	0.994
Cu^{2+}	$IS = 0.991 \times IE - 0.050$	32	0.997
Cu^{2+}	$GE = 1.006 \times EC + 0.077$	44	1.000
Cu^{2+}	$EX = 0.937 \times CO + 0.028$	44	0.993
Zn^{2+}	$GE = 1.003 \times EC + 0.034$	12	1.000
Zn^{2+}	$EX = 1.011 \times CO + 0.014$	12	0.992

* $p < 0.001$ in all instances.

and IEE methods and as predicted at four pH values by GEOCHEM and ECCLES. Table 2 shows Cu^{2+}, Zn^{2+}, Mn^{2+} and Mg^{2+} concentrations (to the nearest 0.05 μM) in a third solution, at four pH values; the concentrations given for the experimental methods were obtained by interpolation of solution results over the pH scale as shown in Figs. 1 and 2. (Tables 7–10 in the Appendix give similar data for the other solutions.)

Table 3 shows the relationship between metal ion concentrations determined by ISE and IEE methods, predicted by GEOCHEM and ECCLES and experimentally determined and computed. Concentrations at four pH values from each solution, determined by interpolation (as in Figs. 1 and 2), were used to give these equations. Results from solutions 1, 2 and 3 were used to give equations relating to Cd^{2+} concentrations, those from solutions 5–15 were used for Cu^{2+} equations and those from solutions 3, 14 and 15 were used to generate the equations relating to Zn^{2+} concentrations. Copper and zinc existed almost entirely as complexed forms at all pH values in solution 4 so these results were not used. Zinc existed almost entirely (>98%) as Zn^{2+} in solution 10 at all pH values and manganese and magnesium existed almost entirely as Mn^{2+} and Mg^{2+} in all the solutions in which they were present.

There was very good agreement between the results of the two programs in all instances, and also between the two experimental methods for Cu; ISE results for Cd were about 8% lower than IEE results on average. The results given by the average of the two experimental methods were very slightly lower than those given by the computer programs.

The agreement between the results obtained using independent experimental methods induces confidence that these methods can be used successfully on more complex systems. However, the ISE method can only be used for copper, cadmium and lead at concentrations above 10^{-6} M and is subject to interference by, for example, iron(III) ions, high concentrations of chloride ions[22] and possibly macromolecular organic matter. The IEE method is not subject to these limitations; it can be used for any metal forming a divalent ion at any concentration, if the solution contains a relatively high concentration of a major divalent cation. The method is suitable for use with soil solutions where Ca^{2+} is usually present at concentrations $>10^{-3}$ M.

Neither experimental method depends on prior knowledge of stability constants, and stability constants cannot be measured using them unless the acid dissociation constants are known. However, the fact that both methods gave results that were in general agreement with computer predictions based on independently measured stability constants gave further confidence that the experimental methods were giving true answers. This is particularly important for metals such as zinc, which have no electrode and whose ion concentration was determined only by the IEE method.

Detailed Comparison of Programs

The ionic strengths of solutions as computed by GEOCHEM were not identical with those calculated manually for ECCLES. This was because the GEOCHEM computations allowed for complex formation, which tended to reduce the ionic strength, while the manual calculations were based on the assumption that the only contributions to the ionic strength were from the ions of the completely dissociated background electrolyte. This problem would be more serious if more concentrated solutions were being considered. Activity coefficients of individual ions, and therefore log β values of individual complex species, were not exactly the same in GEOCHEM and ECCLES solutions, although in practice they did not differ by >0.05 unit.

Table 4 gives the concentrations of some individual species and groups of complex ions in solution 3 at pH 5.0 and solution 12 at pH 4.0, as predicted by (a) GEOCHEM and (b) ECCLES, starting from the same database of stability constants at I = 0.0, and (c) ECCLES, with the stability constants adjusted to the same as those of GEOCHEM. Values in columns (a) and (b) differed because of the effect discussed above, but values in columns (a) and (c) were almost identical. Therefore, if identical corrected stability constants were used, the two methods of iteration used in the two programs gave virtually identical results.

Sensitivity of Programs to Values of Stability Constants

Table 5 shows the effect of varying individual stability constants on Cd^{2+} concentrations predicted by ECCLES for solution 3. Predicted Cd^{2+} concentrations were changed by about 5% at pH 3.5 and about 20% at pH 5.0 by a difference of 0.1 in the value used for log β of either $[CdNTA]^-$ or

Table 4. Concentrations of species (μM) in solutions 3 and 12 as predicted by (a) GEOCHEM; (b) ECCLES, using stability constants corrected manually; and (c) ECCLES, using stability constants corrected by GEOCHEM

Solution No.	pH	Species/μM	a	b	c
3	5.0	Ca^{2+}/mM	3.868	3.864	3.868
		Cd^{2+}	0.553	0.528	0.550
		Zn^{2+}	0.189	0.180	0.188
		Ca - NTA (all species)	9.84	10.33	9.88
		Cd - NTA (all species)	4.48	4.47	4.44
		Zn - NTA (all species)	4.84	4.83	4.81
		H - NTA (all species)	20.8	20.4	20.8
12	4.0	Ca^{2+}/mM	3.851	3.847	3.851
		Cu^{2+}	6.382	6.174	6.384
		Ca - CIT (all species)	25.7	26.7	25.8
		Cu - CIT (all species)	3.5	3.7	3.5
		H - CIT (all species)	70.7	69.4	70.6

Table 5. Cd^{2+} concentrations (μM) in solution 3 at four pH values as predicted by ECCLES when the stability constant (log β) of each of four complexes was changed

	Log β				$[Cd^{2+}]$ at pH			
Version	$[HNTA]^{2-}$	$[CaNTA]^{-}$	$[CdNTA]^{-}$	$[ZnNTA]^{-}$	3.5	4.0	4.5	5.0
Original	10.11	7.23	10.73	11.23	3.54	2.20	1.10	0.53
Change 1	10.21	Orig.	Orig.	Orig.	3.72	2.45	1.27	0.61
2	Orig.	7.33	Orig.	Orig.	3.55	2.22	1.13	0.57
3	Orig.	Orig.	10.83	Orig.	3.31	1.94	0.92	0.43
4	Orig.	Orig.	Orig.	11.33	3.55	2.21	1.10	0.53

Table 6. Cu^{2+} concentrations (μM) in solution 12 at four pH values as predicted by ECCLES when the stability constant (log β) of each of four complexes was changed

	Log β values				$[Cu^{2+}]$ at pH			
Version	$[CaCIT]^{-}$	$[CuCIT]^{-}$	[CuCIT(H)]	$[Cu_2CIT_2(OH)_2]^{4-}$	4.0	4.5	5.0	5.5
Original	4.23	6.64	10.13	34.53	6.17	3.14	1.86	1.29
Change 1	4.33	Orig.	Orig.	Orig.	6.21	3.30	2.11	1.54
2	Orig.	6.74	Orig.	Orig.	5.77	2.72	1.56	1.12
3	Orig.	Orig.	10.23	Orig.	6.05	3.09	1.85	1.29
4	Orig.	Orig.	Orig.	34.63	6.17	3.14	1.85	1.24

$[HNTA]^{2-}$. They were also slightly affected by changing log β for $[CaNTA]^{-}$. This happened because the large excess of calcium present in the solution reacted with a considerable proportion of the NTA and left only a small proportion available to form complexes with Cd^{2+} and Zn^{2+}. Cd^{2+} concentrations were almost unaffected by changing the value of $[ZnNTA]^{-}$.

Table 6 shows the effect of varying individual stability constants on ECCLES predictions of Cu^{2+} concentrations in solution 12. As with NTA, variation of the stability constant for $[CaCIT]^{-}$ had an effect on Cu^{2+} concentrations, particularly at high pH where more CIT^{3-} is complexed by Ca. Variation of log β values for the three principal copper citrate complexes also affected Cu^{2+} concentrations, but in different ways. Variation of log β for [CuCIT(H)] had the largest effect at the lowest pH while variation of log β for $[Cu_2CIT_2(OH)_2]^{4-}$ had its greatest effect at the highest pH; this reflected the pH dependence of the concentrations of the individual complex species.

There is disagreement in the literature over the nature of the predominant copper citrate complex at pH values between 6 and 8. If the published value of log β for the mononuclear complex $[CuCIT(OH)]^{2-}$ was used in the computer programs, very small Cu^{2+} concentrations, which disagreed totally with the experimental results, were obtained. Therefore, the dinuclear anion $[Cu_2CIT_2(OH)_2]^{4-}$ was chosen[23] in preference to $[CuCIT(OH)]^{2-}$.

If GEOCHEM, ECCLES or similar programs are to be used for predicting metal ion concentrations in complex solutions it is absolutely essential to know which species are formed and to employ accurate stability constants, not only for complexes formed by the metal itself but also for those formed by major cations in the solution. Computer predictions can be expected to give results comparable to those obtained by direct measurements only when such detailed knowledge of the composition and complex chemistry of a solution is available.

We thank Professors P. W. Linder (University of Cape Town), G. Sposito (University of California, Riverside) and D. R. Williams (UWIST, Cardiff) for discussions about the programs GEOCHEM and ECCLES.

Appendix

Additional results for the variation of $[M^{2+}]$ for various solutions are given in Tables 7–10.

Table 7. M^{2+} concentrations (μM) in solutions containing nitrilotriacetic acid (NTA)

Solution No.	Composition (M)	M	pH	ISE	IEE	GEOCHEM	ECCLES
1	$CdCl_2$ (10^{-5}), KNO_3 (10^{-2}), NTA (4×10^{-5})	Cd	3.5	6.6		7.1	6.9
			4.0	3.4		4.3	4.1
			4.5	1.5		2.0	1.9
			5.0	0.6		0.7	0.7
2	$CdCl_2$ (10^{-5}), $Ca(NO_3)_2$ (4×10^{-3}), NTA (4×10^{-5})	Cd	3.5	6.4	7.0	7.2	7.0
			4.0	4.2	4.2	4.5	4.3
			4.5	2.2	2.4	2.3	2.2
			5.0	1.2	1.1	1.1	1.0
3	$CdCl_2$ (5×10^{-6}), $ZnSO_4$ (5×10^{-6}), $Ca(NO_3)_2$ (4×10^{-3}), NTA (4×10^{-5})	Cd	3.5	3.45	3.60	3.65	3.55
			4.0	2.20	2.30	2.30	2.20
			4.5	1.15	1.30	1.15	1.10
			5.0	0.60	0.65	0.55	0.55
		Zn	3.5		2.05	2.30	2.20
			4.0		1.00	1.05	1.00
			4.5		0.40	0.45	0.40
			5.0		0.15	0.20	0.20
4	$CuSO_4$ (5×10^{-6}), $ZnSO_4$ (5×10^{-6}), $MnSO_4$ (5×10^{-6}), $MgSO_4$ (5×10^{-6}), $Ca(NO_3)_2$ (4×10^{-3}), NTA (10^{-4})	Zn	3.5		0.90	1.25	1.20
			4.0		0.20	0.45	0.40
			4.5		<0.10	0.15	0.15
			5.0		<0.10	<0.10	<0.10
		Mn	3.5		4.95	4.95	4.95
			4.0		4.90	4.95	4.90
			4.5		4.70	4.85	4.85
			5.0		4.55	4.70	4.65
		Cu	all			<0.10	<0.10
		Mg	all			>4.95	>4.95

Table 8. M^{2+} concentrations (μM) in solutions containing salicylic acid (SAL)

Solution No.	Composition (M)	M	pH	ISE	IEE	GEOCHEM	ECCLES
5	$CuSO_4$ (10^{-5}), KNO_3 (10^{-2}), SAL (2×10^{-3})	Cu	4.5	8.6		8.8	8.8
			5.0	6.9		7.1	7.0
			5.5	3.9		4.5	4.3
			6.0	1.9		2.0	2.0
6	$CuSO_4$ (10^{-5}), $Ca(NO_3)_2$ (4×10^{-3}), SAL (2×10^{-3})	Cu	4.5	8.6	8.7	8.9	8.8
			5.0	7.0	7.1	7.3	7.2
			5.5	4.3	4.4	4.6	4.5
			6.0	2.2	2.2	2.1	2.0

Table 9. M^{2+} concentrations (μM) in solutions containing glycine (GLY)

Solution No.	Composition (M)	M	pH	ISE	IEE	GEOCHEM	ECCLES
7	$CuSO_4$ (10^{-5}), KNO_3 (10^{-2}), GLY (4×10^{-4})	Cu	4.5	6.5		7.1	7.0
			5.0	4.0		4.3	4.2
			5.5	1.5		1.8	1.7
			6.0	0.4		0.5	0.5
8	$CuSO_4$ (10^{-5}), $Ca(NO_3)_2$ (4×10^{-3}), GLY (2×10^{-4})	Cu	4.5	8.0	8.0	8.3	8.2
			5.0	5.4	5.4	6.2	6.0
			5.5	2.0	2.2	3.3	3.1
			6.0	0.8	1.2	1.2	1.1
9	$CuSO_4$ (2×10^{-5}), $Ca(NO_3)_2$ (10^{-2}), GLY (2×10^{-4})	Cu	4.5	15.6	16.0	16.6	16.6
			5.0	10.6	11.4	12.8	12.6
			5.5	6.2	7.0	7.2	7.0
			6.0	2.6	3.6	2.8	2.6
10	$CuSO_4$ (5×10^{-6}), $ZnSO_4$ (5×10^{-6}), $MnSO_4$ (5×10^{-6}), $MgSO_4$ (5×10^{-6}), $Ca(NO_3)_2$ (4×10^{-3}), GLY (2×10^{-4})	Cu	4.5	4.00	3.85	4.15	4.10
			5.0	2.75	2.70	3.10	3.00
			5.5	1.35	1.50	1.65	1.55
			6.0	0.40	0.60	0.60	0.55
		Zn	All			>4.90	>4.90
		Mn	All			>4.90	>4.90
		Mg	All			>4.90	>4.90

Table 10. M^{2+} concentrations (μM) in solutions containing citric acid (CIT)

Solution No.	Composition (M)	M	pH	ISE	IEE	GEOCHEM	ECCLES
11	$CuSO_4$ (10^{-5}), KNO_3 (10^{-2}), CIT (10^{-4})	Cu	4.0	4.7		5.6	5.4
			4.5	2.0		1.8	1.6
			5.0	0.5		0.4	0.4
			5.5	0.1		0.1	0.1
12	$CuSO_4$ (10^{-5}), $Ca(NO_3)_2$ (4×10^{-3}), CIT (10^{-4})	Cu	4.0	6.4	6.1	6.4	6.2
			4.5	3.9	3.8	3.3	3.1
			5.0	2.2	2.2	1.9	1.9
			5.5	1.0	1.0	1.4	1.3
13	$CuSO_4$ (2×10^{-5}), $Ca(NO_3)_2$ (10^{-2}), CIT (10^{-4})	Cu	4.0	14.0	13.6	14.4	14.2
			4.5	9.6	9.4	9.6	9.4
			5.0	5.8	5.8	7.0	6.8
			5.5	3.0	3.2	4.8	4.6
14	$CuSO_4$ (5×10^{-6}), $ZnSO_4$ (5×10^{-6}), $MnSO_4$ (5×10^{-6}), $MgSO_4$ (5×10^{-6}), $Ca(NO_3)_2$ (4×10^{-3}), CIT (10^{-4})	Cu	4.0	3.35	3.20	3.20	3.05
			4.5	2.00	2.00	1.60	1.55
			5.0	1.20	1.20	0.95	0.95
			5.5	0.60	0.65	0.70	0.70
		Zn	4.0		4.30	4.55	4.50
			4.5		3.90	3.85	3.80
			5.0		3.45	3.25	3.20
			5.5		3.10	2.95	2.95
		Mn	4.0		4.90	4.90	4.90
			4.5		4.85	4.80	4.75
			5.0		4.75	4.65	4.65
			5.5		4.65	4.55	4.55
		Mg	All			>4.90	>4.90

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Measurement in the Non-linear Region of the Calibration Graph of the Fluoride Ion-selective Electrode by Blank Adjustment

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A method is described that permits the determination of low levels of fluoride with a fluoride ion-selective electrode, in the non-linear response region, but avoids the use of non-linear calibration. An estimate of the background response of the fluoride ion-selective electrode is obtained by multiple standard additions. Adjustment of the fluoride standard values with this background estimate results in a linear calibration graph. Fluoride levels in samples are then easily determined by linear interpolation and correction for background response, irrespective of whether this is due to fluoride contamination of reagents or hydroxide interference from buffer pH.

Keywords: *Fluoride determination; fluoride ion-selective electrode; background response; non-linear response region*

The lower limit of Nernstian response for the fluoride ion-selective electrode (ISE) has been shown to be about 10 μM of fluoride.[1] The deviation from Nernstian response of the fluoride ISE at low fluoride levels is suggested to be due to various factors, including fluoride contamination of reagents,[2-5] sensitivity of the fluoride ISE to hydroxide ion activity,[6] solubility of the LaF_3 crystal[7] and adsorption of fluoride on the LaF_3 surface.[8] Indeed, the Nernstian response has been observed to extend below 0.5 μM fluoride with the use of ultra-pure reagents,[3,5,9] with pre-equilibration of the fluoride ISE[10] and at low pH.[7,9] Midgley[2] has established that modified Nernst equations can be developed theoretically to define the response of various ISEs near the limit of detection. With respect to the fluoride ISE, it is known that the non-Nernstian response is independent of the solubility of the LaF_3 crystal.[2] Parthasarathy's group[4,8] has shown that the deviation from Nernstian behaviour near the limit of detection of the fluoride ISE is due to a "background" response. For a given fluoride ISE in a solution of a particular pH, this "background" response remains constant.[4,8]

The limit of detection of a given fluoride ISE is influenced not only by the deviation of the electrode response from linearity, but also by the precision of measurement.[2] Apart from the volumetric variability of samples, the precision of measurement with the fluoride ISE is influenced by variation in temperature,[2] proximity to equilibrium potential[11] and the process of calibration drift.[12] This drift in calibration, sometimes called the "memory effect",[13] is probably due to adsorption of fluoride on to the LaF_3 crystal surface.[8]

The direct measurement of low levels of fluoride in the presence of fluoride-chelating ions (*e.g.*, aluminium, iron, magnesium and calcium) requires the use of a total ionic strength adjustment buffer (TISAB) containing a suitable decomplexation agent.[5,14] Fluoride recovery data[14,15] and stability constant values[16] indicate that for the efficient decomplexation of fluoride ions, particularly from aluminium ions, an alkaline TISAB is required. Orion[17] recommend the use of their TISAB IV for such a purpose, but it has been considered unsuitable for the accurate measurement of low fluoride concentrations.[5,17]

The aim of this study was to establish whether a precise measurement of the "background" response of the fluoride ISE would permit the linearisation of the fluoride calibration graph in the region of non-Nernstian response. If it were possible, it would then permit easy calculation by linear interpolation, thereby avoiding the mathematical complexity of using non-linear parameters.[18] The subtractability of the "background" response should be valid for a given fluoride ISE regardless of the cause and should be limited only by the sensitivity of that electrode. To test this hypothesis, data were obtained from fluoride standards and from tap water samples with two fluoride ISEs. The "background" responses were varied by differences in the composition of the TISABs used for the measurements.

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Experimental

Apparatus

Two combination fluoride ISEs (96-09, Orion Research, Cambridge, MA, USA) were used simultaneously with a high-impedence ($>10^{12}$ Ω) digital voltmeter (TPS, Brisbane, Australia) with long-term stability and resolution of ±0.1 mV. All samples were analysed in flat-bottomed polystyrene screw-capped vials (Disposable Products, Adelaide, Australia), which fitted loosely into copper wells (25 mm deep) water-jacketed at 23.0 ± 0.1 °C. Samples were stirred magnetically at constant speed with PTFE-coated followers, and with the fluoride electrodes immersed at a constant depth (5 mm). The air temperature was maintained at 23 ± 1 °C by the laboratory air conditioner. All calibrated flasks and storage bottles were made of polypropylene or polymethylpentene (Nalgene, Rochester, NY, USA). Aliquots of TISAB solutions were dispensed with a high-precision volumetric apparatus (Digital Diluter, Hamilton, Reno, NV, USA). Aliquots of tap-water samples and standard solutions were taken using a manual pipette with disposable polyethylene tips (Pipetman P5000, Gilson, Villiers Le Bel, France). Statistical analyses were performed using an interactive statistical computer program (GLIM, Royal Statistical Society, London, UK) stored on a PDP 1090 computer system (Digital Equipment Corp., Maynard, MA, USA).

Reagents

Fluoride standard solutions. Prepared from sodium fluoride (Suprapur, Merck, Darmstadt, FRG), dried to constant mass and dissolved in de-ionised, distilled water (≤10 nM fluoride). Stock solutions (20.0, 40.1, 60.0, 80.0, 100.0 and 120.0 mM fluoride) contained precisely known (relative standard deviation <0.1%) concentrations of fluoride. Their concentrations were determined gravimetrically, *i.e.*, weighed amounts of fluoride contained in solution volumes determined from

solution mass and specific density (calculated in triplicate). Working standard solutions (2.0, 4.0, 6.1, 8.2, 10.2 and 12.2 μM and 0.20, 0.40, 0.60, 0.81, 1.01 and 1.21 mM fluoride) were weighed dilutions of each stock standard solution.

TISAB solutions. Three solutions were prepared: A and B as formulated by Orion[17] for their TISAB II and TISAB IV, respectively, and C, as for A but with an additional 4.8 ± 0.2 μmol l^{-1} of fluoride. When diluted 1 + 1 with de-ionised, distilled water, the pH values of these buffer solutions were 5.10 ± 0.02 for A and C and 8.34 ± 0.02 for B (mean ± 95% confidence limits).

Procedure

Tap-water samples from local supplies were collected in dry, leak-proof, screw-capped plastic bottles, previously washed and thoroughly rinsed with de-ionised, distilled water. The water samples were roughly sorted for fluoride content according to their measured response potential at 3 min with a fluoride ISE, pre-equilibrated with approximately 1 μM fluoride (no buffer), by comparison with similar measurements on neat fluoride standards (2, 10 and 12 μM). For all accurate fluoride analyses, samples were diluted with an equal volume of a TISAB solution. Provided that the fluoride ISE response was Nernstian in the range of the calibration graph, sample concentrations were determined using linear regression analysis. For determinations where the calibration graph deviated markedly from Nernstian behaviour, an estimate of background fluoride concentration was determined. This "background" value was added to the nominal fluoride standard values. Linear regression analysis of these modified standards permitted the prediction of sample + background values, which after subtraction of the background fluoride concentration gave the sample fluoride concentration.

Analysis for background fluoride was carried out by multiple standard addition[19] using a sample of TISAB diluted with an equal volume of de-ionised, distilled water. Using a slope estimate from the linear range of the calibration graph, the background fluoride concentration was calculated using weighted linear regression analysis of the Gran transformation data. Suitable volumes and fluoride concentrations[20] and an adequate number of titration points ($n = 12$)[19] were chosen to minimise the error in individual measurements of background fluoride.

To maintain a high precision of measurement with the fluoride ISEs, the following procedure was adopted. (a) All samples were pre-heated and their temperatures kept constant to within ±0.1 °C during analysis. (b) Data for electrode response were taken when the rate of potential change was 0.1 mV min^{-1} (three successive identical 30-s readings and ≤0.2 mV change over 2 min). (c) Memory effects were minimised by (i) bracketing all samples within a calibration graph of narrow range (*ca.* 1 decade), (ii) measuring all samples and standards in a sequence corresponding to an increase in activity, *i.e.*, all equilibrium potentials were approached in the same direction, from high to low potential, (iii) pre-equilibrating electrodes in stirred de-ionised, distilled water by multiple changes of water until a constant potential was achieved (corresponding to about 10 nM fluoride by extrapolation of Nernstian response), (iv) running preliminary standards to condition the electrode to lower fluoride activities than those of the calibration graph and (v) using only calibration graph data measured at the same time as the sample data for the determination of fluoride concentrations. All fluoride analyses were measured in triplicate on both fluoride ISEs.

All tap-water samples and fluoride standard solutions were analysed by inductively coupled plasma (ICP) emission spectrometry for aluminium, calcium, magnesium and iron concentrations.

Results

As no significant difference was detected between values of fluoride concentration measured with each fluoride ISE for any given sample using a particular TISAB, the data were pooled ($n = 6$). The three TISAB solutions A, B and C, diluted 1 + 1 with de-ionised, distilled water, contained estimated background fluoride concentrations of 70.4 ± 5.6 nM, 2.41 ± 0.17 μM and 2.38 ± 0.10 μM (mean ± 95% confidence limits), respectively.

In Fig. 1, the pooled data from triplicate calibrations, for *final* nominal fluoride concentrations (*i.e.*, 1–6 μM fluoride), for each electrode using each of the TISAB solutions, are shown as open symbols. Correspondingly, closed symbols are used for the same data for TISAB solutions B and C, using fluoride concentrations increased by the respective background fluoride values. These calibration graphs using nominal values (open symbols, Fig. 1) for fluoride standards with TISAB solutions B and C deviate markedly from Nernstian behaviour. However, when the same response data are re-plotted with fluoride values corrected for the background response (closed symbols, Fig. 1), the calibration graphs are

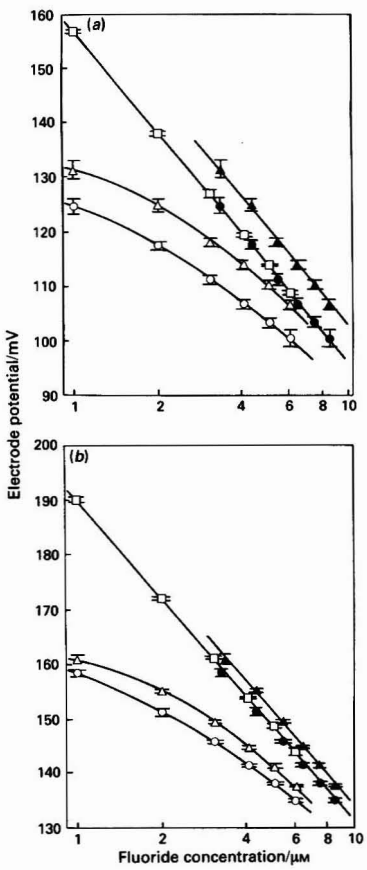

Fig. 1. Calibration graphs for fluoride ion-selective electrodes (*a*)1 and (*b*) 2, using any one of three TISAB solutions, A, B and C, in the range 1–10 μM fluoride. Nominal fluoride standard values (final concentration): A (□), B (△) and C (○). Nominal standard fluoride values plus background fluoride: B (▲), C (●). Data points are means ± 2 S.E. ($n = 3$). Analysis of covariance regression lines:

(*a*) A (□), C(●): $y = -61.2(\pm 0.5)x - 210(\pm 3)$
B (▲): $y = -61.2(\pm 0.5)x - 203(\pm 3)$
(*b*) A (□), C (●): $y = -58.7(\pm 0.3)x - 163(\pm 2)$
B (▲): $y = -58.7(\pm 0.3)x - 160(\pm 2)$

linear with slopes not significantly different from those measured for TISAB A. The slope of the linear calibration graphs for each fluoride ISE calculated by analysis of covariance were -61.2 ± 0.5 mV per decade for electrode 1 [Fig. 1(*a*)] and -58.7 ± 0.3 mV per decade for electrode 2 [Fig. 1(*b*)].

Table 1 gives the levels of fluoride measured in tap-water samples and in solutions containing known amounts of fluoride. All fluoride concentration values correspond to data from six assays (three with each electrode), each involving a separate linear calibration graph. There were no significant differences between the measurements of the fluoride concentration in the synthetic fluoride water samples using any of the three TISAB solutions. With the tap-water samples, although comparable values of fluoride concentration were obtained using all three of the TISAB solutions, significantly higher fluoride concentrations were obtained using TISAB B.

Table 2 summarises the levels of calcium, magnesium, aluminium and iron for each sample as measured by ICP emission spectrometry. The relative standard deviations (%) were determined from three 5-s integrations of the emission readings.

Discussion

The validity of the blank adjustment method relies on the ability to measure accurately the background response. The multiple standard additions method is considered to yield the most precise results,[21] but in practice the results for the background response were significantly variable (the 95% confidence limits being $\leqslant \pm 8\%$ of the mean). In fact, within the limits of the sensitivity of measurement with the fluoride ISE, this variability had no significant effect on the accuracy and precision of the values of fluoride concentration calculated by blank adjustment. Preliminary calculations, using the limiting values for background fluoride for TISAB B, *viz.*, 2.2 and 2.6 μM, to calculate the fluoride concentration in triplicate measurements on the tap-water samples, both gave results that were not significantly different. Consequently, all calculated values of fluoride concentration were obtained using "error-free" values of background fluoride; for TISAB B and C this was 2.4 μM fluoride.

As predicted by Midgley[2] and as observed by Parthasarathy *et al.*,[4] the response of the fluoride ISE in the non-Nernstian region was due to the nominal fluoride ion activity plus a background response. This is demonstrated in Fig. 1, where

Table 1. Fluoride levels in tap-water samples and test standard solutions determined with different TISAB solutions

Sample	Fluoride concentration/μM* TISAB A†	TISAB B‡	TISAB C§
Tap water—			
Maleny (Landsborough Shire)	2.04 ± 0.02	2.18 ± 0.04¶	2.05 ± 0.06**
Sandgate (Brisbane City)	2.54 ± 0.12	2.81 ± 0.13¶	2.50 ± 0.03**
Auchenflower (Brisbane City)	4.46 ± 0.06	4.82 ± 0.14¶	4.57 ± 0.24**
Springwood (Logan Shire)	4.76 ± 0.07	4.98 ± 0.05¶	4.87 ± 0.14**
Toowoomba (Toowoomba City)	5.27 ± 0.08	5.67 ± 0.03‖	5.29 ± 0.23**
Dayborough (Pine Rivers Shire)	5.32 ± 0.09	5.69 ± 0.20‖	5.32 ± 0.23**
Narangba (Caboolture Shire)	5.58 ± 0.13	5.87 ± 0.11‖	5.62 ± 0.05**
Fluoride standard—			
3.0 μM	3.01 ± 0.06	2.95 ± 0.10**	3.06 ± 0.08**
5.0 μM	5.04 ± 0.04	5.01 ± 0.06**	5.07 ± 0.08**
7.1 μM	7.09 ± 0.04	7.07 ± 0.12**	7.15 ± 0.09**

* All data given as means ± 95% confidence limits ($n = 6$).
† TISAB A (reference TISAB) composition as for Orion Research TISAB II.[17]
‡ TISAB B composition as for Orion Research TISAB IV.[17]
§ TISAB C composition as for TISAB A with added 4.8 ± 0.2 μmol l^{-1} of fluoride.
¶ Significantly different ($P < 0.01$) from corresponding data for TISAB A (Student's t test).
‖ Significantly different ($P < 0.001$) from corresponding data for TISAB A (Student's t test).
** Not significantly different from corresponding data for TISAB A (Student's t test).

Table 2. Levels of calcium, magnesium, aluminium and iron in tap-water samples and test standard fluoride solutions determined by ICP emission spectrometry

Sample	Calcium Concentration/μM	RSD,* %	Magnesium Concentration/μM	RSD,* %	Aluminium Concentration/μM	RSD,* %	Iron Concentration/μM	RSD,* %
Tap water—								
Maleny	73.3	0.37	65.8	0.18	0.00	0.51	4.30	0.50
Sandgate	534	0.42	307	0.29	12.2	0.28	2.69	0.00
Auchenflower	437	0.19	399	0.21	18.5	0.46	3.94	0.00
Springwood	522	0.41	407	0.48	14.1	0.72	35.8	0.51
Toowoomba	837	0.10	1208	0.15	41.9	0.48	14.5	0.00
Dayborough	347	0.62	352	0.52	9.64	1.01	3.22	2.62
Narangba	406	1.24	618	0.91	20.8	0.27	5.90	0.46
Fluoride standard—								
3.0 μM	0.00	0.00	0.00	0.00	0.37†	0.29	0.00	0.00
5.0 μM	0.00	2.94	0.00	0.42	0.37†	0.29	0.00	0.67
7.1 μM	0.00	0.00	0.00	0.46	0.00	0.30	0.00	1.33

* Relative standard deviation.
† Below the lower limit of detection for aluminium (0.74 μM).

linearisation of the calibration graphs for TISAB solutions B and C was achieved by adjustment of the fluoride standard values (at the final concentration) with values for background fluoride. The slopes for the linearised calibration graphs approximated the theoretical Nernstian slope for the fluoride ISE. The significantly higher slope (-61.2 ± 0.5 mV per decade) observed for fluoride ISE 1 [Fig. 1(*a*)] is explicable by its age and in the use of data measured under non-equilibrium conditions (*i.e.*, a potential change of 0.1 mV min^{-1}) (the fluoride ISE 1 was over 7 years old, and at low fluoride concentrations gave significantly higher response times and a lower precision of response than the relatively new 18-month-old fluoride ISE 2). Despite this apparent difference, use of either fluoride ISE with any sample resulted in values of fluoride concentration that were not significantly different. With millimolar fluoride standard solutions (0.20–1.21 mM), both fluoride ISEs had calibration graphs of nominal fluoride values with regression coefficients not significantly different from those of fluoride ISE 2 in Fig. 1(*b*).

The corrected data for each electrode with TISAB B (closed triangles, Fig. 1) show a small but significant constant elevation in potential compared with those for TISAB A and C (*viz.*, 7 mV, electrode 1; 3 mV, electrode 2). This decrease in fluoride activity with each fluoride ISE is due to the increase in the ionic strength of TISAB B (almost two-fold) compared with TISAB A and C. As the calculation of fluoride concentration with blank adjustment involves measurement in a solution of constant ionic strength, the activity coefficient is constant and the measured activity is directly proportional to the concentration, whether this activity is fluoride or background.

The comparable values of fluoride concentration for all samples in Table 1, using all three TISAB solutions, suggests that the use of blank adjustment is a valid approach to the problem of the measurement of low fluoride concentrations in the non-linear region of the calibration graph. Irrespective of the TISAB used, the values obtained for fluoride concentration (Table 1) all had confidence limits $\leq \pm 5\%$ of the mean. With TISAB solutions B and C these estimates of variance are possibly underestimated, as no allowance was made for the variance in the value of the background response. The tap-water data (Table 1) compare well with previously reported fluoride values for these particular water supplies and others in the same geographical region.[22]

The significantly higher fluoride concentrations in the tap-water samples determined using TISAB B (Table 1) probably result from the superior decomplexing ability of this TISAB compared with TISAB A.[17] Analysis of all the samples for levels of calcium, magnesium, aluminium and iron (Table 2) showed that whereas none of these ions is detectable in the synthetic fluoride solutions, the tap-water samples contained significant amounts of these fluoride-complexing ions. This contrast is just as marked in the analysis for fluoride (Table 1), where no significant difference was observed in the values of fluoride concentration for the fluoride standard solutions, using either TISAB A or B.

It is reasonable to conclude that the background fluoride response attributable to contamination (TISAB C) and hydroxide ions (TISAB B) is an additive response. Whether other factors (*e.g.*, dissolution of the LaF_3 crystal[7] and adsorption of fluoride on the ISE sensor[8]) suggested to be contributory to the background response also give an additive response cannot be determined from these data. There is evidence that the low-level non-Nernstian response of the fluoride ISE is unrelated to the dissolution of the LaF_3 crystal.[2,4,8] Adsorption of fluoride by the fluoride ISE appears to be mainly implicated in the time-dependent memory effect[13] or calibration-drift process.[12] Studies using ultra-pure reagents[3,5,9] and low pH[7,9] support the view that the predominant source of background response of the fluoride ISE is fluoride contamination and hydroxide ions.

Other workers have tried to avoid the problem of background fluoride in the determination of low levels of fluoride by changing the composition of the TISAB solution[3,5] or by increasing the fluoride content of samples either by preconcentration[23] or by spiking with a known amount of fluoride.[24] With the method reported here, these steps are not necessary.

Statistical analyses were performed with the advice of Mr. A. Barnes, Statistical Advisor, Faculty of Science, University of Queensland. Analyses by ICP emission spectrometry were carried out with the help of Mr. G. Kervin, Department of Agriculture, University of Queensland.

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Simple Rapid Method for the Determination of Dissolved Oxygen by Potentiometric Stripping Analysis

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A method is described for the determination of dissolved oxygen in natural water by potentiometric stripping analysis. Oxygen standards are prepared by the reaction of permanganate with peroxide and are used to oxidise the Cd - Hg formed at the glassy carbon electrode from a standard solution of Cd^{2+}. The quantitative reduction in the stripping time is used to measure the oxygen concentration in the water. The method is simple and rapid, with a higher correlation coefficient between added and measured oxygen levels than is obtainable using the Winkler procedure. The use of the method in acidic solutions is also an advantage for the measurement of dissolved oxygen in natural and waste waters.

Keywords: *Dissolved oxygen determination; potentiometric stripping analysis; natural water analysis; waste water analysis*

A simple and rapid method for the determination of dissolved oxygen in natural water is essential. It is a vital parameter for the assessment of water quality and studies of the biodegradation of detergents in natural water, aquatic ecology, sewage, blood and other fluids. The projected use of surface waters for domestic consumption in Jordan necessitates regular monitoring of parameters for water purity. This study thus assumes a particular local significance.

The usual methods are still based on several modifications of the old Winkler method[1-4] and the Fisher procedure.[5] These are time consuming, tedious and subject to several errors. Those based on redox titrations[6] and measurement of liberated oxygen[4] are not practical to use, especially in the field. Other methods reported include the use of gas chromatography[7] and mass spectrometry,[8] which, besides being of academic interest, require careful calibration. Electroanalytical techniques of measurement require calibration and, in general, give a result proportional to the oxygen concentration.

Polarography[9] and the use of the oxygen-selective electrode[10] fall into this category and necessarily require an electrolytic process. The latter, although suitable for use in the field, suffers from "fouling" with use, contamination of the sensing element, electrode sensitivity variation with salt concentration, permeability of the membrane electrode system to a variety of other gases, and the need for reliable standards and frequent calibration.[11] The former suffers from electrode "poisoning" with waste waters and other interferences.[12]

Garoff[13] has reported a polarographic procedure for the preparation of oxygen standards, for use in the calibration of the oxygen-selective electrode. However, the reported procedure requires the presence of current carrying electrolytes. It is also reported that the electrolyte concentration greatly affects the results obtained and, if no electrolytes are added, the error in fresh water is found to be as great as 50%.

Much work has been reported on the use of potentiometric stripping analysis for the determination of trace metals in a variety of matrices.[14,15] Recent studies have also been reported on the use of intermetallic compound formation to overcome overlapping peaks.[16] However, no study has attempted to use the stripping sequence of amalgamated metal by dissolved oxygen as a technique to determine dissolved oxygen itself. The use of reductants and an interfering analyte stripping sequence, to determine the reductants and interferents themselves, is an interesting study currently being pursued in our laboratories.

Unlike other electroanalytical techniques for dissolved oxygen, potentiometric stripping analysis does not require current carrying electrolytes as in polarography, because the stripping process is a non-electrolytic step, as the pre-plated metals are oxidised back into solution by dissolved oxygen. The method is operational below pH 2, at which pH the polarographic procedure is not suitable. At a pH of around 4, as required for a polarographic measurement, the rate of reaction of $KMnO_4$ with H_2O_2 to liberate O_2 is reduced, thus enabling organics, which may be present in a water sample, to compete with H_2O_2 for the consumption of $KMnO_4$. However, at pH values below 2, as required in the potentiometric stripping analysis, the rate of reaction of $KMnO_4$ with H_2O_2 is sufficiently fast to preclude the use of $KMnO_4$ by the organics present in the water sample.

The adaptation of the potentiometric stripping analysis method for the determination of dissolved oxygen in natural water is thus dependent on adjustment with HCl to a pH of around 2, optimisation of the potentiometric electrolysis step and purging of the solution in the sealed cell with a continuous stream of oxygen-free nitrogen, prior to recording the quantitative reduction of the stripping time of a standard solution of Cd^{2+} by dissolved oxygen.

Experimental

Apparatus and Reagents

Measurements were made in polyethylene disposable cups used as cells, containing 20 ml of solution. The working electrode was a mercury film deposited on a highly polished carbon disc, mounted on a PTFE rod. A saturated calomel reference electrode and a platinum wire counter electrode completed the three-electrode system. The voltage - stripping time data were recorded on thermal recording paper with a Tecator Striptec System, equipped with a strip-chart recorder.

All solutions were prepared from de-ionised water, Aristar mineral acids, standard solutions for atomic absorption spectrometry (AAS) and analytical-reagent grade chemicals. Permanganate and peroxide solutions were freshly prepared and standardised before use. A 100 mg l^{-1} mercury plating solution was prepared by dissolving mercury(II) nitrate in 0.1 M HCl. The reagent stock solutions were stored in polyethylene bottles and diluted as required for standard additions purposes.

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Procedure

The glassy carbon electrode was polished with the Striptec polishing kit, first with ethanol and then with acetone, by wetting the polishing cloth with these solvents in turn and moving the electrode with smooth, circular movements over the polishing cloth. The polished electrode was finally washed with de-ionised water.

The working electrode was "pre-plated" by immersing the electrode system in the mercury(II) solution and applying a potential of −0.5 V at the working electrode for 60 s, while stirring the solution. This was followed by applying gradually increasing potentials of −0.6, −0.7 and −0.8 V, applying each potential for 60 s. A potential of −0.9 V was finally applied four times successively, for 60 s each time. This procedure gave a stable mercury film on the glassy carbon electrode. The electrode was then washed with de-ionised water. With this conditioning, the electrode was ready for use in the measurement cycles.

Measurements were performed by applying −0.95 V as the deposition potential for 2 min, for a concentration level of 250 p.p.b. of Cd^{2+} acidified with HCl to pH 1–2. The stirring was stopped and the voltage - stripping time graph recorded on the Striptec system. (A suitable background was selected for the stripping time of at least 14 s, for the de-aerated Cd^{2+} solution, with a continuous atmosphere of oxygen-free nitrogen, over the solution in the cell throughout the measurements.) The cell was adequately sealed from atmospheric oxygen. The mercury film was removed at the end of the experiment by polishing the electrode with the polishing cloth as described previously.

Oxygen Standard Solutions

The quantitative reaction of hydrogen peroxide with permanganate was used to liberate an equivalent amount of oxygen in acid solution.

$$5H_2O_2 + 2MnO_4^- + 6H^+ \rightleftharpoons 5O_2 + 2Mn^{2+} + 8H_2O$$

Oxygen standards were prepared in the following way: 6–125 µl each of 0.0200 M $KMnO_4$ and 0.0504 M H_2O_2 (an excess of H_2O_2) were added to de-aerated 20-ml aliquots of the water sample acidified with concentrated HCl to pH 1–2. The Winkler method and the potentiometric stripping analysis method were compared, the latter using a 250 p.p.b. reducible Cd^{2+} solution.

Winkler Method

A 25–100 µl volume of each of 0.0504 M H_2O_2 and 0.0200 M $KMnO_4$ solutions were added to 20-ml portions of sample acidified with HCl to pH 1–2 in Quickfit flasks, under a steady stream of pure nitrogen. When the reaction to generate O_2 was complete, determined from the decolourisation of $KMnO_4$, 2 ml of 1.07 M $MnSO_4$ solution and 1 ml of 0.9 M KI solution in 33% NaOH solution were added. A 1-ml volume of concentrated H_2SO_4 was then added to dissolve the $Mn(OH)_3$ precipitated. The liberated iodine was titrated with 0.025 M $Na_2S_2O_3$.

Potentiometric Stripping Analysis

A 5-µg mass of 1000 p.p.m. AAS standard Cd^{2+} solution was added to 20-ml portions of the water sample, acidified with HCl to pH 1–2, containing the three-electrode system, whose glassy carbon electrode had previously been pre-plated as described. The solution was contained in the cell in an atmosphere of oxygen-free nitrogen. A potential of −0.95 V was applied for 2 min and the stripping time recorded after equilibrium had been reached (by recording the second stripping time). The above procedure was repeated in successive cells containing the same reagents but with 6–125 µl of 0.0504 M H_2O_2 and 0.0200 M $KMnO_4$ solutions added in turn to the successive cells, so as to provide quantitative oxygen standards from 0.5 to 10 mg l^{-1} of O_2. The recorded stripping times were used to calculate the concentration of oxygen in real samples, from the calibration graph of oxygen standards.

Results

This paper is based on an interesting physico-chemical principle, namely the measurement of dissolved oxygen by measuring the time needed for re-oxidation of a constant amount of amalgamated cadmium. According to potentiometric stripping theory, the time for re-oxidation (the stripping time) is constant, *i.e.*, $t_s C_{O_2}$ = a constant. If t_s is the stripping time, C_{O_2} the concentration of O_2, dn/dt the flux of O_2 to the electrode, N_{Cd} the number of equivalents of Cd and K the proportionality constant, then $dn/dt = K C_{O_2}$ and by Fick's law $N_{Cd} = t_s dn/dt$ for the total stripping of Cd from the electrode. Hence, $N_{Cd} = t_s K C_{O_2}$, or $t_s C_{O_2} = N_{Cd}/K$ = a constant, for constant Cd deposition conditions. Plotting $1/t_s$ *versus* C_{O_2} yields a straight line (Fig. 1). The sensitivity of the oxygen measurement between 0.5 and 10 mg l^{-1} of O_2 was found to be 0.0375 s^{-1} (p.p.m.)$^{-1}$.

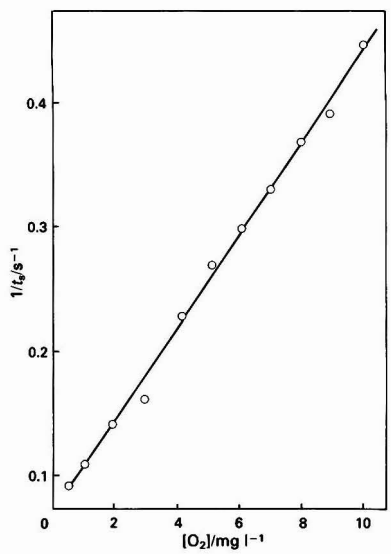

Fig. 1. Variation of the inverse of the stripping time ($1/t_s$) in s^{-1} with concentration of oxygen (mg l^{-1} O_2) added to an acidic solution (pH 2) containing 250 p.p.b. of Cd^{2+}. Electrolysis time, 120 s; and applied deposition potential, −0.95 V

Table 1. Measurement of dissolved oxygen using the Winkler method

Oxygen added/ mg l^{-1}	Oxygen found/ mg l^{-1}
0.8	1.1
1.4	1.6
2.2	2.0
2.8	2.8
3.3	3.6
4.1	4.0
5.5	5.0
8.3	8.3

Table 2. Measurement of oxygen added to a natural water sample using the Winkler method

Oxygen added/ mg l^{-1}	Oxygen found/ mg l^{-1}
1.7	1.5
2.1	1.9
2.8	2.7
3.5	3.3
4.2	3.9
5.2	5.6
5.6	5.2

Table 3. Measurement of oxygen added to a natural water sample by potentiometric stripping analysis

Oxygen added/ mg l^{-1}	Oxygen found/ mg l^{-1}
1.6	1.7
2.0	1.9
3.2	2.9
4.8	4.6
5.6	5.9
6.0	6.4
7.2	7.6
8.0	7.9

The standard additions measurements were carried out in separate cells in the potentiometric stripping procedure, as the added oxygen is consumed with each addition (Tables 1, 2 and 3).

A higher correlation coefficient of 0.9960 was obtained between added O_2 and measured O_2 for real samples as opposed to the Winkler method where the correlation coefficient was 0.9910. The mean deviation in both methods was within experimental error and usually under 0.1 mg l^{-1}.

Precision and Accuracy

The method was evaluated with respect to precision and accuracy. The use of samples of de-ionised water, with added oxygen standards, gave a high correlation coefficient (0.9960) between the values for oxygen as found and added. An equally high correlation coefficient (0.9978) was found when these experiments were repeated with a natural water sample.

At lower oxygen levels of less than 5 mg l^{-1} a relative standard deviation of 1.31% and at higher levels a value of 3.08% were obtained.

Discussion

Measurements using the potentiometric stripping method were carried out with Zn^{2+} and Cd^{2+} as the reducible ions. Although the results were comparable, Cd^{2+} was chosen as the preferred reducible ion for oxidation of the resulting Cd - Hg by oxygen standards, as it possessed a greater solubility in mercury: 10.0 atom-% of Cd^{2+} in mercury, compared with 5.83 atom-% for Zn^{2+}.[17] Also, the allowed level of Cd^{2+} in "natural" water is 6 p.p.b., which is much lower than that quoted for Zn^{2+} (5 p.p.m.).[18] HCl was used in preference to H_2SO_4 as the mineral acid for the preparation of oxygen standards, as there is a possibility of adsorption of HSO_4^- on to the platinum electrode. Such adsorption is induced by the electrodeposition of metal ions such as Cu^{2+} on to platinum electrodes.[19] The kinetics of reaction of Cl^- and H_2O_2 with $KMnO_4$ show that the latter is several hundred times faster, thus precluding any interference from HCl in the generation of oxygen by the reaction of H_2O_2 and $KMnO_4$.

Other species, *e.g.*, Fe(III) and Cr(VI), which can function as oxidising agents in pH 1–2 solution will act as chemical stripping agents, but their concentration in natural waters is very low and hence they do not interfere in the determination of dissolved O_2.

The potentiometric stripping procedure is simple and rapid compared with the Winkler method. Unlike the polarographic procedure, no electrolyte solution is required for the stripping analysis. The electrolyte concentration greatly affects the results when measuring the oxygen content by the latter method.

We have shown that the use of lower concentrations of Cd^{2+} (as low as 50 p.p.b.) allows the measurement of oxygen levels lower than 0.4 mg l^{-1}. The method is capable of distinguishing between samples whose concentrations of dissolved oxygen differ by as little as 0.1 mg l^{-1}.

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SHORT PAPERS

Anaerobic Fluorescence Titration of Dithionite Solutions

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A dithionite stock solution can readily be assayed by pressurising the storage tube and allowing a small portion of the solution to enter a syringe containing an oxygen-free solution of flavin mononucleotide (FMN) of known volume and concentration. The disappearance of the FMN fluorescence is the end-point of the titration. The change in mass of the storage tube is used to calculate the dithionite molarity.

Keywords: *Dithionite titration; flavin mononucleotide indicator; anaerobic titration; fluorescence*

Dithionite ($Na_2S_2O_4$), with its standard reduction potential of 0.48 at pH 7, may be used to exclude oxygen rigorously or to act as a reductant in various types of experiments. For instance, microorganisms can be grown anaerobically in culture tubes sealed with butyl rubber stoppers and manipulated with syringes.[1,2] For certain experiments the tubes must be kept highly reduced with dithionite.[3] Dithionite stock solutions can be conveniently prepared in the same type of butyl rubber-sealed culture tubes. It is necessary, however, to assay such dithionite stock solutions before use, because of uncertainties concerning the purity of commercial dithionite, decomposition of the solutions at room temperature and the rapid decomposition caused by random amounts of oxygen inadvertently introduced during preparation or sampling or through the increasing number of punctures in the butyl rubber seal resulting from use of the stock solution. Although many methods of assay are available (see especially the definitive work of Dixon[4]), there is a need for a quick and simple assay method requiring only a minimum of specialised equipment. This paper describes a syringe method for measuring the concentration of dithionite solutions involving titration into deoxygenated flavin mononucleotide (FMN) solutions using ultraviolet light to detect the end-point. FMN was chosen because it is commonly available in biochemistry and microbiology laboratories, its fluorescence permits the relatively sensitive assay of dilute dithionite solutions and the fluorescence end-point is sharp.

Experimental and Results

All work with FMN was carried out in subdued light. A 4×10^{-4} M solution of FMN (Sigma, labelled 95–97%) was deoxygenated by boiling and cooling under nitrogen (purified by passage over copper beads at 350 °C) and 10 ml were dispensed into each dry nitrogen-flushed 16 × 125 mm screw-capped culture tube using a dry glass - PTFE syringe (Hamilton Gas-Tight) that had also been flushed with nitrogen. The tubes were sealed with butyl rubber stoppers and screw-caps with holes allowing access by syringe (Bellco). The tubes were pressurised by the injection of 10 ml of nitrogen and were stored in the dark under refrigeration. Solutions of dithionite were prepared similarly by boiling a 1% *m/V* sodium hydrogen carbonate solution under nitrogen, cooling, then dispensing 10 ml each into tubes containing 0.01 g each of sodium dithionite. The tubes were sealed and pressurised as above and stored in a refrigerator. For maximum sterility, all tubes, closures, syringes and solutions were autoclaved before use but the dithionite was used directly from the reagent bottle.

A 10-ml glass - PTFE syringe containing a small oval PTFE-covered magnetic stirring bar and fitted with a No. 26 needle was weighed, then gassed with nitrogen. The needle was inserted into a tube of deoxygenated FMN, which was held upside down, allowing the FMN solution to be forced into the syringe. The solution was de-gassed by rapid back-and-forth movements of the plunger, then withdrawn with a final volume of about 5 ml and re-weighed. The filled syringe was attached to a holder designed to restrain the plunger and allow its incremental movement. Commercial micrometer syringe titration assemblies may be used, or the syringe may be clamped to a suitable brass block and the plunger restrained either with a micrometer (as in this work) or a simple finely threaded bolt. The syringe assembly was placed horizontally on a magnetic stirring apparatus under an ultraviolet light (Mineralight, long-wave) in a dimly lit room. A dithionite stock tube was weighed and rapidly attached to the needle of the syringe, with the bottom end of the tube slightly elevated so that none of the pressurising gas would escape into the syringe. The micrometer was turned, thus allowing the dithionite solution to enter the syringe, the pressure in the tube being sufficient to move the plunger of the syringe and keep it snug against the micrometer. The disappearance of the fluorescence marked the end-point. The dithionite tube was removed and re-weighed. The molarity of the dithionite solution was calculated from the decrease in mass of the dithionite tube and the mass and molarity of the FMN solution. A small correction was applied to account for the dead volume in the syringe needle. The dead volume was measured by comparing the result of a simple acid - base titration, using the same syringe assembly, with that obtained with a normal titration. The stirring rate and the speed of the titration were kept constant, as faster stirring and slower titration reduced the effective dead volume.

In a typical titration, 4.981 g of 4.00×10^{-4} M FMN solution required 0.419 g of dithionite stock solution. Subtracting the dead volume of 0.068 g gave 0.351 g of dithionite solution used. As the reaction involved is[5]

$$S_2O_4^{2-} + \underset{\text{(fluorescent)}}{\text{FMN}} + 2H_2O \rightarrow \underset{\text{(non-fluorescent)}}{\text{FMNH}_2} + 2HSO_3^-$$

and as the densities of the solutions were essentially 1.00 g ml^{-1}, the dithionite concentration was 5.65×10^{-3} M, and therefore the stock solution (0.01 g of sodium dithionite in 10 ml; expected to be 5.73×10^{-3} M) had, essentially, not decomposed.

The end-point was sharp with complete loss of fluorescence unless the FMN had not been stored in the dark and had consequently undergone some photodecomposition. In that event there would be residual fluorescence. Repeated titrations showed an average deviation of about ±3%. The precision was limited mainly by the escape of small amounts of the solutions during the attachment to and removal from the syringe.

The method always tends to underestimate the molarity of the dithionite solution, as air leaking into the FMN tube or into the titration syringe, losses of dithionite while inserting or

removing the needle of the titration syringe and overshooting of the end-point would all lead to a lower calculated concentration. The purity of the FMN used is of little importance as commercial samples of FMN, although they may contain only as little as 70% of FMN itself, contain hydrolysis products and isomers of FMN as impurities.[6] Such impurities have essentially the same relative molecular mass and the same fluorescence properties as FMN and therefore do not affect the outcome of the titrations.

Once the anaerobic tubes have been prepared, each titration takes less than 10 min. Therefore, this method of titrating dithionite solutions is quick, cheap, reasonably accurate and can be accomplished with simple equipment.

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Spectrophotometric and Fluorimetric Determination of Clonidine Hydrochloride

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Two methods are described for the determination of clonidine hydrochloride: a simple and rapid spectrophotometric method based on the reaction of this drug with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to form a coloured product with maximum absorption at 455 nm; and a very sensitive fluorimetric method based on the reaction of clonidine hydrochloride with 1-dimethylaminonaphthalene-5-sulphonyl chloride (dansyl chloride) to give a highly fluorescent derivative. The relative standard deviation for both methods was less than 2%. The proposed methods have been applied to the determination of clonidine in pure and in tablet form.

Keywords: *Clonidine determination; 2,3-dichloro-5,6-dicyano-p-benzoquinone; 1-dimethylaminonaphthalene-5-sulphonyl chloride (dansyl chloride); spectrophotometry; fluorimetry*

2-(2,6-Dichloroanilino)-2-imidazoline hydrochloride (clonidine hydrochloride) is a highly effective antihypertensive drug activating α-adrenergic receptors in the central nervous system.[1] The main problem in the determination of clonidine is the precise, specific and easy measurement of very low concentrations of the drug in dosage forms. In addition, clonidine only possesses a very low absorption in the ultraviolet region with $A_{1cm}^{1\%} = 14.0$.[2] This weak absorption means that a conventional ultraviolet spectrophotometric assay of clonidine is susceptible to interference from excipients.

The literature presents few methods for the determination of clonidine in biological fluids, these being radioimmunoassay[1] or gas chromatography.[3-6] However, these methods require either derivatisation of the compound or a selective detector and elaborate multi-step extraction procedures. Therefore, it was felt useful to develop spectrophotometric and fluorimetric methods for its determination in pharmaceutical preparations.

2,3-Dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) has been reported[7] to form condensation products with imidazoline derivatives. The reaction has been described as an $n \rightarrow \pi$ charge-transfer complex formation. Being an imidazoline derivative, clonidine reacts as an n-electron donor with DDQ as a π-acceptor to give a coloured product with a maximum absorption at 455 nm, which is the principle of the spectrophotometric method.

It was found that primary and secondary amines, imidazoles and phenols[8,9] react quantitatively with 1-dimethylaminonaphthalene-5-sulphonyl chloride (dansyl chloride) under suitable conditions to produce the corresponding sulphonamides or phenolic esters. Dansyl derivatives exhibit an intense yellow fluorescence and can be detected in very small amounts.[8] Based on this principle, a fluorimetric method has been developed for the determination of clonidine based on the reaction of clonidine with dansyl chloride to form a highly fluorescent product.

Experimental

Apparatus

A Shimadzu Model UV-120-02 UV - visible spectrophotometer with 1-cm quartz cuvettes and a Perkin-Elmer Model 650-10S fluorescence spectrophotometer were used. The temperature of the fluorimetric cell compartment was controlled by circulation of water at room temperature.

Reagents

All reagents and solvents were of analytical-reagent grade. Whatman No. 1 qualitative-grade filter-papers were used.

DDQ solution, 2 mg ml^{-1} *in acetonitrile*. Prepared freshly.

Dansyl chloride solution, 1 mg ml^{-1} *in acetone*. This was kept in a refrigerator.

Catapres tablets. Obtained from Boehringer, Ingelheim, FRG, and labelled to contain 150 μg of clonidine hydrochloride per tablet.

Spectrophotometric Method

Reference drug solutions

Transfer an accurately weighed amount of clonidine hydrochloride equivalent to 50 mg of the base into a separating funnel. Dissolve in about 20 ml of distilled water, make alkaline with a few drops of 10% *m/V* sodium hydroxide solution and extract with five successive 10-ml portions of chloroform. Pass the chloroform extracts sequentially over anhydrous sodium sulphate and collect the combined chloroform extracts in a 50-ml calibrated flask. Dilute to volume with chloroform.

Sample preparation

Extract an accurately weighed amount of the tablet powder, equivalent to about 2 mg of clonidine base, with 20 ml of distilled water by shaking for 20 min. Filter into a separating funnel. Then proceed as described under *Reference drug solutions* starting from "make alkaline with . . ."

Procedure

Transfer different portions of reference or sample solution (in the concentration range 0.1–0.4 mg) into 10-ml calibrated flasks. Heat on a boiling water-bath to remove the solvent and dissolve the residue in 4 ml of acetonitrile. Add 1 ml of DDQ reagent and dilute to 10 ml with acetonitrile. Measure the absorbance at 455 nm against a blank.

Fluorimetric Method

Reference drug solutions

Prepare a solution containing 50 mg of clonidine hydrochloride in a mixture of 10 ml of acetone and 40 ml of 0.5 M sodium carbonate solution. Transfer a 10-ml portion of this solution into a 50-ml calibrated flask and make up to the mark using the same solvent mixture.

Table 1. Determination of clonidine hydrochloride using the proposed and official methods

Clonidine HCl sample	Recovery, %* Spectrophotometric method	Fluorimetric method	Official method[10]
Powder	99.4 ± 1.0	100.2 ± 0.86	—
Tablets (Catapres)	99.8 ± 1.5	99.6 ± 1.0	99.2 ± 1.7

* Average of five determinations ± standard deviation.

Sample preparation

Extract an amount of the powdered tablets equivalent to about 2 mg of clonidine hydrochloride with two 10-ml portions of ethanol. Filter the combined extracts into a 25-ml calibrated flask. Wash the filter with 5 ml of ethanol and evaporate the combined ethanolic extract to dryness on a boiling water-bath. Cool, dissolve the residue and dilute to volume with a mixture of 5 ml of acetone and 20 ml of 0.5 M sodium carbonate solution.

Procedure

Transfer different portions of sample or reference solution in the concentration range 0.02–0.1 mg into 25-ml calibrated flasks and add 0.7 ml of dansyl chloride and 1 ml of acetone. Allow the mixture to stand at room temperature for 20 min. Make up to volume with 4-methylpentan-2-one. Measure the fluorescence intensity after 10 min at 445 nm using an excitation wavelength of 345 nm. Protect the solutions from light throughout the procedure. Correct the observed fluorescence by subtracting the fluorescence intensity, measured using the same procedure for a reagent blank.

Results and Discussion

Clonidine reacts with DDQ to form an intense red compound with a maximum absorption at 455 nm. Dansyl chloride reacts with clonidine to form a highly fluorescent derivative with a maximum fluorescence at 445 nm using an excitation wavelength at 345 nm. In both methods, the reaction time, the concentration of the reagents, the selectivity of the solvents and the final dilution were optimised so as to produce the best sensitivity and stability, a minimum blank reading and adherence to Beer's law.

In the spectrophotometric method, the best solvent was established after studying various solvents. It was found that non-polar solvents were unsuitable, in contrast to polar solvents such as acetonitrile, which were considered to be better as they produced a higher yield of DDQ radicals. The red coloration produced under the conditions described was found to be stable for at least 1 h and obeyed Beer's law over a concentration range of 0.01–0.04 mg ml^{-1}. The apparent molar absorptivity, ε, was found to be 5276.7 l mol^{-1} cm^{-1}.

In the fluorimetric method, the dansylation of clonidine was performed in acetone and 0.5 M sodium carbonate solution at about pH 10, which is the optimum pH for dansylation.[8] 4-Methylpentan-2-one was used to dilute the dansylated product as it produced the highest fluorescence intensity.[8]

Under the described experimental conditions, the linear equations for the relationship between absorbance (A) and concentration (C) over the concentration range 0.01–0.04 mg ml^{-1} was as follows:

$$A_{455\ nm}^{1\ cm} = -0.00319 + 22.9C$$

and that for the relationship between fluorescence (F) and concentration was

$$F_{445\ nm} = 0.27 + 7.6C$$

where C is in mg ml^{-1}. The correlation coefficient was 0.9993 for both methods.

Five separate determinations were carried out using different concentrations by the two methods to test the reproducibility; the relative standard deviations (coefficient of variation) were found to be less than 2%. The proposed methods were applied to the determination of clonidine hydrochloride in tablet formulations. The results obtained are shown in Table 1. For the purpose of comparison, the proposed methods were compared with the official method.[10] These results are also shown in Table 1.

The proposed methods offer distinct advantages in rapidity, simplicity and sensitivity and can be easily used for the routine determination of clonidine hydrochloride in its tablet formulations.

At the normal instrumental gain setting used, the spectrofluorimetric method was about 10 times more sensitive than the spectrophotometric method. Accordingly, it can be recommended for the determination of clonidine hydrochloride in biological fluids after applying a suitable separation technique and using a higher instrumental gain setting.

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Mononitration of Toluene and Quantitative Determination of the Three Isomeric Products by Reversed-phase Liquid Chromatography

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Three isomeric nitrotoluenes, obtained from a laboratory-scale synthesis, were separated and quantitatively determined by reversed-phase liquid chromatography. The proportions of *ortho*, *meta* and *para* isomeric products were found to be 58.5, 4.5 and 37.0%, by mass, respectively.

Keywords: *Nitrotoluene determination; toluene mononitration; reversed-phase liquid chromatography*

Reversed-phase liquid chromatography (RPLC) is recognised as a versatile and rapid separation technique. Although the mobile phase in RPLC is limited to a few solvents and their mixtures, its scope is broadened by the use of numerous chemically bonded phases. In this study, a column packed with a relatively new 3 μm particle size octadecylsilyl (ODS) bonded phase was employed to separate and quantitatively determine the products from the nitration of toluene.

Mononitration of toluene yields *o*-, *m*- and *p*-nitrotoluene in different amounts. Although the relative amounts of isomeric products depends on the reaction variables such as nitrating agent, temperature and reaction time, typical yields of *o*-, *m*- and *p*-nitrotoluene are 60 ± 5%, 3 ± 1% and 37 ± 5%, respectively.[1,2]

This work illustrates two important aspects: firstly it provides a direct analytical method to demonstrate the directionality of electrophilic substitution; and secondly, it applies a popular and simple chromatographic method to the identification and quantitative determination of the reaction products.

Experimental

Nitration of Toluene

The method for the nitration of toluene was adapted from that of Jones and Russell.[3] Their procedure was modified to obtain reaction products on a laboratory scale.

The nitration of toluene was carried out in a 1-l, three-necked round-bottomed flask fitted with a reflux condenser and a dropping funnel reaching near the bottom. The mononitration acid mixture was prepared by the addition of 100 ml of concentrated sulphuric acid and 15 ml of concentrated nitric acid to 50 ml of water contained in the round-bottomed flask. A 20-ml volume of toluene was next introduced dropwise from the dropping funnel over a period of 20 min into the flask. The contents of the flask were stirred continually with a magnetic bar placed inside it and the reaction was continued for an additional 70 min after the addition of all the toluene.

The reaction mixture was then poured into about 200 ml of ice-cooled water in a separating funnel and to this about 200 ml of diethyl ether were added. The contents were shaken until the lighter organic layer was separated; the aqueous layer was discarded. The diethyl ether layer was washed with water until it was free of acid (tested with litmus paper). Finally, the diethyl ether was evaporated by leaving the ethereal solution of the product in a fume cupboard overnight.

Caution—The concentrated acids should be handled carefully and the addition of diethyl ether should be carried out in a fume cupboard.

Chromatographic Procedure

The chromatographic system consisted of a Waters Model 510 solvent delivery system, a Rheodyne Model 7125 sample injection valve and a Waters Model 481 variable wavelength detector. The detector outputs at 254 nm were monitored with a Waters 730 Data Module. A 3.0-cm 3-μm ODS column, commercially available from Perkin-Elmer, was used. The solvents were spectrophotometric grade water and methanol. The mobile phases were prepared by transferring, separately, appropriate volumes of water and methanol into a container. An amount of eluent equivalent to about 20 column volumes was allowed to flow through the column before any injection was made. The injection volume of the standard and unknown solutions of the isomeric nitrotoluenes was 2–3 μl.

Results and Discussion

Representative chromatograms for *o*-, *m*- and *p*-nitrotoluenes are shown in Fig. 1(*a*) and (*b*) with 40 + 60 and 65 + 35 *V/V* water - methanol mixtures as the mobile phases, respectively. The chromatograms of the products formed after the nitration are shown in Fig. 2(*a*) and (*b*) for total reaction times of 1.5 and 2.5 h, respectively. In Fig. 2, peaks 3, 4 and 5 have been identified as *o*-, *p*- and *m*-nitrotoluene, respectively, peak 6 as unreacted toluene and peaks 1 and 2 have not been identified. (The larger sizes of the peaks with longer reaction times indicate that peaks 1 and 2 are for higher nitration products.)

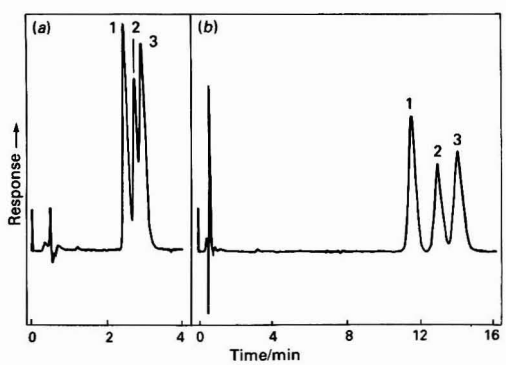

Fig. 1. Chromatograms of a standard solution of isomeric nitrotoluenes. Detector, 254 nm UV, 0.01 a.u.f.s. Peaks: 1, *o*-nitrotoluene; 2, *p*-nitrotoluene; and 3, *m*-nitrotoluene. Amounts of *o*-, *p*-, and *m*-isomers are in the ratio 36.6 : 31.3 : 32.1 *m/m*, respectively. Mobile phases: (*a*) 40 + 60 *V/V* water - methanol and eluent flow-rate, 0.7 ml min^{-1}; (*b*) 65 + 35 *V/V* water - methanol and eluent flow-rate, 1.0 ml min^{-1}

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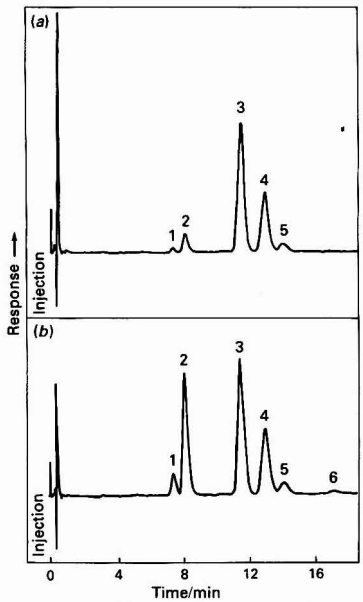

Fig. 2. Chromatograms of the nitration products of toluene with 65 + 35 *V/V* water - methanol as the mobile phase and an eluent flow-rate of 1.0 ml min^{-1}. Detector, 254 nm UV, 0.01 a.u.f.s. Peaks: 1 and 2, unknowns; 3, *o*-nitrotoluene; 4, *p*-nitrotoluene; 5, *m*-nitrotoluene; and 6, toluene. Total reaction times for nitration are (*a*) 1.5 h and (*b*) 2.5 h.

Determination of the Ratio of Isomers in the Mononitration Products

Let the concentration of any isomer, i, injected as a dilute solution of the reaction product be $W_{i(p)}$ and that in any standard solution (where the amount of the isomer is known) be $W_{i(s)}$. The detector response, in terms of the peak area A_i, with a spectrophotometric detector is directly proportional to the concentration of isomer in both instances provided that the concentration is in the linear range of the detector. With a full-loop injection of both product and standard solution, the following equation is obtained:

$$W_{i(p)} = \frac{W_{i(s)} \cdot A_{i(p)}}{A_{i(s)}} \quad .. \quad .. \quad .. \quad .. \quad (1)$$

If $\Sigma W_{i(p)} = 1$, the relative amount of *ortho*-, *meta*- and *para*-isomers produced during the nitration of toluene can be easily found.

To avoid the use of an external standard, the fractional area of the peak, defined by $a_i = A_i/\Sigma A_i$, together with the corresponding values for W_i (*e.g.*, $w_i = W_i/\Sigma W_i$) in the standard solution are recommended for use in the calculations. This procedure will essentially produce the same ratio of the isomeric products. This also allows the introduction of the incomplete loop injection or further dilution of the standard solution to take advantage of the higher detector sensitivity.

Using the latter procedure, the ratios of the isomeric products formed after mononitration were calculated from at least five replicate measurements. The ratios of the isomeric products are given in Table 1. These values are in excellent agreement with the literature values.[2]

The following equations were used to calculate $w_{i(p)}$ of an isomeric product, p, and the estimated standard deviation (e.s.d.) in $w_{i(p)}$:

$$w_{i(p)} = \frac{w_{i(s)} \cdot a_{i(p)}}{a_{i(s)}} \quad .. \quad .. \quad .. \quad .. \quad (2)$$

Table 1. Amount of isomeric products (% *m/m*) obtained after the nitration of toluene

	Total reaction time/h	
Isomer	1.5	2.5
o-Nitrotoluene	58.5 ± 0.9	56.6 ± 0.6
m-Nitrotoluene	4.5 ± 0.3	5.1 ± 0.4
p-Nitrotoluene	37.0 ± 0.8	38.4 ± 0.5

$$\text{E.s.d.} = w_{i(p)}\sqrt{\left(\frac{\sigma_{a_{i(p)}}}{a_{i(p)}}\right)^2 + \left(\frac{\sigma_{a_{i(s)}}}{a_{i(s)}}\right)^2} \quad .. \quad .. \quad .. \quad (3)$$

where, σ represents the standard deviation in the corresponding parameter given as a subscript.

To prepare a standard solution of three isomeric nitrotoluenes, about 100 μl of each was transferred and weighed accurately into a 25-ml calibrated flask, which was then filled with methanol. This solution was diluted to about 2% of its original concentration by additional methanol prior to injection. A dilute solution containing the reaction products was prepared and the major peaks in the chromatograms for standard and product samples were observed to have approximately the same height.

The base line separation for three isomeric nitrotoluenes was achieved in a reasonable time, with the RPLC system containing large amounts of water in the aqueous - organic mobile phase and an efficient column with a small particle size packing. A 15 cm long 6-μm ODS column was found to be inadequate for this study. The peaks of the isomers were resolved with 65 + 35 *V/V* water - methanol as the mobile phase, but with the disadvantage of exceedingly large retention times. Again, columns packed with a 5-μm cyclodextrin bonded phase (25 cm long) and two with acetylated cyclodextrin bonded phase (10 and 25 cm long) failed to resolve all the isomers with mobile phases containing 65% *V/V* or higher water compositions. The peaks for these compounds were not resolved in the normal-phase liquid chromatographic study by Kiselev *et al.*[4]

In RPLC, the difference in the extent of retention of the three isomers would result from the competitive interactions of these solutes with both the octadecylsilane bonded stationary phase and the relatively polar mobile phase. Dipole moments of *o*-, *m*- and *p*-nitrotoluenes, for example, in benzene are 3.78, 4.23 and 4.47 D,[5] respectively. These values may reflect a measure of the strength of the interaction of these isomers with the polar mobile phase. The modes of interaction and orientation of the aromatic ring with the stationary phase and the steric hindrance, due to the presence of the $-NO_2$ group in the interaction of the CH_3 group with the stationary phase are also expected to play a dominant role.

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Comments on Theoretical Considerations on the Measurement of Nebuliser Suction

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Comments by Gustavsson on a previous paper by the authors are critically discussed. It is demonstrated that the authors' initial conclusions are valid and not significantly modified by the changes suggested by Gustavsson.

Keywords: *Suction; nebuliser; pressure fall; Bernoulli*

Recently Gustavsson[1] commented on the equations that were used[2] in an appraisal of three methods for the measurement of nebuliser suction. He suggested that some of the components of the pressure-drop equations were in error and that only static pressure had been considered. He went on to infer that our conclusions were, therefore, probably invalid, without offering alternative equations.

When numerical values are substituted into the pressure-drop calculations, it becomes clear that the main pressure drops encountered are those along the lengths of aspiration and nebuliser tubing and not those due to sudden expansion or contraction. As discussed later, the results in Fig. 8 of reference 2 show that the pressure-drop calculations must be approximately correct. The expansion/contraction components are only a very small fraction of the total pressure fall. Therefore, there would need to be a really gross error, *e.g.*, *ca.* one order of magnitude or more, in these components for our conclusions to be invalidated.

Gustavsson's main criticism was that equations (3) and (4) in reference 2 were applicable only for turbulent flow, whereas the flow encountered in our experiments was said to be generally laminar. Perry and Chilton[3] gave the following equation for frictional loss for a sudden enlargement with turbulent flow:

$$F = \frac{V_1^2}{2g}(1 - A_1/A_2)^2 \quad .. \quad .. \quad .. \quad (1)$$

where V_1 is the velocity in the smaller diameter tubing, and A_1 and A_2 are the cross-sectional internal areas of the smaller and larger tubing, respectively. This is the same as equation (3) in our earlier paper,[2] but differs from Gustavsson's equation (6) in that $(1 - A_1/A_2)$ is raised to the power 2. We do not know the source of Gustavsson's equation (6). Perry and Chilton[3] suggested that for laminar flow the result obtained should probably be doubled, giving some insight into the approximate and empirical nature of the equation. Even when this advice is followed, the pressure-drop component is still a very small part of the total pressure loss. Equation (7) in reference 2, for example, becomes

$$\text{Suction change (cmHg)} = 1.057Q + 0.0031Q^2 \quad .. \quad (2)$$

instead of

$$\text{Suction change (cmHg)} = 1.057Q + 0.0030Q^2 \quad .. \quad (3)$$

No equation is given in reference 3 for laminar flow for the pressure drop due to a sudden contraction. We assume that equation (4) in our original paper[2] provides a reasonable estimate of this component. An experiment was performed in which methylene blue solution was pumped through a narrow-bore tube immediately after water so that the laminar flow pattern could be clearly seen. When a sudden expansion or contraction point was reached, the flow clearly became turbulent over a short distance beyond the connector. We therefore believe that equations (3) and (4)[2] provide an estimate of these pressure drops, which is adequate in the context in which they are used, especially bearing in mind the natural variability of nebulisers.

Gustavsson[1] correctly pointed out that pressure losses due to changes in kinetic energy should be considered, and not just the pressure losses due to friction and height. This component has now been calculated using the Bernoulli equation for each of the nebulisers studied. For the Perkin-Elmer nebuliser the loss is $6.27 \times 10^{-5}Q^2$, and the corrected equation finally becomes:

$$\text{Suction change (cmHg)} = 1.057Q + 0.00316Q^2 \quad .. \quad (4)$$

The change is insignificant; we have re-calculated all the results and re-plotted all the graphs from reference 2, and found that even in the worst instance the changes are virtually imperceptible.

Therefore, the conclusion drawn in reference 2 still holds, in that suction decreases, sometimes substantially, at high aspiration rates. Gustavsson has not presented theoretical or empirical evidence that supports his contrary viewpoint. On the other hand, Fig. 8 in our earlier work[2] offers strong experimental evidence for the validity of our conclusions. For the larger diameter tubing (*i.e.*, at higher aspiration rates), when the correction equations are applied, the suction does not reach the same values as when the dry method of measuring nebuliser suction has been used. If narrow-bore aspiration tubing is used with the Baird and Perkin-Elmer nebulisers and the corrections are applied, the suction values found are very close to those obtained without any solution flowing, suggesting that the equations applied must be reasonably reliable. The same lengths and diameters of tubing were used for all three nebulisers, but for the EEL nebuliser the corrections clearly showed the large decrease in suction at higher aspiration rates, and this effect *must*, therefore, be a genuine one. Again, we wish to stress that nebulisers stop working at higher pressures and high aspiration rates because of a decrease in suction.

Gustavsson's comments concerning the third method for the measurement of suction presumably arose because he did not realise the relative lack of importance of the components that he suggested were in error.

Conclusion

It must be assumed from the above discussion that, although the equations used were not as precise as they might have been, they were certainly sufficiently accurate to be applicable for the purpose for which they were developed. Moreover, since this initial work,[2] we have applied our results both to explain the apparently low sample temperature dependence of aspiration rate[4] and also to compensate for changes in aspiration rate.[5] Neither of these developments could have been successful if our initial conclusions were invalid.

We are grateful to Mr. Jim Plunkett for his helpful comments, and to the SERC for financial support for this work.

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COMMUNICATION

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

Manuscripts are usually examined by one referee and inclusion of a Communication is at the Editor's discretion.

Simple Method for Minimising the Effect of Chloride on the Chemical Oxygen Demand Test Without the Use of Mercury Salts

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Keywords: *Chemical oxygen demand; mercury free method; chloride suppression; chromium(III) addition*

The chemical oxygen demand (COD) test is widely used to determine the organic content of wastewaters, including trade effluents.[1-3] The main problem with the standard COD test is that the oxidising agent, potassium dichromate, in the strong sulphuric acid matrix used oxidises chloride ion to chlorine:

$$Cr_2O_7^{2-} + 6\,Cl^- + 14\,H^+ \rightarrow 3\,Cl_2 + 2\,Cr^{3+} + 7H_2O$$

Samples containing high chloride concentrations (especially those with low COD values) can be subject to a positive bias.

The current UK Department of the Environment/National Water Council Standing Committee of Analysts standard method[2] uses mercury(II) sulphate, which reacts with chloride ion to form undissociated mercury(II) chloride. Even with this addition of mercury(II) sulphate there is still a significant positive bias at chloride ion concentrations over 1000 μg ml^{-1}. This bias is greater for low sample COD values. An apparent COD value of 59 μg ml^{-1} was reported for a 2000 μg ml^{-1} chloride solution.[2]

Mercury is a List 1 substance[4] and it is not desirable that routine methods should utilise significant amounts of mercury. An alternative approach is to eliminate the mercury and precipitate the chloride as silver chloride. This can be achieved by increasing the concentration of the silver sulphate in the silver sulphate - sulphuric acid catalyst solution from 1 to 8% *m/V*.[5] Alternatively, silver nitrate can be added to the sample prior to the addition of potassium dichromate and sulphuric acid reagents.[6,7] However, these "mercury-free" techniques still suffer bias from chloride interference at low sample COD values.

Following a conversation with Mr. A. Lloyd of Southern Water Authority, a small amount of chromium(III) was added to the digest prior to heating. This was found to decrease significantly the response of the method to chloride. It is thought that the chromium(III) complexes any free chloride ion in the digest solution.

Experimental

Apparatus

Quickfit TJ 15/16/1 16 mm o.d. digestion tubes fitted with 14/23 cones of length 180 mm acting as air condensers were used. The tube contents were mixed using a Hook and Tucker Rotamixer. The digestions were carried out on a Grant BT4 block digester. Titrations were carried out in 100-ml white plastic disposable cups.

Reagents

Digestion solution (0.03473 M $K_2Cr_2O_7$). Dissolve 10.216 ± 0.001 g of potassium dichromate (previously dried at 105 °C for 1 h) in 500 ml of distilled water. To this solution add 167 ± 1 ml of concentrated sulphuric acid (sp.gr. 1.84) with stirring. Cool the solution and dilute to 1 l with distilled water in a calibrated flask. Mix thoroughly.

Catalyst solution (5% *m/V* Ag_2SO_4 *in* H_2SO_4). Dissolve 125 ± 0.5 g of silver sulphate (general-purpose reagent) in 2.5 l of concentrated sulphuric acid. Great care must be exercised; a shatter-proof Winchester bottle must be used. Allow to stand overnight to facilitate dissolution, and invert the bottle to mix the contents before use. To aid the initial dissolution, the Winchester bottle can be placed in an ultrasonic bath.

Iron(II) ammonium sulphate solution, 0.025 M. Dissolve 9.80 ± 0.01 g of iron(II) ammonium sulphate (analytical-reagent grade) in about 200 ml of distilled water and slowly add, with stirring, 25 ml of concentrated sulphuric acid (sp.gr. 1.84). Cool the solution and dilute to 1 l in a calibrated flask.

Standard reference solution of potassium hydrogen phthalate, (800 μg ml^{-1} *COD*). Dissolve 0.680 ± 0.001 g of potassium hydrogen phthalate (previously dried at 105 °C for 2 h) in water and dilute to 1 l in a calibrated flask.

1,10-*Phenanthroline - iron(II) complex*. Available as ferroin indicator (BDH Chemicals).

Silver nitrate solution, 50% *m/V*. Place 25 ± 0.1 g of silver nitrate in a 50-ml measuring cylinder and dilute to 50 ± 0.5 ml with water. Store in a dark bottle.

Chromium(III) potassium sulphate dodecahydrate solution (25% *m/V*). Place 25 ± 0.1 g of $CrK(SO_4)_2.12H_2O$ in a 100-ml measuring cylinder and dilute to 100 ± 1 ml with hot water. This solution is at the solubility limit of chromium(III) potassium sulphate. The solution container should be placed in a beaker of warm water (50 °C) and well shaken prior to use.

Sodium chloride (for chloride interference studies). Ash at 500 °C to ensure the absence of organic matter.

Modified Procedure

Protective clothing, gloves and full face protection must be worn when carrying out this procedure. [*Note:* In the original procedure, step 3, the addition of the chromium(III) solution, was omitted.]

1. Pipette 2.5 ± 0.02 ml of sample or diluted sample into a digestion tube. (The range of the method is 0–800 μg ml^{-1} COD.)
2. Add 0.1 ± 0.005 ml of 50% *m/V* silver nitrate solution.
3. Add 0.1 ± 0.005 ml of 25% *m/V* $CrK(SO_4)_2.12H_2O$ solution. Mix well using a suitable mixer and allow to stand for 5 min.
4. Dispense 1.5 ± 0.01 ml of digestion solution into the digestion tube.
5. Dispense 3.5 ± 0.05 ml of catalyst solution carefully down the side of the tube and not on the ground-glass joint.
6. Add two anti-bumping granules and fit an air condenser to the tube.
7. Mix the contents thoroughly for 10 s using a suitable agitator.
8. Place in a block heater which is set to 150 ± 2 °C and heat the contents for 2 h ± 5 min. Then allow to cool for at least 30 min.
9. Transfer the refluxed solution together with condenser and digestion tube rinsings into a 100-ml disposable white plastic cup. The total volume of solution and rinsings should not exceed 30 ml.
10. Add one drop of ferroin indicator and immediately titrate the residual dichromate with 0.025 M iron(II) ammonium sulphate solution.

Results

$CrK(SO_4)_2.12H_2O$ Concentration

Initially 0.2 ml of a 25% *m/V* solution was added in step 3 of the modified procedure. This significantly decreased the effect of chloride but because of the green colour of the resulting solution slightly decreased the accuracy of the end-point determination. For all further work, 0.1 ml of a 25% *m/V* solution was used and this did not significantly affect the determination of the end-point. The chromium(III) added is approximately equivalent to half of the original chromium(VI) present in the digest. With this amount of chromium(III), chloride interference was effectively suppressed up to chloride levels of approximately 3500 μg ml^{-1}.

Effect of Chloride

The effect of chloride is most significant at low COD values,[2,5,6] so most work in this preliminary study was carried out at zero COD concentration.

The effect of chloride (added as sodium chloride) is shown in Table 1. It can be seen that if the chromium(III) addition is omitted (original procedure) the chloride interference is

Table 1. Comparison of modified procedure and original procedure

		Determined mean COD value/μg ml^{-1}			
		Modified procedure with addition of 0.1 ml of 25% *m/V* $CrK(SO_4)_2.12H_2O$		Original procedure without addition of chromium(III)	
Chloride concentration/ μg ml^{-1}	COD value/ μg ml^{-1}	Lab. 1*	Lab. 2*	Lab. 1*	Lab. 2*
1000	0	<15 (5)†	<15 (5)	34 (38)	35 (10)
2000	0	<15 (6)	<15 (5)	40 (10)	43 (5)
3500	0	16 (17)	19 (5)	60 (10)	62 (5)
5000	0	34 (34)	28 (5)	67 (5)	
5000	100	132 (27)		161 (8)	
5000	400	411 (10)		437 (7)	

* Lab. 1, Charlotte Road, Yorkshire Water; Lab. 2, Gelderd Road, Yorkshire Water.
† Figures in parentheses are the number of replicates.

Table 2. Effect of chloride on a number of COD methods (no added COD)

	Determined mean COD value/μg ml^{-1}				
	Method described in this paper				
Chloride concentration/ μg ml^{-1}	Lab. 1*	Lab. 2*	SCA Method[2]	Open tube, Hg free[5]†	Closed tube, Hg free[7]
500	<15 (5)‡		10	35 (17)	15 (2)
1000	<15 (5)	<15 (5)	23	43 (23)	19 (2)
2000	<15 (6)	<15 (5)	59	51 (38)	32 (2)
5000	34 (34)	28 (5)		79 (11)	
10000	97 (11)	89 (5)		165 (11)	
10000	37 (12)§	40 (6)§			

* Lab. 1, Charlotte Road, Yorkshire Water; Lab. 2, Gelderd Road, Yorkshire Water.
† Determined by Lab. 1.
‡ Figures in parentheses are the number of replicates.
§ 0.2 ml of 25% *m/V* $CrK(SO_4)_2.12H_2O$ and 0.1 ml of 100% *m/V* $AgNO_3$.

significantly worse. It has been observed by numerous workers that the effect of chloride significantly decreases as the COD increases. This can be attributed to formation of chromium(III) and subsequent complexation of chloride ion.

Table 2 gives a comparison of the effect of various chloride concentrations with no added COD for the proposed method and previously published COD methods. It can be seen that with respect to chloride interference the new method is a significant improvement over existing methods. At chloride concentrations above 3500 μg ml^{-1} a measurable interference effect was observed, the magnitude increasing with chloride concentration. Table 2 shows that the effect at 10000 μg ml^{-1} of chloride was significantly reduced if the volume of the chromium(III) solution (step 3 in the modified procedure) was increased from 0.1 to 0.2 ml and the concentration of the silver nitrate solution was increased from 50 to 100%. This modification should be useful for samples with a low COD that contain chloride concentrations in excess of 3500 μg ml^{-1}. Initial studies indicate that the modified method described in this paper gives results for real samples (that contain low levels of chloride) comparable to those obtained using a previously published method.[5] A paper giving full performance characteristics of the method is in preparation.

The views expressed in this paper are those of the authors and are not necessarily those of Yorkshire Water.

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BOOK REVIEWS

Gas-Chromatographic Retention Indices of Toxicologically Relevant Substances on SE-30 or OV-1. Second Edition
Report II of the DFG Commission for Clinical-Toxicological Analysis, Special Issue of the TIAFT Bulletin. Pp. 175. VCH Verlagsgesellschaft. 1985. DM68. ISBN 3 527 27335 2.

This publication consists of a collection of GC retention indices (RIs) of some 1500 compounds, mostly drugs, which could be encountered by chemical toxicologists working in a range of disciplines from pesticides to hospital and coroner's work and forensic sciences, using SE-30 or OV-1 packed columns.

The data have been collected from the literature and private sources, using alkane standards, or by calculating the RIs from other (*e.g.*, drug) standards. Compounds are listed alphabetically, using a logical system of priority names (International non-proprietary, CA, "commonest practice," etc.) and the data are logically cross-indexed, under the following: Alphabetical names; Numerical RI; CAS Index No.; and CAS Index name. Literature sources are cited, and the number of individual values used in calculating the RIs are recorded. There is a short introductory section on GC conditions, standard mixtures for column evaluation and calculation of RIs.

The principal value to the reader of this publication is in the preparation of a short list of possible identities from a measured RI of an "unknown" compound and, to a lesser extent in the elimination of a named substance. Analytical chemists will use such a list with discretion as they will generally have information in addition to the compounds's GC characteristics. The disadvantage of any such system is that it cannot take account of the variety of derivatisation reactions available, and the inclusion of highly polar drugs such as non-steroidal anti-inflammatories is of limited value. Further, many laboratories will already have changed from packed to capillary systems, although for comparable stationary phases the RIs will probably be very close.

Collection and compilation of data of this kind represents the harvest of a great deal of hard, tedious and rather unrewarding work for which the reader must be grateful. This publication is an essential work of reference for any laboratory charged with the detection and identification of toxic substances. At the price of about £20–25 it must qualify as one of the year's best buys.

M. S. Moss

Annual Reports on Analytical Atomic Spectroscopy, Volume 14. Reviewing 1984
Edited by M. S. Cresser and L. Ebdon. Pp. xiv + 445. Royal Society of Chemistry. 1985. Price £65. ISBN 0 85186 677 8; ISSN 0306 1353.

It was a distinct pleasure to read the 14th volume of this series, which reviews progress in analytical atomic spectroscopy during 1984. My over-all impression is that the reviews have been very professionally presented and correctly reflect the changes in emphasis in the directions of the developmental research. But these reviews go further than that. There is an air of authority about them which clearly emerges in specific parts of the text. It is most evident in the section on electrothermal atomisation (1.4), where the reviewer has been prepared to advance her/his judgement on the relative worth of different developments. It is evident again in the specific advice given to those interested in sample introduction by volatilisation, *viz.*, to read a particular review by Kantor.

Reviews and reports such as this are meant to be authoritative and educative, and this report fulfills that requirement exceptionally well. It is great to sense the authors passing on their experience and judgement in such a professional manner.

This volume brings to an end the original format of these reports on progress in analytical atomic spectroscopy. In the future the reports will appear as an integral part of the new journal, *Journal of Analytical Atomic Spectrometry*. In the years since their inception, the *Annual Reports on Analytical Atomic Spectroscopy* have been a great boon for active spectrocopists. They have contained a wealth of information, much of which, coming from conference papers, is very contemporary even allowing for the inevitable lapse of time between the commencement of the review exercise and publication. To those of us who can attend conferences only very rarely it has been most beneficial. The inclusion of the annual reports in the new Journal can only be an important additional reason to make certain that *Journal of Analytical Atomic Spectrometry* is on your library shelf.

John Aggett

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