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INVESTIGATIONS OF REACTIONS INVOLVED IN ELECTROTHERMAL ATOMIC ABSORPTION PROCEDURES
Part 9. An Atomization System with Controlled Atmosphere and Temperature for the Determination of Volatile Elements in Complex Matrices

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(Received 18th February 1981)

SUMMARY

A controlled temperature controlled atmosphere atomization system is described. The sample is placed on a tungsten wire provided with temperature-controlled heating. After thermal pretreatment of the sample the wire is inserted into a hot quartz tube and rapid vaporization is accomplished by separate electrothermal heating. The pyrolysis products formed are mixed with a gas buffer and are passed through two equilibrium zones. The residence time of the analyte in the system is of the order of seconds so that the probability of attaining a state close to equilibrium is high for high temperatures. A third zone is placed perpendicularly to the others and constitutes the atomic absorption measuring cell. The usefulness of the system is illustrated for the determination of lead, bismuth, cadmium and zinc. The system provides unique possibilities in controlling interference effects in complex matrices. Examples are given for lead in concentrated chloride and sulphate solutions. Good agreement between the experimental results and high-temperature equilibrium calculations was obtained for a large variation in the composition of the gas phase.

Atomic absorption spectrometry (a.a.s.) with electrothermal atomization has, because of its high sensitivity, become one of the most useful techniques for ultratrace determinations. This position has been reached in spite of several drawbacks, such as difficulties in signal evaluation, limited possibilities of controlling the chemical environment in the atomizer, unwanted changes in the gas phase caused by sample constituents, and uncontrollable loss of analyte. For a proper understanding of the aim of the work presented in this paper a brief discussion of each of these items is given below, with special reference to graphite furnaces working under non-isothermal conditions.

A true representation of the atom population is not easily obtained for non-isothermal conditions. Peak height evaluation is based on the assumption that the maximum concentration of free atoms in the cell represents the total number of atoms. However, the maximum concentration will be influenced by, for example, changes in the rate of atom formation and the

temperature interval within which the atomization occurs. Such changes can be observed even if minute amounts of concomitants are present [1]. Peak area measurement is not the solution to this problem because the time integral of the absorbance is only proportional to the total number of atoms when they are measured at constant temperature [2].

Another disadvantage associated with graphite furnaces is the limited possibility of controlling the chemical environment. For example, the atmosphere of the graphite tube at high temperatures is strongly reducing and problems may be caused by the formation of metal carbides and metal monocyanides [3]. An oxidizing atmosphere at high temperature is sometimes desirable during the ashing step for decomposition of the sample, but such conditions cannot be maintained in the graphite tube without causing problems.

The third point is that the gas phase inside the graphite tube under normal conditions has a very low "buffer" capacity because the main gaseous constituent is an inert gas such as argon. This means that the sample components determine, to a large extent, the composition of the gaseous phase. The small volume of the graphite tube constitutes another problem for concentrated samples. The gas volume corresponding to a 1-mg sample at a high temperature will be several times larger than that of a normal graphite tube.

Many elements form stable, volatile compounds at temperatures well below their atomization temperature. This is a serious drawback for atomizers working under non-isothermal conditions because the analyte can be lost as molecules before a sufficiently high temperature is reached for their decomposition.

From a thermodynamic point of view, the extent of chemical interference effects is generally expected to be less at higher temperatures. An ideal atomization system for a.a.s. should therefore be able to vaporize the sample in an environment kept at a high and constant temperature with possibilities of controlling the composition of the gas atmosphere. Moreover, such a system should provide a maximum of flexibility with respect to the choice of conditions for thermal pretreatment.

The atomization system described below permits the temperature as well as atmosphere to be controlled. For simplicity, this first version of the instrument was constructed from quartz which means that the maximum allowable temperature is restricted to 1500 K. Therefore, the number of elements which theoretically can be atomized in this system is limited to about twenty, using the criterion that the vapour pressure of an element should be at least 10 mm of mercury. However, the primary aim of the construction of this system was to investigate the possibilities offered by a controlled atmosphere atomization system with respect to minimization of chemical interference effects.

EXPERIMENTAL

Instrumentation

A laboratory-assembled (from commercially-available "modules") spectrometer was used for all measurements.

Three electrically-heated home-made ovens, each made of two semi-circular Alsint profiles (Export Technische Keramik, Waldkraiburg, F.R.G.) were arranged as shown in Fig. 1. All ovens could be opened easily to provide fast access to the T-shaped quartz tube. The ovens were provided with PtRh-Pt thermoelements connected to thermoregulators (Staticor model SYC2, Coreci, France) which kept the temperature constant within 10°C.

With the Pythagoras tube (see Fig. 2) in the position shown in Fig. 1, 5 μl of the solution was pipetted onto the tungsten wire through an injection

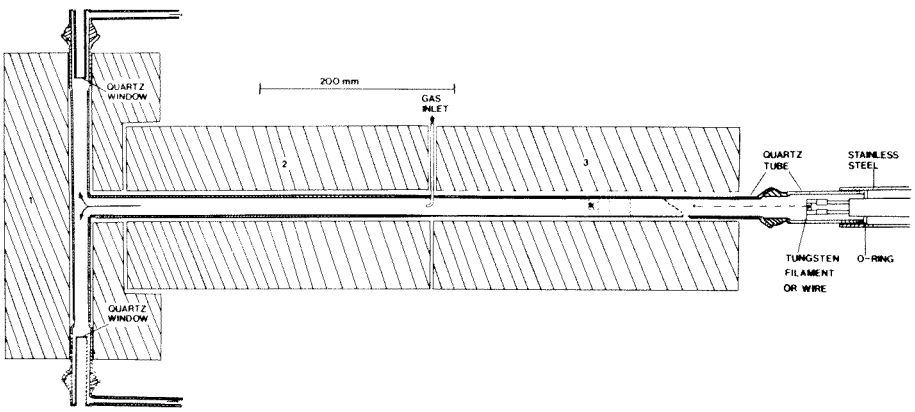

Fig. 1. Arrangement of furnaces and vaporization system (dashed lines indicate the position of the vaporizer when fully inserted). The inner diameter of the horizontal quartz tube was 19 mm, and that of the vertical quartz tube 15 mm. 1, 2, 3 are temperature-regulated furnaces (300—1500 K).

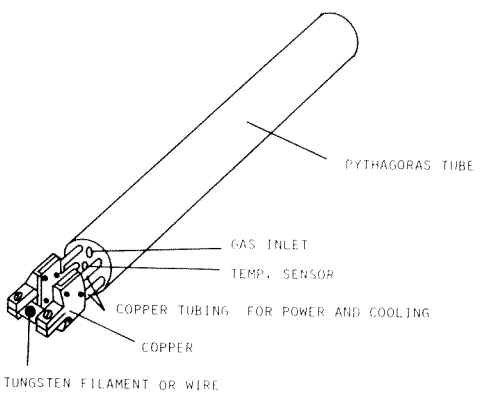

Fig. 2. Close-up of the vaporizer.

port that was located on the short quartz tube. Thermal pretreatment was accomplished by applying a low voltage to the wire. The Pythagoras tube was positioned manually in the middle of the third oven, as indicated by the dashed line in Fig. 1. This position was sensed by a micro-switch, which immediately initiated the vaporization step. In this step, the wire was heated rapidly to a preset final temperature by means of temperature-controlled heating [4]. The vaporization products formed were mixed with the auxiliary gases introduced through the inlets seen in Figs. 1 and 2. All gases were taken from high-pressure cylinders via reduction-valves, needle-valves and rotameters. The rotameters were calibrated with soap-bubble flowmeters.

After passing through ovens 3 and 2, the gas mixture entered the measuring cell, oven 1, which was mounted on the optical bench of a normal atomic absorption spectrometer. In order to prevent condensation and facilitate cleaning, the quartz windows (Suprasil I, Heraeus Schott, Hanau) were mounted as shown in Fig. 1. The spectrometer was provided with background correction and a 2-channel strip-chart recorder, on which the atomic absorption and background signals were monitored simultaneously. The height and the area of each peak were obtained digitally from a peak reader module [5]. When the absorption signal had returned to the baseline, the Pythagoras tube was positioned outside the oven. In order to prevent backflow, a check valve was connected to the gas outlets. A new sample injection could be made immediately, because of the effective water cooling of the vaporizer. The instrumental parameters are given in Table 1.

During this study the quartz ware was cleaned twice in hot aqua regia, followed by a thorough rinsing in ethanol and distilled water. Further details of the components used in this atomization system including electrical connections, can be obtained from the authors on request.

TABLE 1

Instrumental parameters[a]

	Pb	Cd	Bi	Zn
Temperature, oven 1 (°C)	800	800	800	700
Temperature, oven 2 (°C)	1130[b]	1200	1100	1000
Temperature, oven 3 (°C)	1130[b]	1180	1100	1000
Argon flow (ml min^{-1})	425	425	580	730
Hydrogen flow (ml min^{-1})	20—70	18	38	38
Carbon dioxide flow (ml min^{-1})	0—100	—	—	—
Oxygen flow (ml min^{-1})	0—45	—	—	—

[a]Filament temperature: drying at 100° C for 30 s, vaporization at 1800° C for 4 s. [b]Varied for some experiments.

High-temperature equilibrium calculations

The calculations were done as described in Part 1 [6]. The elements and compounds considered are given in Table 2. The input amounts of the elements used to calculate the theoretical signal depressions (Tables 3 and 4) were estimated by assuming the sample constituents to be evenly distributed in a gas volume corresponding to a flow time of 5 s.

TABLE 2

Species considered in the equilibrium calculations

Gaseous:	Ar, H_2, H, O_2, O, Cl_2, Cl, S_2, S, Fe, $FeCl_2$, $FeCl_3$, Pb, Pb_2, PbCl, $PbCl_2$, PbS, PbO, HCl, CO, CO_2, H_2O, H_2S, CS, CS_2, SO, SO_2, COS, C, Na, NaCl, Na_2Cl_2, NaO, Si, SiO, SiS, Si_2, $SiCl_2$, CH_2, CH_4
Condensed:	C, SiO_2, NaCl, Na_2S, Na_2SO_4, Na_2O, Pb, PbO, FeO, Na, Na_2CO_3

TABLE 3

Comparison between calculated and experimental signal depressions for 25 pmol of lead in concentrated sulphate solutions, at 1420 K

Sulphur added (nmol)	Gas mixture (ml min^{-1})			Calc. p_{O_2} (atm)	Signal depression (%)	
	H_2	Ar	CO_2		Found	Calc.
125	61	425	0	4×10^{-17}	3 ± 4	6
125	61	325	100	3×10^{-12}	8 ± 4	17
500	61	425	0	4×10^{-17}	23 ± 4	20
500	61	375	50	5×10^{-13}	38 ± 3	34
500	61	325	100	3×10^{-12}	50 ± 3	45

TABLE 4

Comparison between calculated and experimental signal depressions for 25 pmol of lead in chloride matrices
(The flows of hydrogen and argon were 45 and 425 ml min^{-1}, respectively)

Matrix (μmol)	Signal depression (%)			
	1400 K		1500 K	
	Experimental	Calc.	Experimental	Calc.
$FeCl_3$ (0.25)	3 ± 3.5	2	1 ± 3.0	1
NaCl (1.5)	0 ± 4.5	0	1 ± 3.9	0

RESULTS AND DISCUSSION

General performance of the system

Typical peak shapes for lead, cadmium, zinc and bismuth are shown in Fig. 3. The signal for each element returns to the baseline within a reasonable time, which indicates small retention effects. Such effects might arise from interactions with the tungsten vaporizer or the quartz. The variation in the residence time seen for the elements can be attributed partly to differences in their diffusion coefficients. Antimony was studied in some separate experiments, the results of which were erratic. This could be explained by the fact that this element is soluble in tungsten [7]. The sensitivities for the elements can be estimated from the data given in Fig. 3. These sensitivities are slightly poorer than for conventional graphite furnace a.a.s. (see Fig. 4 in [8]) as long as peak area values are compared. As indicated in the introductory discussion, the primary aim of the present system was not to optimize the sensitivity. However, in order to improve the sensitivity, the cross-section of the measuring cell could be made smaller. For example, a decrease from the present 15 mm to 6 mm should increase the sensitivity about 6 times. The light flux would decrease only slightly because of the smaller cross-section and hence the signal to noise ratio should not be significantly poorer.

Typical values of the relative standard deviations for lead in pure as well as complex solutions were found to be 5 and 2% for peak height and peak area measurements, respectively. The day-to-day variation for a 5-ng sample of lead was as low as 2% relative for peak area values. Calibration curves for lead and bismuth are given in Fig. 4. It should be observed that the dynamic range for lead is as large as from 0 to 8 A × s.

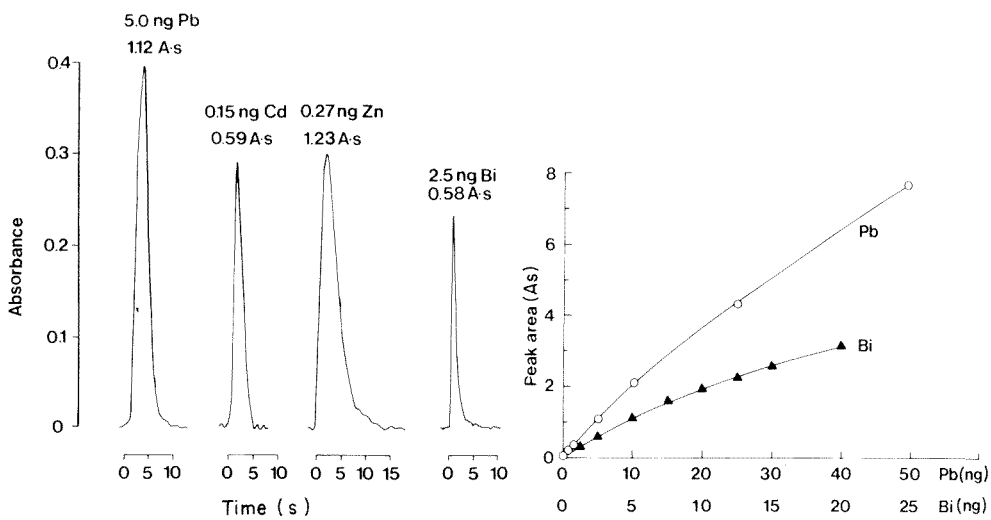

Fig. 3. Typical absorption pulses obtained for Pb, Cd, Zn and Bi.

Fig. 4. Calibration curves for lead and bismuth.

Experimental and theoretical studies of interference effects on lead

The complicated chemistry of lead in sulphate matrices using graphite furnaces was discussed in Part 6 [9]. An illustrative diagram of the lead–sulphur system is given in Fig. 5. It shows the calculated distribution of lead species at 1500 K for a large variation in the partial pressures of oxygen and hydrogen. The results indicate that, independent of the composition of the atmosphere, gaseous lead will not be formed quantitatively. As can also be seen, small changes in the partial pressure of oxygen strongly influence the proportion of lead atoms formed. A maximum will be obtained for a partial pressure of oxygen around 10^{-8} atm. It is interesting to note that even for extremely reducing conditions the calculated fraction of lead atoms never exceeds 70%.

In order to verify experimentally the theoretical results given in Fig. 5, the interference effects caused by sulphur were studied for large variations in the composition of the gas mixture. The results obtained are shown in Fig. 6. By comparisons with Fig. 5, it can be seen that the shape of the curve representing the theoretical recovery of lead was verified experimentally. The calculated equilibrium partial pressures of oxygen corresponding to these experimental conditions vary in the range 10^{-3}—10^{-17} atm. However, the uncertainty of the results given by the points marked D, E and F in Fig. 6 was large, for the following reasons. The tungsten vaporizer was found to require a hydrogen-rich atmosphere for proper functioning. As a consequence, it was difficult to achieve a well-defined gas mixture covering a range of oxygen partial pressures from 10^{-10} to 10^{-3} atm, because small and

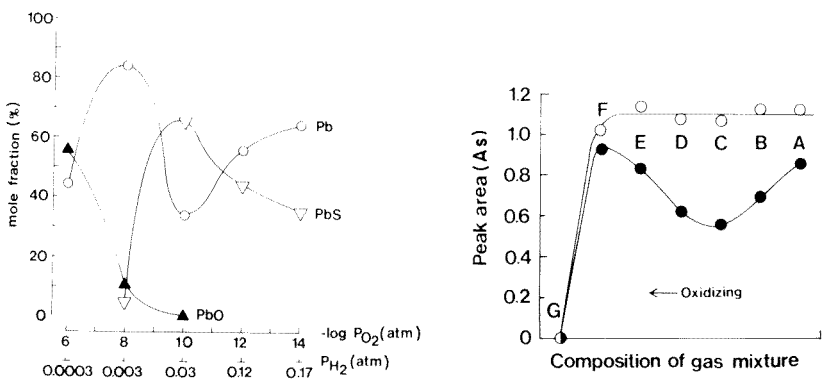

Fig. 5. The distribution of lead compounds at 1500 K as a function of the partial pressures of oxygen and hydrogen. The input amounts (μmol) used in the calculations were: Ar = 4, C = 0.4, H = 2, S = 0.006 and Pb = 1×10^{-5}.

Fig. 6. Experimental results obtained at 1420 K for 25 pmol of lead: (○) in water and (●) in the presence of 0.5 μmol of sulphur as $MgSO_4$. The flow of hydrogen was 61 ml min^{-1}. Flow rates of argon, carbon dioxide and oxygen were as follows (ml min^{-1}): A = 425, 0, 0; B = 375, 50, 0; C = 325, 100, 0; D = 325, 100, 10; E = 325, 100, 35; F = 325, 100, 40; G = 325, 100, 41.

therefore uncertain flows of oxygen had to be added to a gas mixture with poor "buffer" capacity. By use of a vaporizer material other than tungsten, for example glassy carbon, hydrogen could have been excluded and in this way a well-defined gas mixture with good buffer capacity could have been obtained by a suitable mixing of argon, carbon monoxide and carbon dioxide. A comparison between the experimental and theoretical results should be based on the results given in Table 3. As can be seen, the agreement is good and this shows that a state close to equilibrium was obtained. These results are promising, for they indicate the possibilities of establishing optimum conditions for atom formation in complex matrices by use of high-temperature equilibrium calculations.

Interference studies of lead in iron and sodium chloride are shown in Table 4. Owing to the large dilution of the sample constituents with the auxiliary gas buffer, interference effects from chlorides are expected to be less compared with graphite furnace a.a.s. For a graphite tube of normal size, the expected signal depression for lead in the presence of sodium chloride, as defined in Table 4, is about 10% at 1500 K. The agreement between the theoretical and experimental results in Table 4 again indicates that equilibrium conditions are attained even though the temperatures used are relatively low.

It should be mentioned that background signals caused by chloride and sulphide matrices were very low, in the order of 0—0.03 A × s. This means that accurate measurements in such matrices are possible without using a background corrector.

Conclusions

As is evident from the above discussion the system described offers unique possibilities for studying and controlling interference effects. It should be emphasized that the element atoms are always measured at constant temperature. This means that the influence of concomitants on the relative number of free atoms can be established adequately for various gas phase conditions provided that peak areas are evaluated.

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DECOMPOSITION OF BIOLOGICAL MATERIALS, ROCKS, AND SOILS IN PURE OXYGEN UNDER DYNAMIC CONDITIONS FOR THE DETERMINATION OF SELENIUM AT TRACE LEVELS

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SUMMARY

Selenium is evolved from organic materials and from rocks and soils, after admixture with silicic acid and with silicic acid/cellulose, respectively, by combustion in oxygen under dynamic conditions in a special combustion apparatus. While concomitant elements that form sparingly volatile oxides remain in the ash on the sample holder, selenium dioxide volatilizes and condenses on a cold finger, whence it is dissolved off with hydrochloric acid (1 + 1) or nitric acid (65% w/v) by boiling under reflux. The isolated selenium is determined either by hydride-generation a.a.s. or by differential pulse cathodic stripping voltammetry. Detection limits for the overall procedures lie at 3 and 30 ng g^{-1}, respectively.

Selenium plays an important role in biochemistry because it is both essential and toxic to human and animal metabolism within a narrow concentration range [1, 2]. As of yet, no one is in a position to give precise statements on borderline concentrations. Recent studies suggest, however, that they are of the order of ng g^{-1} to μg g^{-1} [2, 3]. These lower levels necessitate sensitive and dependable analytical procedures to determine the true value in the often very complex matrices. Direct procedures, such as instrumental activation analysis [4—6] may avoid a range of difficulties, in that only minimal sample preparation is necessary [7, 8], but they lack the required sensitivity or are not sufficiently reliable at ng g^{-1} levels, unless post-irradiation separation is employed [9]. The satisfactory results thus obtained are paid for, however, by the time and high costs per analysis. Therefore, chemical procedures applicable to large numbers of samples must be given priority, wherein the biological matrix is decomposed prior to the determination step. In such methods, there is still the inclination to save time. Mostly the matrix still contains numerous concomitant elements which interfere with the commonly used spectrophotometric [10], spectroscopic [11—13], chromatographic [14, 15], and electrochemical [16—18] methods for

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the determination, thus necessitating separation steps involving, for instance, solvent extraction [19], co-precipitation [20], volatilization [20], ion-exchange [21], and hydride generation [22—24]. Attention has been focussed recently on interferences associated with the most familiar hydride-generation technique which fails in the presence of some elements such as Cu, Ag and Pb [25, 26].

Volatilization in the presence of oxygen offers a possibility for separating selenium as selenium dioxide from sparingly fugacious matrices such as metals [27], and rocks [20] with relatively simple means. For biological materials, the volatilization has to proceed via a combustion; in most cases, such methods demand elaborate combustion and trapping gadgets, which for the most part are not commercially available [11] and require skilled personnel. Nevertheless, in ultratrace analysis a combustion which separates SeO_2 simultaneously from matrix elements has to be preferred to wet decomposition methods. The latter call for relatively large volumes of strongly oxidizing acids, e.g. $HClO_4$, $HClO_3$, H_2SO_4 and HNO_3, and reagents such as magnesium nitrate [28—32] to prevent selenium from being volatilized and to ensure complete mineralization. There is, therefore, a serious risk of introducing blanks which may cause the analytical result to be incorrect by orders of magnitude if ng g^{-1} levels have to be determined [7, 8]. Closed systems require less acid, but even under pressure many stable organic complexes are not completely mineralized at temperatures below 200°C [33].

This paper deals with the decomposition of organic and some inorganic samples for the determination of traces of selenium. For the opening-out, a commercially available apparatus (Trace-O-Mat; Fig. 1) was used; this has already been tested comprehensively on numerous organic samples [34]. During combustion of the sample in pure oxygen under dynamic conditions, selenium volatilizes as SeO_2 and condenses on a cold finger. If silicic acid is added to the sample to be burnt, sparingly volatile concomitant elements are fixed in the slug which remains on the sample holder. An admixture of silicic acid with cellulose even allows the decomposition of comminuted rocks and soils, which are heated by the combustion of cellulose to a sufficiently high temperature for liberation of selenium. After the combustion, the sample holder with the ash is withdrawn from the apparatus, thus providing a separation from interfering elements. The selenium dioxide is dissolved off the cooled areas with a minimum of acid and can then, in isolated form, be determined by any sensitive method. In this study, the familiar hydride-generation—atomic absorption spectrometric (a.a.s.) method was used, partly because a great deal of experience had been gained in its use [23, 26] and partly because of its ease of handling. For comparison, a disparate procedure was used, involving wet digestion [31] and/or static volatilization [20] in combination with differential pulse cathodic stripping voltammetry (d.p.c.s.v.) to confirm the accuracy of the results.

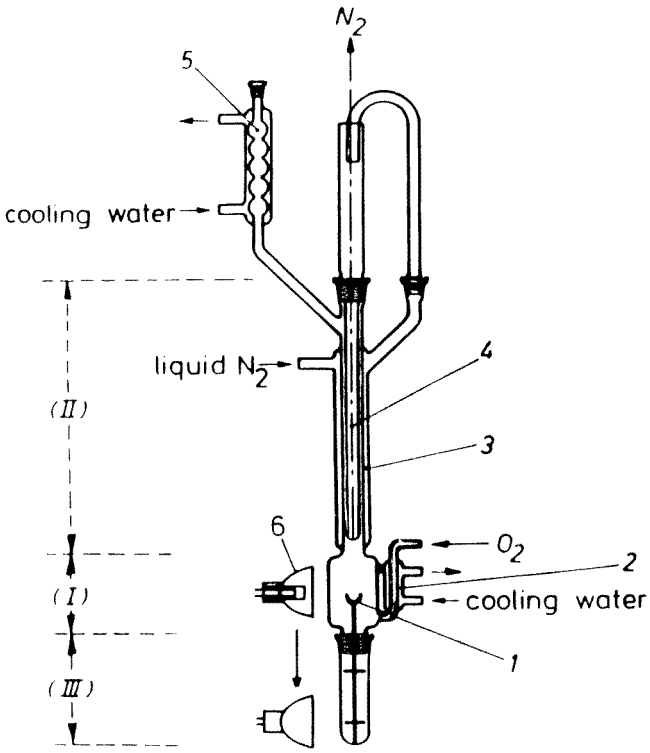

Fig. 1. Quartz combustion device of the Trace-O-Mat. (I) Combustion chamber (ca. 75-ml capacity); (II) cooling system (liquid N_2); (III) quartz test tube; (1) sample holder; (2) oxygen inlet; (3) cooling mantle; (4) cold finger; (5) condenser; (6) i.r. lamps.

EXPERIMENTAL

Apparatus

The decomposition apparatus used was a Trace-O-Mat [34] (H. Kürner, Neuberg 1, F.R.G.). The die for pelletting the samples was home-made from PTFE with a brass piston coated with PTFE.

For a.a.s., a Perkin-Elmer spectrometer (type 303) was used with deuterium background correction and the hydride MHS-1 system (Bodenseewerk Perkin-Elmer, Überlingen, F.R.G.). The quartz cell (60 mm long, 12 mm o.d., 10 mm i.d.) was employed with a vertical slit (slit width 6 mm), and an electrodeless discharge lamp, type BLM 77, with power supply (Meßtronic, Stuttgart). For d.p.c.s.v., a multipolarograph type 471 was used with a hanging mercury drop electrode and a saturated calomel electrode (Amel, Mailand, Italy); the home-made microcell held 5 ml of solution.

The pressure decomposition apparatus comprised PTFE vessels with steel housing, an aluminium oven and electronically controlled temperature setting [35] (H. Kürner, Neuberg 1). Automatic micropipettes and continuously adjustable pipettes were from Eppendorf (Hamburg).

Reagents

All reagents were of analytical grade (E. Merck) and all gases were from Messer Griesheim (Düsseldorf) if not stated otherwise. Twice-distilled water was used for all solution preparations.

For the dynamic combustion process, the oxygen was 99.995% pure. Silicic acid (Riedel de Haen AG, Seelze-Hannover) was heated to 1150°C for 2—3 h in a stream of oxygen to remove selenium blanks ($\geqslant$1 ng g^{-1}) and pulverized in a mortar before use. The cellulose (Merck Cat. no. 2337) was used as received.

For the hydride-generation—a.a.s. method, the nitrogen was 99.995% pure. The reduction solution, which was freshly prepared before use, contained 2.5% NaOH and 7.5% $NaBH_4$: 6.25 g of NaOH was dissolved in about 100 ml of water and 18.7 g of $NaBH_4$ (98%; Roth, Karlsruhe) was added; this solution was passed through a folded filter paper and made up to 250 ml with water. The rinsing solution was 0.1 M HCl from a PTFE wash bottle.

For the selenium stock solution, 1.64 g of H_2SeO_3 (99%; Fluka Feinchemikalien, Neu-Ulm) was dissolved in water and made up to 1 l (1.0 mg Se ml^{-1}). Working solutions with selenium contents of 0.5—4.0 μg ml^{-1} were prepared by dilution of the stock solution with 3 M HCl.

The base electrolyte for d.p.c.s.v. [36] was prepared by dissolving 33.035 g of ammonium sulfate in water, adding 20 ml of 0.1 M EDTA solution and 1.0 ml of 0.016 M copper sulfate solution, and diluting to 250 ml after adjusting to pH 4.5 with 0.1 M H_2SO_4.

The selenium stock solution for d.p.c.s.v. contained 70.25 mg of selenium dioxide dissolved in 500 ml of water (1 mg Se g^{-1}). For calibration, more dilute solutions with selenium contents of 2—25 ng ml^{-1} were prepared daily.

Standard solutions of selenium-75 were prepared by dilution of $H_2{}^{75}SeO_3$ in 0.5 M HCl (180 μCi μg^{-1}, 0.21 μCi μl^{-1}) with 0.5 M HCl (NEN chemicals, Dreieich, F.R.G.).

Optimization of combustion parameters of the Trace-O-Mat

The combustion parameters were optimized using the radioactive isotope selenium-75.

Sample holder. In the determination of selenium, the choice of an adequate sample holder for combustion of the matrix is less difficult than earlier, because the selenium dioxide formed is easily liberated during combustion, in contrast to the oxides of many other relevant trace elements, e.g. As, Cr, Mn [34]. It has only to be ensured that the ash remains on the holder to guarantee separation of selenium from concomitant elements which interfere with the determination step. A fork-shaped holder, which ensures that the sample is sufficiently fed with oxygen, with a bottom plate and, in some cases, an annular quartz thread at the upper part of the fork, to prevent the pellet and the ash from dropping out, stood the test (Fig. 2).

Oxygen flow rate. The oxygen supply has to be regulated in such a way that the combustion proceeds slowly, uniformly, and completely. Usually, 10—20 s is the standard combustion time for a sample weight of 0.1—0.2 g.

Too little oxygen (<400 ml min^{-1}) can cause soot formation and incomplete ashing, while too much oxygen may give violent combustions with particles flung out of the sample holder. The situation may be helped by using additives which serve two purposes (see below for organic matrices). The appropriate flow rate for unfamiliar materials has to be ascertained by preliminary tests on a small scale to avoid untoward incidents such as violent combustion or even explosions. In practice, the gas flow must be low (about 400 ml min^{-1}) at ignition; immediately thereafter, it has to be raised to higher levels which depend on the sample to be ashed. The gas flow lay between 70 and 120 l h^{-1} with all samples opened out in this investigation.

Dissolution of volatilized combustion products. After the sample holder has been withdrawn from the apparatus when the combustion has finished, selenium dioxide, and a few other volatilized combustion products (e.g., Cd, Sb, Tl, Bi as volatile compounds), have to be dissolved from the cooled areas. Nitric acid (65% w/v) or hydrochloric acid (1 + 1) ensures complete dissolution of selenium dioxide and is compatible with the succeeding determination step; 1.5—2 ml proved to be sufficient. Initially, 1 ml of the acid is placed in the receiver test tube and heated, gently at first, to thaw the combustion products (especially water and CO_2). Another 1 ml is passed through the condenser and has a rinsing effect. After boiling under reflux for about 30 or 40 min with hydrochloric acid (1 + 1) or nitric acid (65%), respectively, ⩾95% of the selenium was recovered in the combustion of, e.g., wheat flour (Fig. 3). In this investigation a boiling time of 45 min was always applied.

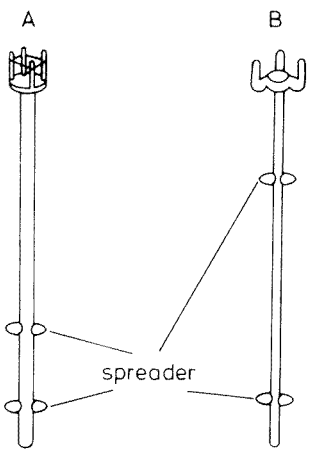

Fig. 2. Quartz sample holders: (A) fork-shaped with bottom plate and quartz thread; (B) fork-shaped. Prong length, 10 mm.

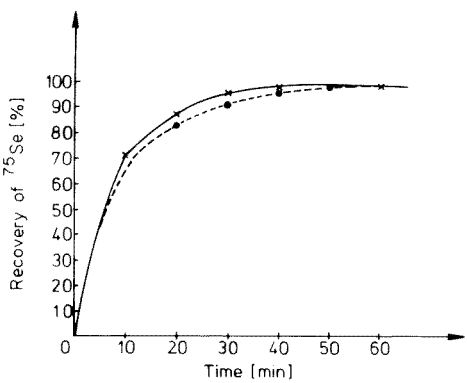

Fig. 3. Recovery of selenium in relation to the duration of boiling under reflux with 2 ml of HCl (×) or HNO_3 (•).

Sample preparation. Undried samples were always used to avoid loss of selenium on drying [37]. Results are, however, reported on a dry basis. The water contents were determined on corresponding samples and were found not to exceed 10% on all the samples under study.

Samples with a relatively high water or fat content, e.g. meat or bacon, are best weighed directly into a cup made of ash-free filter paper. Powdered materials (e.g., cereal flour, milk powder, leaves, grass) are most easily placed on the sample holder either by enveloping them in filter paper (poor in both blank and ash) or by pelletting.

Combustion of organic matrices. In order to avoid too violent a combustion, the samples are diluted with an additive such as silicic acid which can be purified before use by heating in a furnace to 1150°C for about 2—3 h. An admixture of 10—20% of silicic acid ensures slow and uniform combustion, and simultaneously favours the retention of concomitant elements that form sparingly volatile oxides (e.g., Mn, Fe, Cu, Pb) in the slug remaining on the sample holder.

Fusion of inorganic matrices (rocks and soils). Selenium can be liberated from rocks and soils if an auxiliary substance such as cellulose is added which will initiate and sustain a combustion, thus providing temperature conditions to evolve selenium. The optimal proportion of sample to cellulose was found to lie between 1:0.5 and 1:2. With these matrices it is also advisable to add 15—30% of silicic acid to ensure cementation of many interfering elements. The purity of the cellulose poses some limitations to its use, in that it cannot be purified easily, like silicic acid.

Separation of selenium from concomitant elements. The determination of selenium by hydride generation and a.a.s., which is mostly recommended for its speed and ease of handling, suffers interferences from a range of heavy metals [23, 26]. After combustion of the matrix with the additives, the oxides of heavy elements are retained almost completely in the slug on the sample holder, while selenium dioxide is deposited on the cold finger. If the sample holder is removed from the apparatus, an efficient separation is achieved if the following conditions are met: (1) uniform combustion of the sample; (2) quantitative liberation of selenium from the sample; (3) complete retention of the combustion residue on the sample holder.

In order to be certain of selenium being completely liberated from resistant types of bonding, an excess of copper and mercury, which form sparingly volatile selenides, was added to two matrices and left to stand for 2 days. After the combustion, the recovery of selenium both in the slug and in the rinsing acid was determined using ^{75}Se. More than 97% was always found in the acid, regardless of the matrix (Table 1).

Other decomposition methods

Pressure decomposition. A range of investigations has shown that the pressure decomposition [35] fails in as much as the matrix is not completely mineralized. Thus, difficulties are encountered not only in polarography [36]

TABLE 1

Yield of selenium after combustion of some organic matrices spiked with an excess of copper and mercury

Sample	Sample weight (mg)	Element added (μg)		Selenium found (%)	
		Cu	Hg	Solution	Residue on holder
Rice meal	100	—	—	98	—
	200	63	—	98.2	0.6
	200	126	—	97.1	—
	200	126	—	98.2	—
	200	—	0.1[a]	97.1	—
	200	—	0.2[a]	97.5	—
Fish meal	100	—	—	98.1	0.6
	200	—	—	101	—
	200	0.6	—	97.8	0.8

[a]Allowed to stand for 2 days.

but also in electrothermal a.a.s. as well as in hydride-generation a.a.s. [26] on account of high background or strong foaming of the solution [38]. A high-pressure decomposition device made of Cr—Ni—Mo—steel has been developed recently, in which organic samples up to 5 g can be burnt in oxygen at up to 90 bar [39].

Wet decomposition in open systems. Many of these methods are unsatisfactory because of the high fugacity of selenium and many of its compounds unless large volumes of strongly oxidizing reagents are used [29, 30, 40, 41]. Treatment of the sample with magnesium nitrate and nitric acid (65%) in a muffle furnace has proved to be efficient [31] but introduction of blanks by the large quantities of reagents prevents its application if ng g^{-1} levels are to be determined.

Determination of selenium

All determinations were completed by hydride-generation—a.a.s. [23]. For comparison, d.p.c.s.v. was used after volatilization of selenium under static conditions (rocks) [20] and after a wet decomposition with magnesium nitrate and nitric acid [31]. The reasonable agreement of the results obtained by two disparate procedures with the results of standard reference materials (NBS) indicated the accuracy of the results (see below). Some difficulties were encountered in d.p.c.s.v. on samples with a low selenium content because high hydrochloric acid concentrations (>0.15 M) interfered with the electrolysis. The decomposition solution which is normally 6 M in hydrochloric acid has to be diluted or very small aliquots have to be used. Thus, selenium concentrations below about 15 ng/0.5 g can no longer be detected, although the absolute detection limit of d.p.c.s.v. for selenium lies at about 2 ng. Further investigations concerning this subject are under way.

Recommended procedure for combustion under dynamic conditions (Trace-O-Mat)

Sample preparation. To a mixture of ⩽0.5 g of the sample and silicic acid (15—30% of the sample weight) is added about 50 μl (depending on the sample weight) of a 1% solution of cellulose acetate in acetone (binding agent). The mixture is again mixed and pelletted, and the pellet is dried at room temperature under vacuum in a desiccator.

Geological samples (⩽0.3 g) are treated likewise with an additional admixture of cellulose in a 1:1 or 1:2 sample—cellulose ratio depending on the matrix.

Combustion. The pellet is placed on the sample holder, which is fitted into the test tube and carefully inserted in the dry apparatus. After the cold finger and mantle have been filled with liquid nitrogen, the oxygen flow rate is adjusted to 0.4 l min^{-1}. After ignition of the sample with the halogen lamps, the oxygen flow rate is raised to about 1.2—2 l min^{-1}, depending on the matrix and size of pellet. When the combustion has ceased, the sample holder with the ash is withdrawn from the apparatus, and 1 ml of acid is added to the test tube to thaw the condensed reaction products by gentle heating of the acid with the halogen lamps for a few minutes. Subsequently, another 1 ml of acid is added through the condenser and the whole solution is boiled under reflux for 45 min while a gentle oxygen stream is maintained.

After the acid has cooled, the apparatus is rinsed with another 1—2 ml of acid, and the solution is transferred to a graduated flask and made up to 5 or 10 ml with water, depending on the procedure to be used finally.

Procedures for wet decomposition

Decomposition in open vessels [31]. To a 50-ml pyrex beaker containing 0.15 g of the biological material, e.g. Orchard Leaves (NBS SRM 1571) are added 2 ml of nitric acid (65%) and 1.0 g of magnesium nitrate (Merck, cat no. 5853). The mixture is gently heated to dryness on a hot plate, and then the temperature is raised until brown fumes cease to emerge. The residue is heated in a muffle furnace at 500°C for 40 min and allowed to cool. Hydrochloric acid (5 ml, 1 + 1) is then added to dissolve the residue with gentle heating. The solution is transferred to a graduated flask and diluted to 10 ml with water.

Pressure decomposition [35]. The biological sample (0.05—0.2 g) is weighed into a PTFE or glassy carbon vessel to which 1 ml of nitric acid (70% purified by sub-boiling point distillation) is added. The procedure is completely as described elsewhere [35].

Procedures for measurement

Hydride-generation a.a.s. [20]. The acidic decomposition solution is diluted with water to a concentration that is 3 M in hydrochloric acid with a selenium content ranging between 5 and 20 ng/10 ml. This solution is transferred to the reduction vessel containing 10 ml of 3 M HCl added from a piston burette. Addition of 25 ml of reduction solution as well as the nitrogen

flow rate (670 ml min^{-1}) are regulated automatically by the MHS-1 system. Ultraviolet absorption is measured at 196 nm in the quartz cell heated to 1000°C and the height of the absorption peak is evaluated. The calibration is done analogously with 1—40-ng aliquots of the standard solution.

Differential pulse cathodic stripping voltammetry [36, 42]. An aliquot (2 ml) of the decomposition solution, which has been diluted with water to be 0.15 M in hydrochloric or nitric acid, and 2 ml of the base electrolyte (1 M $(NH_4)_2SO_4$, 8 × 10^{-4} M EDTA, 2 μg Cu^{2+} ml^{-1}) are pipetted into the electrochemical cell, and deaerated by passing argon (99.995% pure) through the solution for 5 min. Deposition at the hanging mercury drop electrode is conducted at −0.4 V for 1 min while stirring. After 30 s of quiescence, the cathodic stripping voltammogram is recorded. The selenium standard solution (2—10 ng Se in μl aliquots) is added to the decomposition solution and the selenium content is evaluated by the standard addition method.

RESULTS AND DISCUSSION

The decomposition of biological matrices for the determination of selenium by hydride-generation a.a.s. was investigated with the Trace-O-Mat apparatus. The total procedure was checked for accuracy with standard reference samples (Table 2) as well as by comparing the results obtained with those of an independent procedure, a combination of a wet decomposition and/or volatilization technique under static conditions [20] with d.p.c.s.v. (Tables 3—5).

TABLE 2

Determination of selenium in NBS Standard Reference Materials
(Means, standard deviations, relative standard deviations (%) and number of determinations are given)

Sample	Weight (mg)	Selenium content (μg g^{-1})						
		A.a.s.				D.p.c.s.v.		Reference data
		x	s	s_r	n	$\bar{x}$	n	
Bovine liver (NBS 1577)	20—110	1.09	0.06	5.06	7	1.16	2	1.1 ± 0.1[a] 1.12 [11] 1.02[b]
Wheat flour (NBS 1567)	10—120	1.03	0.04	4.18	9	1.0	2	1.1 ± 0.2[a] 1.01 [11]
Rice flour (NBS 1568)	30—160	0.38	0.01	3.11	11			0.4 ± 0.1[a] 0.34 [11]
Orchard leaves (NBS 1571)	70—150	0.076	0.0013	3.05	12			0.08 ± 0.01[a] 0.078 [11] 0.074[b]

[a]NBS certificate. [b]Instrumental neutron activation analysis; MPI for Metal Research, Laboratory for High-Purity Materials, Schwäbisch Gmünd.

A range of biomaterials (Table 3), and rocks and soils, after admixture of cellulose (Table 4) were analysed for selenium. The investigation showed that the combustion under dynamic conditions constitutes an elegant and rapid method for volatilizing selenium from these matrices. The admixture of silicic acid fixes oxides of heavy metals in the slug that forms on the sample holder, so that they can be separated easily from selenium dioxide by removing the sample holder. The combustion proceeds in a small reaction chamber of about 75-ml capacity and requires only oxygen and 1—2 ml of hydrochloric or nitric acid which can easily be purified. Therefore, introduction of blanks by reagents and interaction with container walls are negligible. Some combustion parameters such as sample weight, amount of additives and oxygen flow rate have, however, to be investigated in preliminary tests if unknown materials have to be analysed. Standard sample weights are 0.5 and 0.3 g of organic and inorganic materials, respectively. The gas flow rate lies between 70 and 120 l h^{-1} in such cases. With a detection limit of 2 ng for the total procedure, the hydride-generation—a.a.s. method is sensitive enough to detect selenium in matrices with $\geqslant 4$ ppb, based on a maximum sample weight of 0.5 g, in that the whole decomposition solution can be transferred to the reduction chamber.

TABLE 3

Determination of selenium in organic materials by the hydride-generation—a.a.s. procedure (Means, standard deviations, relative standard deviations (%), and number of determinations are given)

Sample	Weight (mg)	Selenium content ($\mu g\ g^{-1}$)				Other data
		$\bar{x}$	s	s_r	n	
Fish meal	20—160	2.61	0.1	3.66	9	2.72 [11] 2.23[a] 2.64[b]
Human hair	30—90	1.73	0.02	1.4	4	1.73 [11] 1.77[b]
Soy groats	100—180	0.5	0.02	3.5	4	0.51 [11] 0.51[a]
Milk powder	100—150	0.087	0.002	1.6	4	0.082 [11] 0.096[a] 0.090[c]
Blood meal	30—100	0.63	0.03	5.1	3	0.62 [11] 0.71[c]
Rape groats	20—70	0.91	0.03	3.55	4	0.92 [11] 0.99[a]
Cellulose[d]	400—1000	0.01	0.001	10.1	4	—
Cellulose	1230 + 10 ng Se	0.019				

[a]Hahn-Meitner Institut für Kernforschung, Berlin. [b]The mean of 3 determinations by the d.p.c.s.v. procedure. [c]See footnote b, Table 2. [d]The result obtained by the wet decomposition method [31] on a 1080-mg sample was 0.0085 μg Se g^{-1}.

TABLE 4

Determination of selenium in geological samples by the hydride-generation—a.a.s. procedure

Sample	Weight (mg)	Selenium content (ng g^{-1})				
		A.a.s.				Reference values
		$\bar{x}$	s	s_r	n	
Deep sea basalt A II-92	90.4	165	—	—	—	142 [11], 168 [20], 178 [43]
Basalt I	92.9	122	—	—	—	121 [11], 124 [20], 127 [43]
Basalt III	120—180	63	3.5	5.5	4	62—66 [11], 67 [20], 20 [43]
GSP-1	130—200	63	4.7	7.5	4	62 [11], 64 [20], 58 [44]
Phonolite-NA	90—120	144	2.2	1.5	4	147 [11], 144 [20], 160 [43]
BCR-1		80	—	—	2	70—80 [11], 92 [20], 100 [44, 45], 94 [46], 103 [47], 118 [48]
Soil		340	—	—	—	304—390 [11]

In d.p.c.s.v., which has an absolute detection limit of about 1 ng for selenium, the given conditions of supporting electrolyte, pH, copper(II) concentration, have to be strictly adhered to [36]. Therefore, only μl-aliquots of the decomposition solution can be dispensed into the micro-cell. Hydrochloric acid concentrations exceeding 0.15 M affect the deposition of selenium significantly; this is manifested by a shift of the calibration graph and by a change of its slope.

Direct use of the decomposition solution after the aforementioned pressure decomposition fails because the organic matrices are not completely mineralized. The remaining fragments are absorbed on the surface of the mercury drop and prevent the formation of intermetallic compounds (Hg—Cu—Se) and thus preconcentration of selenium; this has been shown for numerous biomaterials. Even in the hydride-generation method, serious interferences are encountered if the solution obtained by pressure decomposition is used directly. Strong foaming occurs even though only aliquots

TABLE 5

Determination of selenium in Orchard Leaves[a] (NBS 1571) by hydride-generation—a.a.s. after application of different decomposition methods

Method	Se found (ng g^{-1})			Duration of analysis
	$\bar{x}$	s	n	
Combustion in O_2/Ar	78	9.5	28	14 h
Combustion in O_2	76	2.3	12	40 min
Wet decomposition [31]	76	2.5	5	6 h

[a]Certified value of NBS: 80 ± 10 ng g^{-1}; sample weight 100—200 mg.

are employed [38]. The same is true for electrothermal a.a.s., in which anions, especially chloride, also interfere. Some recent investigations with a new plateau-graphite tube promise improvements in this determination of selenium [49]. The notorious problems in the sample preparation remain, however, untouched by this.

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THERMAL STABILIZATION OF INORGANIC AND ORGANICALLY-BOUND TELLURIUM FOR ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY

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SUMMARY

The thermal stabilization of inorganic tellurium(IV) and organically-bound tellurium for electrothermal atomic absorption spectrometric determination of the element was studied with the use of the isotope tellurium-127m. Of the 19 metals and potassium iodide tested, 15 metals had a stabilizing effect on inorganic tellurium; among the 9 metals tested with organically-bound tellurium, only 3 exhibited an effect. The most effective metals for stabilizing inorganic tellurium were cadmium, copper, palladium, platinum and zinc, while the best agents for stabilization of organically-bound tellurium were silver, palladium and platinum; in the presence of palladium and platinum, tellurium in both forms could be heated in the graphite tube to 1050°C without losses. Attempts were made to determine tellurium in human whole blood and garlic, but the concentrations were found to be below the detection limits of 3 ng ml^{-1} and 140 ng g^{-1}, respectively.

In most inorganic materials and in biological samples tellurium is found in very low concentrations. Its quantification is further complicated by its volatility, and the high levels of salts present in biological matrices. The standard methods of trace analysis, such as atomic absorption (a.a.s.) and emission spectrometry, neutron activation analysis (n.a.a.), mass spectrometry and x-ray fluorescence have all been employed in the determination of tellurium. It is not known whether or not tellurium is an essential element to humans and animals.

Previous papers on the content of tellurium in various biological materials give a rather confusing picture. Schroeder et al. [1] tabulated values for a number of human organs and body fluids. These data are high, e.g., 1.07 μg ml^{-1} in serum, 77 μg g^{-1} in bone, and 30—70 μg g^{-1} in garlic. Similar high values for human organs have been reported by Soman et al. [2]. In minerals, rocks, soil, water, etc., the normal tellurium/selenium ratio is of the order of 10^{-2}—10^{-3}. From recent data on the content of selenium in, e.g., human serum (approximately 0.1 μg ml^{-1}), and presupposing that the above ratio is maintained, one would expect a concentration of tellurium of approximately

1 ng ml^{-1}. The suggestion that previous data are too high is supported by recent data published by Van Montfort et al. [3], and the values listed in the compilation by Iyengar et al. [4]; compared to the early data [1, 2], the values obtained in more recent studies are lower by two to three orders of magnitude.

Following previous work on thermal stabilization [5] and direct electrothermal a.a.s. determination of selenium in human serum [6], the present work was started with the purpose of finding agents for thermal stabilization of tellurium, and of developing a direct method for the quantification of the element.

EXPERIMENTAL

Instruments and apparatus

The a.a.s. measurements were made with a Perkin-Elmer (PE) 5000 instrument equipped with deuterium lamps for background correction and a PE 500 graphite atomizer. A PE HGA-76 graphite atomizer was used in the isotopic studies. The radiation source was a tellurium electrodeless lamp run at 9 W, and the wavelength employed was 214.3 nm. All determinations of tellurium were based on measuring peak heights. The graphite atomizers were purged with argon (99.9% pure). The radioactivity was measured with a Packard Auto-Gamma scintillation spectrometer. A polytetrafluoroethylene-lined aluminium bomb was used for decompositions. Weighings were made with a semi-micro balance.

Reagents and solutions

Tellurium standard solutions were prepared from the element (purity 99.999+, American Smelting and Refining Co., U.S.A.). The tellurium-127m, in the form of a solution of tellurous acid in 4 M HCl (New England Nuclear, U.S.A.) had a half-life of 109 days, a total activity of 500 μCi, a specific activity of 0.16 mCi mg^{-1}, and a radiochemical purity of 99.000%.

The concentrated acids were of Suprapur quality (Merck); other chemicals were of reagent-grade quality.

Standard solutions. A tellurium(IV) standard solution (500 μg ml^{-1}) was prepared by dissolving the proper amount of the element in the minimum amount of nitric acid.

The following standard solutions (1000 μg ml^{-1} with respect to the metal; BDH Chemicals) were employed: cadmium, chromium(III), cobalt, copper, iron(III), manganese(II), nickel and zinc, all in the form of their nitrates in 1 M nitric acid; and molybdenum as molybdate in 1 M nitric acid.

Other 1000 μg ml^{-1} solutions were prepared as follows: cerium from cerium(III) sulfate; caesium from the chloride; lanthanum from the oxide; palladium and platinum from the chlorides; and silver, thorium, thallium and zirconium from the nitrates. All these solutions were 5% (v/v) in nitric acid. Finally, a 1000 μg ml^{-1} potassium iodide solution was prepared and a 6% solution of tantalum as described by Zatka [7].

Thermal stabilization of inorganic and organically-bound tellurium

During the measurements of the γ-activity the settings of the scintillation spectrometer were as follows: lower level, 40 scale units (10 KeV); window, 140 scale units (35 KeV); full scale value 1000 units (0.25 MeV).

Stabilization of inorganic tellurium. The tellurium-127m solution was diluted to an activity of approximately 3×10^4 counts per min (cpm) from a 15-μl portion deposited in a graphite tube.

A new graphite tube was transferred to one of the plastic containers of the spectrometer, and the background activity (B) was recorded. The tube was then placed in the atomizer and 15 μl of the diluted tellurium-127m solution and 15 μl of a reagent solution were pipetted into the tube. The mixture was dried at 100°C for 40 s, and the activity (C_1) was measured to correct for possible pipetting errors. The tube was again put into the furnace, heated for 30 s at a preselected temperature, cooled, and transferred to the spectrometer for measurement of the final activity (C_2). The tube was finally heated for 10 s at maximum power, and cleaned by brushing. In all measurements the counting time was 60 s.

The recovery in percent was $(C_2 - B/C_1 - B)$ 100. All experiments were carried out in duplicate.

Stabilization of organically-bound tellurium. Samples were prepared by injecting tellurium-127m solutions into Wistar rats, and taking a 5-ml sample of whole blood from the vena cava after a period of 90 min. As demonstrated by Agnew and Cheng [8], this time is sufficient to transform most of the injected tellurium into organic compounds.

Before the injection it was necessary to adjust the pH of the strongly acidic tellurium-127m solution to about 7.0; this was done by adding 1 M sodium hydroxide. During the neutralization a faintly yellow precipitate was formed, and the activity of the solution was considerably reduced. However, 0.2 ml of the neutralized solution was injected; this volume had an activity of 6×10^6 cpm. This dose was less than one fifth of the lethal dose of tellurite.

From the blood containing the organically-bound tellurium, 20-μl portions were transferred to the graphite tubes. With a counting time of 120 s, this volume gave an activity of about 1200 cpm, and the background activity was below 100 cpm.

Portions (20 μl) of blood and of one of the stabilizing agents were transferred to a graphite tube, and the mixture was dried at 120°C for 30 s. Mixtures which were later to be heated above 600°C, were pyrolyzed in two 30-s steps, first at 450°C, and then at 600°C. The experiments and calculations were done as described above for inorganic tellurium.

RESULTS AND DISCUSSION

Table 1 lists the metals and compounds tested for their stabilizing effect on inorganic and organically-bound tellurium, and the temperatures to which the mixtures can be heated without any appreciable loss, i.e. a loss less than

TABLE 1

The stabilizing effect of metals and potassium iodide on inorganic and organically-bound tellurium

Agent	Maximum pyrolysis temperature (°C) without loss of tellurium present		Agent	Maximum pyrolysis temperature (°C) without loss of tellurium present	
	Inorganic	Organically-bound		Inorganic	Organically-bound
Te alone	400 (400[a])	300	Mn	850 (900[a])	300
Ag	1000 (1000[a])	1100	Mo	400	n.d.
Ca	300	n.d.[b]	Ni	900 (1000[a])	400
Cd	1000	300	Pd	1100	1050
Ce	800	n.d.	Pt	1100	1050
Co	300	n.d.	Ta	300	n.d.
Cs	800	n.d.	Tl	750	n.d.
Cu	1050 (1050[a])	200	Th	900	300
Fe	800	n.d.	Zn	1000	200
KI	150	n.d.	Zr	800	n.d.
La	800	n.d.			

[a]A.a.s. results in parentheses. [b]Not determined.

5%, of tellurium. For comparison, the upper temperature limits are also tabulated for inorganic and organically-bound tellurium, without added stabilizing agent.

A study of the loss of inorganic tellurium, without the use of the isotope, was also made by a.a.s.; the upper temperature limits from this investigation are given in parentheses in Table 1. As is apparent from the Table, these data are in good agreement with those from the measurements of radioactivity.

Figures 1 and 2 show, for some of the systems studied, the recovery of inorganic and organically-bound tellurium as a function of the temperature of pyrolysis. It is clear from these Figures that while a number of metals stabilized inorganic tellurium, the only agents which stabilized organically-bound tellurium in whole blood were the noble metals. Palladium and platinum were also the most effective agents for inorganic tellurium.

The stabilizing effect of nickel on inorganic tellurium has been reported by Ediger [9]. He found that without nickel, tellurium began to vaporize above 800°C, while in the presence of the metal, the pyrolysis temperature could be raised to 1200°C. In the present study (see Fig. 1) tellurium without added metal began to vaporize above 400°C; in the presence of nickel the maximum pyrolysis temperatures, as established with the use of the isotope and a.a.s., were 900 and 1000°C, respectively.

Some of the agents tested increased the volatility of tellurium. Thus potassium iodide, which has been reported [10] to stabilize selenium, made inorganic tellurium more volatile. Likewise, copper and zinc seemed to increase the volatility of organically-bound tellurium.

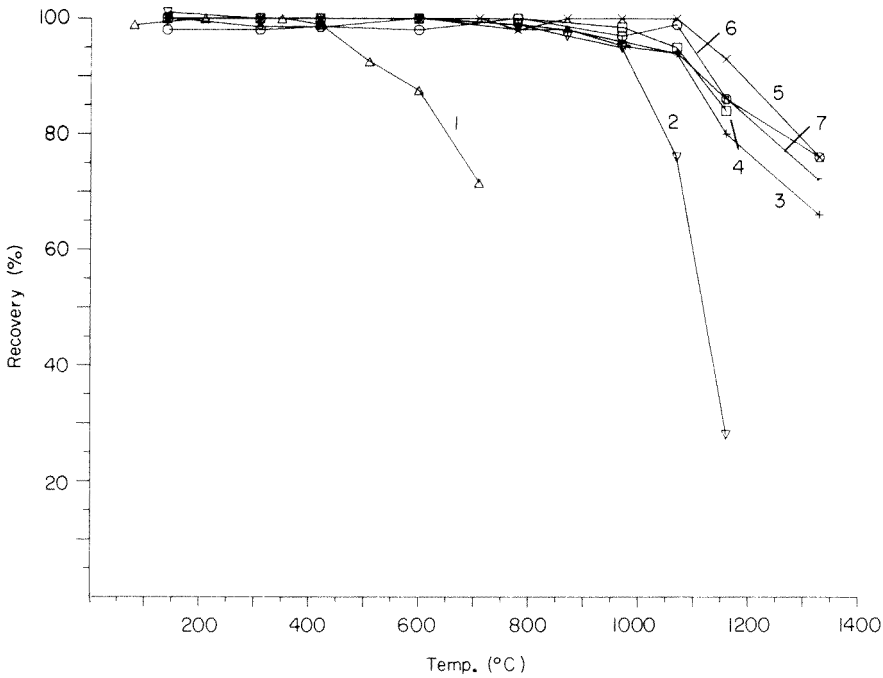

Fig. 1. The recovery of inorganic tellurium without and with added stabilizing agents as a function of the temperature of pyrolysis: (1) tellurium alone; (2) with silver; (3) with cadmium; (4) with copper; (5) with palladium; (6) with platinum; (7) with zinc.

Applications

For a number of biological samples, such as whole blood, plasma and serum, the data reported for tellurium are widely scattered. It was therefore of interest to apply the present technique in the analysis of some biological materials.

From a sample of human whole blood (taken from an unexposed individual), one volume was mixed with one volume of 1000 μg ml^{-1} palladium solution and two volumes of 1000 μg ml^{-1} nickel solution. According to a recent study [11], the addition of the latter solution serves to remove the otherwise uncorrectable positive line interferences from the atomization products of phosphate (P_2, PO and PO^+). From the mixture, 50 μl were pipetted into the atomizer, the flow of argon was adjusted to 30 ml min^{-1}, and the heating program given in Table 2 was applied. No signal from tellurium was recorded; the concentration in the present sample was thus below the detection limit of 3 ng ml^{-1}. In another sample of human whole blood, tellurium was determined by n.a.a., and the content was found to be below the detection limit of 2 ng ml^{-1}.

Finally, a sample of commercial garlic powder was analyzed by the solid sampling technique of a.a.s. Portions of about 5 mg were placed in the

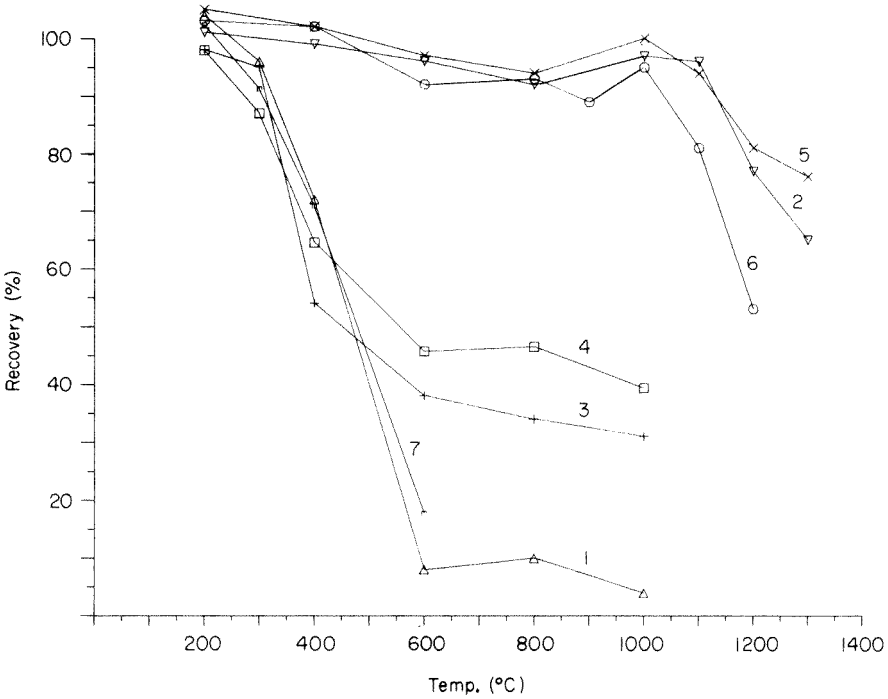

Fig. 2. The recovery of organically-bound tellurium without and with added stabilizing agents as a function of the temperature of pyrolysis. The curves are numbered as in Fig. 1.

TABLE 2

Heating program for the determination of tellurium in human whole blood

Step	1	2	3	4	5	6
Ramp time (s)	5	5	5	40	10	0
Heating time (s)	20	5	20	10	20	5
Temperature (°C)	120	150	300	600	1100	2000

graphite tube, and 20 μl of 1000 μg ml^{-1} palladium solution was added. In this determination of tellurium, a less sensitive spectrometer was applied; again no signal was observed, which meant that the content was less than 140 ng g^{-1}. Now the possibility existed that the garlic powder contained volatile compounds of tellurium which were lost during the initial heating steps; they may of course also have been lost during the production of the powder. A portion of a fresh onion was therefore decomposed in the bomb with sulfuric and hydrochloric acids (1 + 1), and the solution was analyzed by a.a.s. with nickel as the stabilizing agent. No tellurium signal was recorded, again indicating a concentration of less than 140 ng g^{-1}; in a previous paper [1] the concentration range for tellurium in garlic was found to be 30—70 μg g^{-1}.

It thus seems confirmed that some of the early data on the content of tellurium in biological samples are erroneous, and that the recent lower data are more reliable.

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LINE ABSORPTION OF MATRIX ELEMENTS AS A BACKGROUND CORRECTION ERROR IN ATOMIC ABSORPTION SPECTROMETRY

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SUMMARY

Accurate measurement of weak atomic absorption signals is only possible after exact background correction. If the accompanying elements in excess have their own resonance lines in the immediate vicinity of the resonance line of the element to be determined, and these fall within the spectral band transmitted by the monochromator, then the value of the measured apparent background absorbance is higher than the true one. The error may usually be diminished by choosing an appropriate spectral bandwidth. Tables of such bandwidths for a large number of pairs of elements are given.

When low concentrations or elements providing poor sensitivity are to be determined by atomic absorption spectrometry (a.a.s.), it is often necessary to increase the amount of sample. In such cases, the solution introduced into the atomizer may contain an appreciable concentration of metal salts. For this reason, the background absorption value may be so large as to equal or exceed that of the characteristic absorption. It is usually corrected by a continuum light source (H_2, D_2). When very low concentrations are to be determined, the accuracy of correction is of decisive importance.

In analytical practice, it sometimes occurs that the background level is over-compensated by the background correction and the corrected signal may be higher than the true analytical signal. This problem was studied by Marks et al. [1] who attributed this phenomenon to the line absorption of the matrix element. Within spectral bandwidths of 0.3—0.5 mm, they performed measurements on the 217-nm lead line in the presence of various metals without finding any systematic relationship. Manning [2], in the determination of selenium by graphite-furnace a.a.s., demonstrated an iron-matrix line interference for background correction at 196.03 nm.

With a view to determining low concentrations with higher accuracy, the phenomenon of over-correction was studied for a large number of elements with the results described in this paper.

EXPERIMENTAL

For studying various problems associated with automatic background correction, measurements were obtained, partly, with a Varian-Techtron AA-775 spectrometer. However, for systematic measurements, the older Varian-Techtron AA-5 spectrometer was used. The spectral bandwidth of the latter could be changed continuously in the range 0—1.0 nm, and by means of a M-70 wavelength scanner it was possible to scan the spectrum at the required rate in both directions. All measurements were plotted by an X-Y recorder. For the AA-5, the installed slit-width was calibrated by using a gauge strip while the value of background absorption was measured with a hydrogen lamp.

RESULTS

When lead is determined, the background over-correction level in an antimony solution is very marked [3]. By nebulizing a solution containing 100 g Sb l^{-1} into an air—acetylene flame, an absorbance of 0.014 was obtained at a 1-nm spectral bandwidth, using a lead hollow-cathode lamp. With a hydrogen lamp, an absorbance of 0.023 was obtained, from which the over-compensation is evident. Background level measurements were continued with decreasing bandwidths; the results are shown in Fig. 1. With narrowing slit the measured background absorbance tended to decrease until at 0.4 nm it reached a constant value, and over-correction simultaneously ceased. In contrast, the absorption of the light emitted from a lead lamp was independent of the bandwidth.

In Fig. 2 the absorption spectrum of a 100 g Sb l^{-1} matrix is shown in the region of the resonance line of lead, at a 1-nm bandwidth. For comparison purposes the spectrum obtained by nebulizing pure (1 + 1) hydrochloric acid and the emission line profile of lead are shown. The two absorption spectra are two nearly parallel lines. The distance between them corresponds to the true background absorbance. Its slope is due to the fact that the radiation from the hydrogen lamp is wavelength-dependent. On the antimony spectrum, an elongated peak is observed, which has its maximum at 217.6 nm, i.e. it corresponds to the resonance line of antimony. This peak and the line profile of lead overlap to a large extent at the 1-nm bandwidth used. Thus, antimony absorption is detected at the lead wavelength.

The experiment was repeated by narrowing the slit-width. The results are presented in Fig. 3. At 0.66-nm bandwidth the two peaks still overlap, but at less than 0.33 nm they are completely resolved. This is in full agreement with the results shown in Fig. 1. Thus, when measurements were made by lead lamp at 0.2-nm bandwidth, an unchanged 0.014 absorbance was obtained for the above antimony solution, while with a hydrogen lamp the background value was decreased to 0.012; thus a correction could have been made.

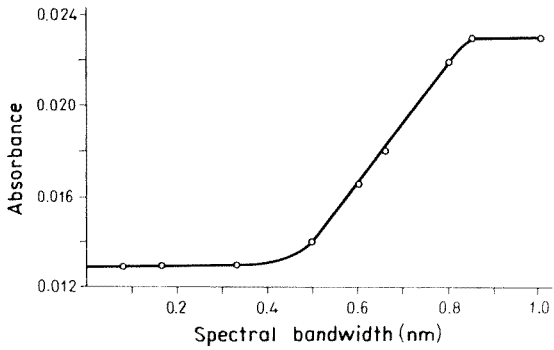

Fig. 1. Variation in the background absorbance with bandwidth at the 217-nm lead line by nebulizing a 100 g Sb l^{-1} solution into an air—acetylene flame (H_2-lamp).

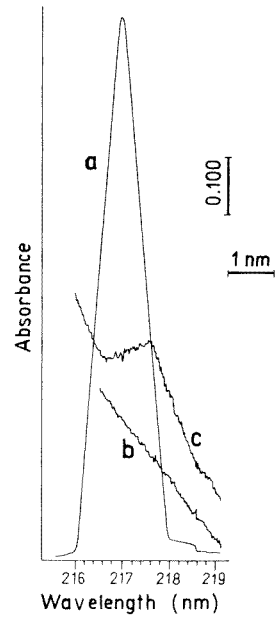

Fig. 2. (a) Emission profile of a lead hollow-cathode lamp (arbitrary units); and the absorbance spectra obtained by using continuous radiation and nebulizing; (b) (1 + 1) hydrochloric acid; (c) 100 g Sb l^{-1}. Spectral bandwidth, 1 nm.

Both the over-correction and its removal can be demonstrated if an automatic background corrector is used. In Fig. 4, the spectra obtained with an AA-775 spectrometer are shown, when a 50 g Sb l^{-1} solution contaminated by lead was measured at 217.0 nm. For a 1-nm bandwidth, the background corrector over-compensates, while at 0.5 nm accurately corrected absorbances are obtained. All these data agree with those obtained by measuring background-only values.

Theoretically, more accurate background correction values will be obtained at larger bandwidths, since the degree of line absorption of the element determined is negligible, and the baseline is more stable, so that the relative standard deviation will be less. For this reason, in cases similar to those quoted above, it is necessary to seek the largest bandwidth at which the line of the matrix element and that to be determined are still resolved. This is the optimal bandwidth for background measurement.

In general, when any trace element is to be determined by a.a.s., it should always be ascertained whether or not any resonance line of the matrix element could be transmitted by the monochromator, after the usual optimal conditions for the element determined have been selected. If it could, the monochromator slit-width must be modified to give the optimal bandwidth described above.

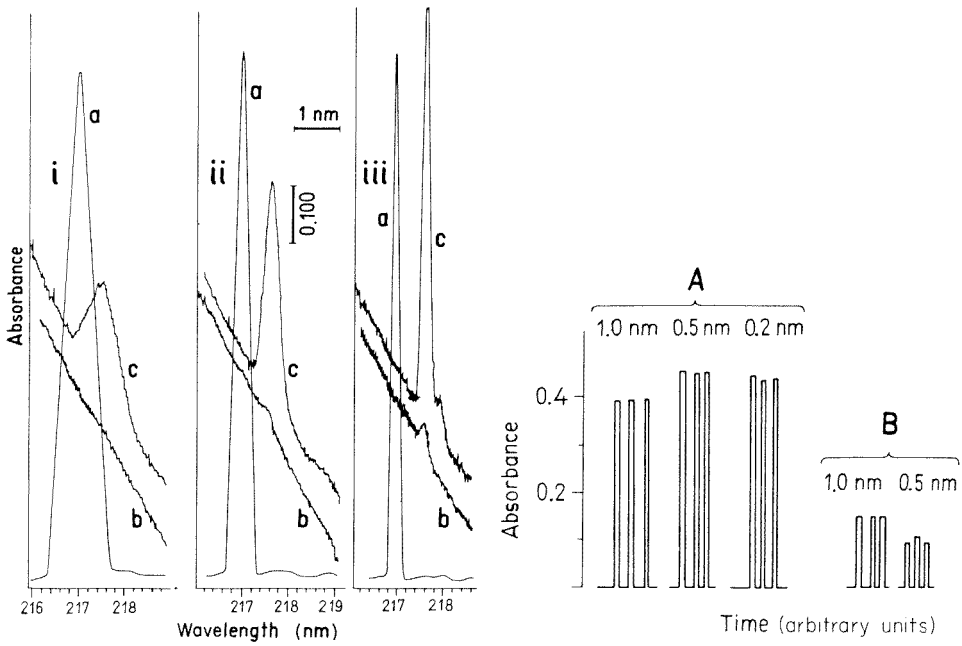

Fig. 3. Spectra obtained as in Fig. 2, but with spectral bandwidths of (i) 0.66 nm; (ii) 0.33 nm; (iii) 0.17 nm.

Fig. 4. (A) Absorbance of lead in an antimony solution by automatic background compensation and (B) background-only measurement, at various bandwidths.

In order to calculate from known data the optimal bandwidth for any pair of interfering elements (without doing separate measurements), several practical cases were studied, starting from the absorption spectra obtained for various elements. For demonstration purposes, the spectral conditions and the variations in the background absorption were represented graphically and, as an example, the determination of zinc in tellurium was chosen. The outcome is shown in Fig. 5. The wavelength axis shows the maxima of the resonance line profiles of both elements. For the two spectral lines, the band half-widths transmitted at the given bandwidths, for spectra with peaks similar to those shown in Figs. 2 and 3, are represented by short vertical lines. (The half-width values in the figure, of course, are not identical with the true half-width of the absorption line profile.) The intersection of their boundary lines (dotted) indicates clearly the point (w') where the two lines become completely separated from each other.

The same Figure also shows the change of background absorbance with bandwidth, as measured by a hydrogen lamp (similar to Fig. 1). The bandwidth at which the absorption became constant (w) is indicated. The bandwidths obtained by these two methods are in good agreement.

In order to investigate to what extent w depends on the sensitivity of the

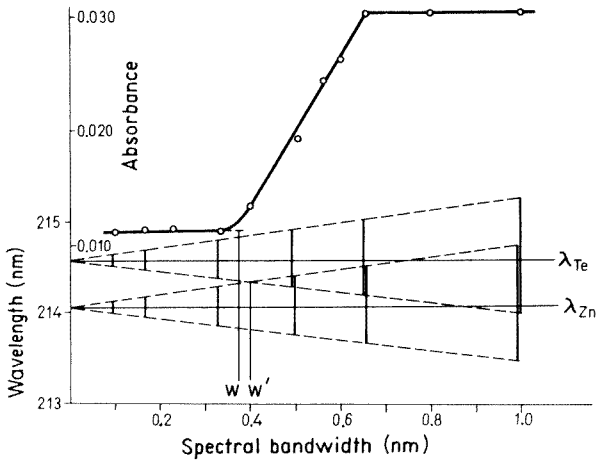

Fig. 5. Variations in the background absorbance at the zinc line (○), and the recorded half-width values as a function of the spectral bandwidth when determining zinc in tellurium at 213.9 nm.

matrix-metal line and on the concentration, the roles of the two metals were exchanged and the background absorbance was measured at the tellurium line in 20, 50 and 100 g Zn l^{-1} solutions. In this way the measurements were carried out at a matrix line 30-times more sensitive, under otherwise the same spectral conditions. The results are summarized in Fig. 6 in a way similar to those presented in Fig. 5. The theoretical value of w' is unaltered. However, practical background measurements provided substantially smaller

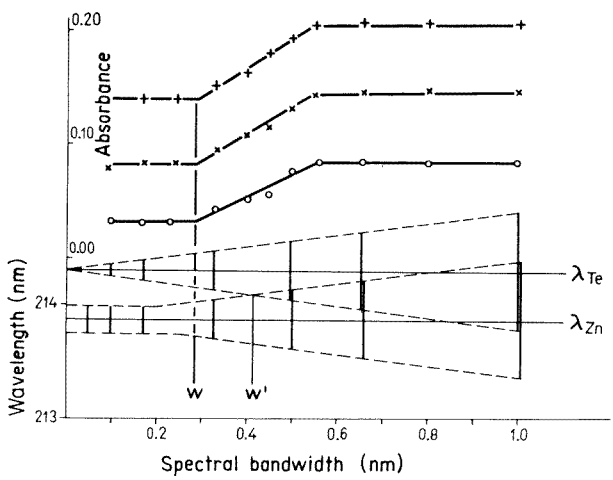

Fig. 6. Variations in the background absorbance on the tellurium line and the measured half-width values as a function of spectral bandwidth at the 214.3-nm zinc line, for (○) 20; (×) 50; (+) 100 g Zn l^{-1}.

w values. For measurements done on the above zinc solutions it was demonstrated that the matrix concentration had an effect on the background absorption, but not on the optimal bandwidth. Figure 7 shows the full peak widths instead of half-width values. This representation gave a value of w' identical to that of w shown in Fig. 6.

Thus, as a result of the theoretical determination of the optimal bandwidth two extreme cases have been obtained, one by using the half-width of the matrix line, and the other by using the full width value.

It can be derived geometrically that the optimal bandwidth is equal to the spectral distance between the two lines ($w = \Delta\lambda$) in the first case and to $w = \Delta\lambda/1.5$ in the second case. From an examination of many pairs of elements, it can be stated that the value of w is always between these limits (Tables 1 and 2). Thus, a knowledge of the distance between two lines allows the approximate value of the optimal bandwidth to be determined, without doing separate measurements.

After studying the absorption lines by the above method, three interesting conclusions were drawn. Firstly, in Figs. 6 and 7 it can be observed that the width of the zinc line does not tend to zero with decreasing bandwidth, but tends toward a finite value corresponding to the width of the resonance line profile, which can be as great as 0.1 nm. Very great line broadening was observed for the most sensitive resonance lines (Cd 228.80 nm, Mg 285.21 nm) as a function of sensitivity and concentration. On account of this broadening, it was not possible to separate any nearby analytical wavelength from the absorption line profile of the matrix element even when the smallest bandwidths were used, e.g., when iridium was determined at 284.97 nm in a solution containing 50 g Mg l^{-1}.

Secondly, a number of sensitive absorption lines which interfere in background correction are not included in tables of the atomic absorption literature, because they are unimportant from an analytical point of view. Among them are some which can be resolved into several lines by using higher resolution. Their half-width does not depend in a regular way on the mono-

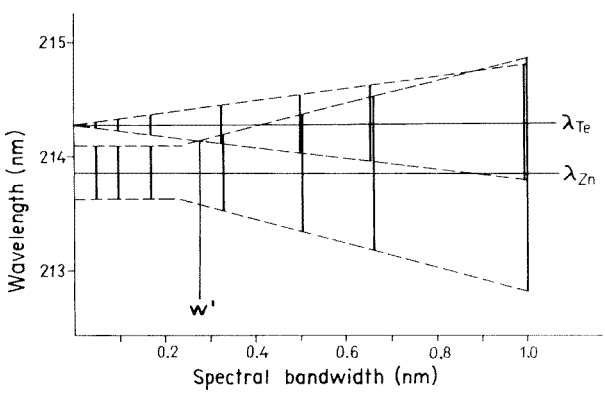

Fig. 7. Absorption lines as for Fig. 6, but displaying full wave widths.

TABLE 1

Pairs of elements for which background over-correction is possible
(λ_o, nominal wavelength of spectral line; $\Delta\lambda_o$, spectral distance apart; w, optimal spectral bandwidth; w_x is the spectral bandwidth generally recommended for the element to be determined. All wavelengths and bandwidths are in nm.)

Matrix element	λ_o	Element to be determined	λ_o	$\Delta\lambda_o$	w	w_x	Remarks
Ag	193.23	As	193.70	0.47	0.47	1.00	a
As	228.81	Cd	228.80	0.01	0.01	0.50	a,b
As	234.98	Be	234.86	0.12	0.1	0.50	a
B	208.96	Ir	208.88	0.08	0.1	0.10	a,c,d
Ba	350.11	Rn	349.94	0.17	0.15	0.20	a,d
Bi	195.96	Se	196.03	0.07	0.07	1.00	a
Co	217.46	Sb	217.59	0.13	0.11	0.20	a
Co	242.49	Au	242.80	0.31	0.26	0.50	e
Co	252.14	Si	251.61	0.53	0.50	0.20	a,c
Co	345.52	Re	346.05	0.53		0.20	a,c
	346.58			0.47			
Co	349.57	Ru	349.89	0.32		0.20	a,c
	350.23			0.34			
Cr	360.53	Zr	360.12	0.41	0.4	0.20	c,e
Cs	459.32	Eu	459.40	0.08		1.00	e
Cu	216.51	Pb	217.00	0.49	0.45	1.00	e
Cu	217.89	Sb	217.59	0.30	0.28	0.20	c,e
Cu	223.01	Bi	223.06	0.05	0.035	0.20	e,f
Cu	224.43	Sn	224.61	0.18	0.11	0.50	e
Cu	244.16	Pd	244.79	0.63	0.50	0.20	c,e
Cu	249.22	B	249.68	0.46	0.35	0.10	c,e
			249.77				
Cu	249.22	Fe	248.33	0.89	0.70	0.20	c,e
Cu	327.40	Ag	328.07	0.67	0.50	0.50	c,e
Dy	422.51	Ca	422.67	0.56	0.56	0.50	a,c
Eu	390.71	Sc	391.18	0.37	0.37	0.20	a,c
Fe	216.68	Pb	217.00	0.32	0.25	1.00	e
Fe	217.81	Sb	217.59	0.22	0.18	0.20	e
Fe	249.06	B	249.68	0.68	0.70	0.10	c,e,g
	250.11		249.77				
Fe	251.81	Si	251.61	0.20	0.20	0.20	a,c
	252.28				0.67		c,e
Fe	253.56	Hg	253.65	0.09	0.08	0.50	a
	254.10						a
Fe	254.60	W	255.14	0.54	0.20	0.10	c,e,g
	254.96			0.18			a

TABLE 1 (continued)

Matrix element	λ_o	Element to be determined	λ_o	$\Delta\lambda_o$	w	w_x	Remarks
Fe	294.79	Ga	294.36	0.43	0.4	0.50	a
Fe	303.74	In	303.94	0.20	0.20	0.20	a
Fe	358.12	Cr	357.87	0.25	0.20	0.20	e
Fe	358.12	U	358.49	0.37	0.28	0.10	c,e,g
Fe	371.99	Tm	371.79	0.20	0.17	0.50	e,g
Ho	410.38	Y	410.24	0.14	0.14	0.50	e
In	410.17	Ho	410.38	0.21	0.18	0.28	e
In	410.17	Y	410.24	0.07	0.06	0.50	e
Ir	266.48	Pt	265.95	0.53	0.50	0.20	c,e
Ir	314.04	Mo	313.26	0.78	0.77	0.50	a,c
Mg	279.55	Mn	279.48	0.07	0.06	0.20	a
Nb	358.03	Cr	357.87	0.16	0.16	0.20	a,d
Ni	216.56	Pb	217.00	0.44	0.43	1.00	a
Ni	303.79	In	303.94	0.15	0.15	0.20	a
Ni	313.41	Mo	313.26	0.15	0.13	0.50	e
Ni	324.31	Cu	324.75	0.44	0.44	0.50	a
Ni	343.35	Rh	343.49	0.14	0.14	0.50	a
Ni	349.29	Ru	349.89	0.60	0.46	0.20	c,e
Ni	359.77	Zr	360.12	0.35	0.35	0.20	a,c
Pb	217.00	Sb	217.59	0.59	0.38	0.20	c,e
Pb	224.69	Sn	224.61	0.08	0.07	0.50	a
Pb	280.20	Mn	279.48	0.72	0.70	0.10	a,c
Pb	368.35	Gd	368.41	0.06	0.06	0.10	a
Pd	276.31	Tl	276.79	0.49	0.37	0.50	e
Pd	324.27	Cu	324.75	0.48	0.35	0.50	e
Pt	216.52	Pb	217.00	0.48	0.42	1.00	e
Pt	248.72	Fe	248.33	0.39	0.35	0.20	c,e
Rb	421.55	Dy	421.17	0.38	0.38	0.10	c,e
Rh	328.06	Ag	328.07	0.02	0.02	0.50	a,f
Ru	240.27	Co	240.73	0.46		0.10	c
Ru	245.55	Pd	244.79	0.76		0.20	c
Ru	343.67	Rh	343.49	0.18		0.50	
Sb	271.58	Pb	217.00	0.58	0.47	1.00	e
Sn	214.87	Te	214.28	0.59	0.58	0.50	a
Sn	231.72	Ni	232.03	0.31	0.30	0.20	a,c
Sn	242.95	Au	242.80	0.15	0.12	0.50	e
Sn	254.65	W	255.14	0.49	0.43	0.10	c,e
Sn	266.12	Pt	265.95	0.17	0.14	0.20	e
Sn	303.41	In	303.94	0.53	0.53	0.20	a,c
Te	214.28	Zn	213.86	0.42	0.37	0.50	e
Ti	398.97	Yb	398.80	0.17	0.17	0.50	a

TABLE 1 (continued)

Matrix element	λ_o	Element to be determined	λ_o	$\Delta\lambda_o$	w	w_x	Remarks
Tm	410.58	Ho	410.38	0.20	0.20	0.20	c,e
Tm	410.58	Y	410.24	0.34	0.37	0.50	e
V	251.96	Si	251.61	0.35	0.35	0.20	a,c
V	294.15	Ga	294.36 294.42	0.21	0.21	0.50	a
V	368.81	Gd	368.41	0.40	0.40	0.10	a,c
W	400.87	Er	400.80	0.07	0.07	0.50	e,f
Y	410.24	Ho	410.38	0.14	0.12	0.20	e
Yb	328.94	Ag	328.07	0.87	0.50	0.50	c,e
Zn	213.86	Te	214.28	0.42	0.29	0.50	e
Zn	307.59	Hf	307.29	0.30	0.30	0.20	a,c,g
Zn	328.23	Ag	328.07	0.16	0.16	0.50	a

[a]On account of the low sensitivity of the interfering matrix line, any error of background over-correction can be neglected. [b]The difference in wavelengths is small and no optimal bandwidth can be chosen. It is necessary to make a preliminary separation of the matrix or a separate measurement of the background level at an adjacent wavelength (automatic background correction may not be used). [c]Within the bandwidth recommended, no background over-correction generally occurs. [d]As a lower atomizing temperature is used for the determined element, there is no absorption of matrix line at this temperature. [e]Because of the atomic absorption of the matrix metal, substantial errors may occur in the background correction. [f]The difference in wavelengths is small and no optimal bandwidth can be chosen. Consequently, another analytical line should be used. [g]The possibility of background over-correction should be considered on account of the relatively low sensitivity for the determined element, even when relatively large concentrations are to be determined.

chromator bandwidth. For larger monochromator apertures it is smaller, and for smaller apertures, it is greater, than for single lines.

This phenomenon can be illustrated with a practical example. In Fig. 8, the half-width values for the 216.5-nm and 218.0-nm lines of copper at various spectral bandwidths are shown as in the previous Figures. The latter line, a close doublet, interferes in the background correction of antimony, while the former interferes with that of lead (Fig. 9).

Thirdly, for lines lying very close together, the plot of background absorbance against bandwidth has a maximum, as seen for the resonance line of bismuth in a copper matrix (Fig. 10). The lines are only 0.06 nm apart. Thus, with decreasing bandwidth, the intensity of the copper line increases up to the point where the maximum of the line profile falls within the transmitted band. Above this point, the intensity tends to be diminished, theoretically up to the point at which the two lines are completely resolved. The possibility of detecting this phenomenon is limited by the resolving power of the monochromator.

TABLE 2

Alternative analytical lines for some elements and matrix elements interfering with background correction
(Symbols and units as in Table 1)

Element to be determined		Matrix element		$\Delta\lambda_o$	w	w_x	Remarks
λ_o		λ_o					
Ag	338.28	Ni	338.06	0.22	0.18	0.50	e
Al	308.22		—			0.50	h
Al	396.15		—			0.50	h
B	208.89	Ir	208.88	0.01	0.01	0.10	b,e,g
	208.95						
Bi	227.66	Re	227.53	0.13	0.13	0.50	a,d,g
		Sn	226.89	0.77	0.70		c,e
Co	242.49	Au	242.80	0.31	0.22	0.50	e
		Sn	242.17	0.32	0.29		e
		Sn	242.95	0.46	0.40		e
Co	252.14	Fe	252.28	0.14	0.12	0.20	e
		V	251.96	0.18	0.18		a,d
Cr	359.35	Ni	359.77	0.42	0.40	0.50	a
Cr	360.53	Ni	361.05	0.52	0.45	0.50	a,c
Cr	427.48		—			0.50	h
Cu	216.51	Fe	216.68	0.17	0.15	0.50	e
		Pb	217.00	0.49	0.34		e
		Pt	216.52	0.01	0.01		e,f
Cu	217.89	Fe	217.81	0.07	0.04	0.20	e,f
		Pb	217.00	0.89	0.60		c,e
		Sb	217.59	0.30	0.23		c,e
Cu	327.40	Ag	328.07	0.67	0.45	0.45	e
		Rh	328.06	0.66	0.64		a
Dy	419.49	Tm	418.76	0.73	0.70	0.10	c,e
Er	248.81	Cu	249.21	0.40	0.35	0.20	c,e
		Pt	248.72	0.09	0.07		e
Fe	271.90	Ga	271.97	0.07	0.05	0.20	a
		Ta	271.47	0.43	0.43		c,e,d
Ga	287.42	Pb	287.33	0.09	0.08	0.50	a,f
Ga	417.21		—			0.50	h
Gd	405.82	Nb	405.89	0.07	0.07	0.20	a,g
		Pb	405.78	0.04	0.03		a,f,g
Ir	363.97		—				h
Ir	284.97	Mg	285.21	0.24	—	0.1	c,i
Lu	356.78	Ni	356.64	0.14	0.14	0.50	a,g
		Rh	357.02	0.24	0.24		a,g

TABLE 2 (continued)

Element to be determined λ_o		Matrix element λ_o		$\Delta\lambda_o$	w	w_x	Remarks
Mn	279.83	Pb	280.20	0.37	0.36	0.20	a,c
Mn	280.11	Pb	280.20	0.09	0.08		a
Ni	341.48	Co	341.23	0.22	0.18	0.50	e
			341.26				
		Pd	342.12	0.64	0.60		e
Ni	352.45	Co	352.68	0.23	0.18	0.50	e
		Os	352.86	0.41	0.33		e
Pb	283.30	Os	283.86	0.56	0.55	0.50	a,c
		Pt	283.03	0.27	0.27	0.20	e
		Sn	284.00	0.70	0.70		c,e
Pd	247.64	Fe	247.98	0.34	0.27	0.20	c,e
Pt	299.80	Fe	299.44	0.36	0.30	0.50	c,e
		Fe	300.10	0.30	0.23		c,e
		Ni	300.25	0.45	0.42		a
		Pd	300.27	0.47	0.47		a,c
Re	345.19	Co	345.35	0.16	0.16	0.20	a,c
Re	346.47	Co	346.58	0.11	0.10	0.20	a,g
Sb	306.83	Bi	206.17	0.66	0.60	0.50	c,e
Sb	231.18		—				h
Sc	327.36	Ag	328.07	0.71	0.47	0.20	c,e
		Cu	327.40	0.04	0.03		e,f
		Rh	328.06	0.70	0.62		a,c
Si	250.69	Fe	250.11	0.58	0.45	0.50	e,g
		Fe	251.08	0.39	0.33		e
Si	251.43	Fe	251.08	0.35	0.30	0.20	c,e,g
Si	252.41	Co	252.14	0.27	0.22	0.50	e,g
		Fe	252.28				
			252.43	0.02	0.02		e,f
Sn	235.48	As	235.00	0.48	0.48	0.50	a
Sn	286.33						h
Ti	364.27	Pb	364.00	0.27	0.26	0.50	a
V	306.64	Bi	306.77	0.13	0.10	0.50	e,g
		Pt	306.47	0.17	0.15		e,g
W	400.88	Er	400.80	0.08	0.06	0.50	b,e,g
Y	414.24		—			0.50	h
Zr	468.78		—			0.10	h

[a–g]See Table 1. [h]On account of the atomic absorption of the matrix element, no background over-correction is to be expected. [i]A higher matrix concentration interference is probable, because of line broadening.

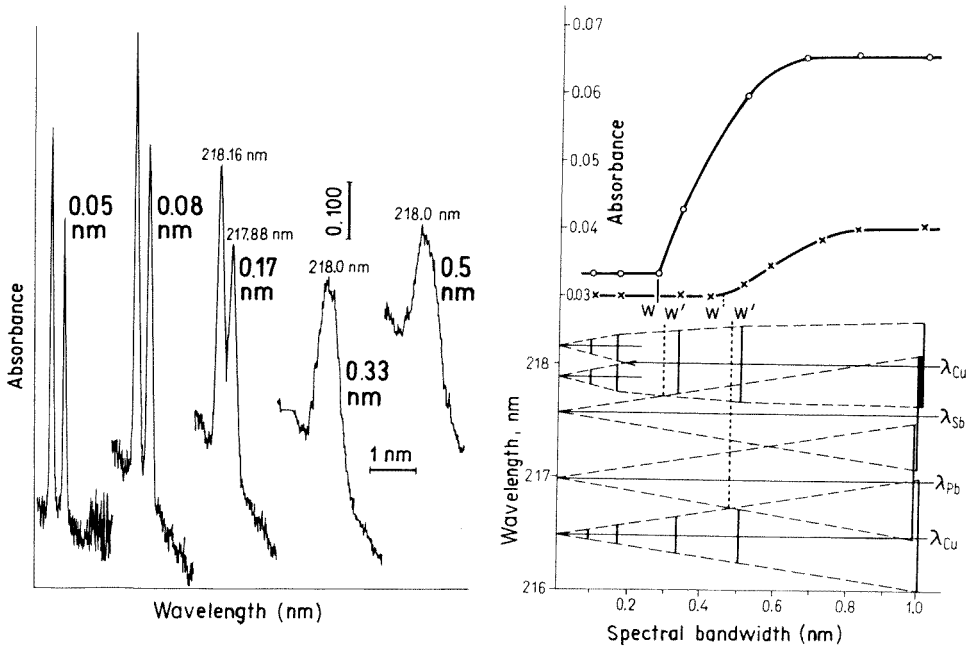

Fig. 8. Absorption of hydrogen lamp radiation in nebulization of a 50 g Cu l^{-1} solution, at the spectral bandwidths indicated.

Fig. 9. Background absorbance vs. spectral bandwidth at (○) the 217.6-nm antimony line and (×) the 217.0-nm lead line, in nebulization of 100 g Cu l^{-1} solutions.

DISCUSSION

For every analytical case in which the magnitude of the background absorbance is commensurate with that of the atomic absorption signal, it is recommended that it should be ascertained whether the accompanying element(s) produce line absorption at the given wavelength, within the given spectral bandwidth. The possible cases are included in Table 1, based on test results. This Table omits any spectral lines for which no absorption of continuous radiation was detected on nebulizing into the flame a solution of the relevant matrix element at a concentration of 50 g l^{-1}. For this reason, the pair of elements (Se, Fe) reported by Manning [2] has been omitted.

Some comments on the correction errors are provided at the end of Tables 1 and 2. Sometimes these remarks are, apparently, contradictory. To which of them priority should be given depends on the conditions of the relevant test (e.g., the relative concentrations).

For several reasons, analytical lines other than the most sensitive ones, are used. One of the potential reasons is that a particular line is too close to one of the matrix lines. Table 2 includes data on alternative lines for which the characteristic concentration of the relevant element is not more than ten

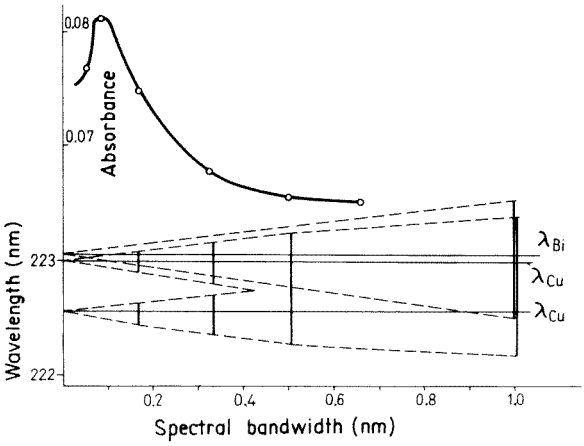

Fig. 10. Plots of background absorbance at the Bi line (○), and the recorded half-width values vs. spectral bandwidth in the determination of bismuth at 223.1 nm in a 50 g Cu l^{-1} solution.

times the value measured at the most sensitive line, together with the possible background correction interferences.

Conclusions

When small analytical atomic absorption signals are measured, the line absorption of the matrix element present at a concentration exceeding 1 g l^{-1} leads in several cases to substantial inaccuracies. The errors are due to the inexact measurement of the value of the background absorption. Each of the pairs of elements included in Tables 1 and 2 may occur in routine or specialized analytical work. A knowledge of the possibilities of background over-correction then allows the analytical error to be substantially diminished by using optimal bandwidth values or other solutions to the problem.

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DETERMINATION OF SUB-NANOGRAM AMOUNTS OF MERCURY BY COLD-VAPOUR ATOMIC FLUORESCENCE SPECTROMETRY WITH AN IMPROVED GAS-SHEATHED ATOM CELL

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SUMMARY

An improved gas sheathed cell for the cold-vapour determination of mercury by atomic fluorescence spectrometry is described. Mercury ions in aqueous solution are reduced by tin(II) chloride, and the mercury flushed from solution by argon is discharged from a tube situated adjacent to the spectrometer entrance slit and a mercury electrodeless discharge lamp. A second stream of argon is directed up small capillaries arranged around this outlet tube to provide a laminar sheath of argon around the atom cell. At optimised flow rates, the signal is about 10 times greater than when no laminar sheath is provided. Precision is also improved. The limit of detection (2σ) is 0.01 ng of mercury for a 0.5-ml aliquot of sample (i.e. 20 ng l^{-1}). The accuracy of the system is demonstrated by the determination of mercury in NBS Orchard Leaves and in barley seeds. Typically relative standard deviations are in the range 0.6—3%.

Environmental and toxicological concern over mercury has been reflected well in recent years in the large volume of analytical literature presenting methods for the determination of mercury at trace levels and below. Atomic absorption spectrometry (a.a.s.) and atomic fluorescence spectrometry (a.f.s.) are commonly used to determine mercury at the trace level with both flame and flameless atom cells. In flames, typical detection limits are 0.5 mg l^{-1} for a.a.s. [1] and 0.1 mg l^{-1} for a.f.s. [2]. When the cold-vapour reduction/aeration method is used, the reported detection limits are much lower, e.g., 0.6 ng for a.a.s. for 5-ml samples [3] and 0.02 ng for a.f.s. for 1-ml samples [4].

The comprehensive review by Ure [5] summarised the various approaches to the determination of mercury by cold-vapour a.a.s. and a.f.s. Two methods are commonly used to generate mercury vapour for subsequent delivery into the optical path of an atomic spectrometer. The first involves amalgamation of the mercury, electrolytically onto copper wire [6], or directly from solution onto silver screens or coils [7, 8] or from the vapour onto gold wool [9], followed by desorption of the mercury vapour on heating. Such

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methods have the advantage of introducing both a preconcentration step and a selective separation step, whereby potential interferences may be rejected, but they can be quite complex. The second method, the cold-vapour reduction/aeration technique pioneered by Hatch and Ott [10], is therefore quite generally preferred for the determination of mercury by a.a.s. The cold-vapour a.a.s. method, however, suffers from some disadvantages which may be overcome by using a.f.s. With cold-vapour a.a.s. at low mercury levels, fogging of the cell windows by moisture necessitates drying the vapours prior to their entry to the atom cell, and the desiccant can be both a source of contamination and mercury loss [11]; when a windowless cell is used in a.f.s., such problems are avoided. Molecular absorption interferes with cold-vapour a.a.s. and background correction is highly desirable; in a.f.s. molecular absorption poses a smaller problem. Advantages of improved sensitivity and greater linear range are also available with a.f.s.

The design of the atom cell is of critical importance if the full advantages of a.f.s. over a.a.s. are to be achieved. Thompson and Reynolds [12] recognised this when they used a cell without windows, later modified by using a simple "sheath unit" constructed from acetyl resin [4]. Full details of the construction of the sheath, which was designed to produce a laminar shield of argon around the atom cell, were not given but an improvement in the signal of a factor of two, attributed to decreased quenching by entrained air, was reported. Cavalli and Rossi [13] reported a "gas-shielded mercury vapour sprayer" for a.f.s. This was manufactured from perspex and the central mercury vapour channel was protected by a laminar flow of argon issuing from a 1-mm annulus. An improvement in the intensity of the fluorescence signal of 28-fold was obtained but the device appears to require careful engineering.

In this paper, the construction of a simple device from routinely available laboratory glass-ware is described; this device allows the determination of mercury by a.f.s. using a windowless cell with a protective laminar shield of argon. Optimisation of the shielding gas flow-rate and the total system are reported. This improved fluorescence cell has been used for a variety of practical analyses with a conventional dispersive spectrometer. It should be equally suitable for use with a non-dispersive system of the types recently reported [14, 15] but not widely available. The performance of the system is evaluated by the determination of mercury in barley seeds and NBS Orchard Leaves.

EXPERIMENTAL

Equipment

The reduction vessel and fluorescence cell are shown in Fig. 1. The former is a 3-necked pear-shaped flask of 25-ml capacity. The fluorescence cell was constructed as follows. Approximately 150 capillary tubes (60 mm long, 0.7 mm i.d.) of the type commercially available as melting-point tubes, were

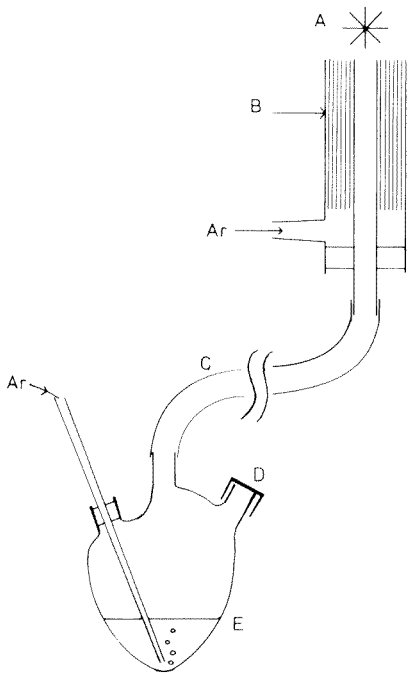

Fig. 1. Diagram of reduction vessel and fluorescence cell. (A) Fluorescence zone; (B) capillary tubes; (C) polypropylene tubing; (D) rubber septum; (E) reduction cell plus tin(II) chloride solution.

glued round the outside of a pyrex glass tube (6 mm i.d.). This arrangement was secured inside a shortened Hirsch filter tube (22 mm i.d.), using the side arm as the gas inlet. The bottom of the tube was sealed with a rubber bung. The glassware was painted matt black on the outside.

A syringe was used to inject sample aliquots via the rubber septum (Fig. 1) into tin(II) chloride solution. Mercury vapours were removed by a flow of argon into the central tube, while a second flow of argon was passed through the surrounding capillary tubes to produce a laminar stream of argon around the central flow. The fluorescence was excited and measured in the volume of gas 2—25 mm above the device. The laminar stream eliminated the effects of draughts and lateral diffusion of sample vapour, and prevented entrainment of atmospheric oxygen which effectively quenches mercury fluorescence.

The cell was mounted vertically in an atomic absorption spectrometer in place of the burner/nebulizer assembly, and at the focal point of the spectrometer lens. The spectral source, a mercury electrodeless discharge lamp, was arranged so that the volume immediately above the cell was irradiated and fluorescence radiation passed through the spectrometer focussing lens to the monochromator. The geometry of this arrangement is shown in Fig. 2. The distances were: light source to cell 65 mm; cell to spectrometer lens 78 mm;

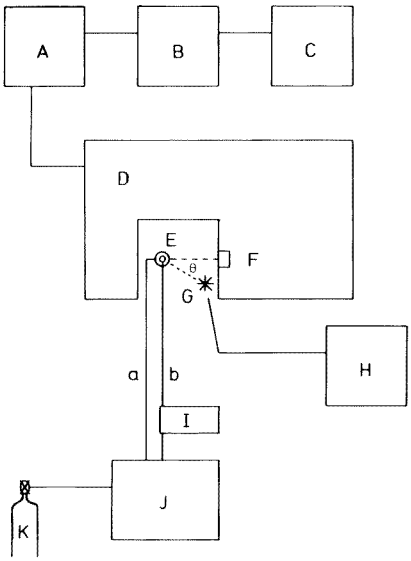

Fig. 2. Block diagram of instrumentation. (A) Linear amplifier; (B) precision integrator; (C) chart recorder; (D) atomic spectrometer; (E) laminar flow device; (F) lens to spectrometer entrance slit and monochromator; (G) electrodeless discharge lamp in microwave cavity; (H) microwave generator; (I) reduction cell; (J) gas pressure and flow controller; (K) argon supply. (a) Argon for laminar flow; (b) argon for flushing Hg to atom cell; (θ) angle subtended by source, atom cell and slit, 45°.

an angle of 45° was subtended. The mercury lamp was air-cooled by directing the air stream from the spectrometer compressor down through the top of the microwave cavity. The spectrometer was operated in its "emission" mode and fluorescence was detected at 253.7 nm with a spectral bandpass of 2.0 nm. Fluorescence peaks were obtained on a strip-chart recorder and an integrator provided peak area measurements. Instrumentation and operating conditions are given in Table 1.

Reagents

Tin(II) chloride solution. Shake tin(II) chloride (40 g; general-purpose reagent) with 100 ml of 1 M hydrochloric acid. Shaking the suspension before use yielded a suitably homogeneous mixture. Add a few granules of tin to prevent aerial oxidation. Purge the solution with argon before use to remove any absorbed mercury.

Potassium dichromate solution. Dissolve potassium dichromate (0.3 g; analytical-reagent grade) in 1 l of 1.6 M nitric acid (analytical-reagent grade).

Phenylmercury(II) acetate solution. Phenylmercury(II) acetate was prepared as described by Makarova and Nesmeyanov [16], and twice recrystallised. For the stock solution, dissolve 0.4197 g in potassium dichromate solution and dilute to 1 l with this solution. By appropriate dilution, prepare a 0.25 mg Hg^{2+} l^{-1} solution.

TABLE 1

Instrumentation and optimum operating conditions

Component	Model	Supplier	Operating conditions
Spectrometer	IL151	Instrumentation Laboratories, Lexington, MA	Flame emission damping factor 1 s; scale expansion ×5; PMT voltage 700; wavelength 253.7 nm; spectral bandpass 2.0 nm
Excitation source	Mercury EDL	EDT Research Ltd., London NW10	36 W incident power cooled by an airflow of ca. 3 l min^{-1}
Microwave cavity	Broida ¾-wave cavity, 210 L powered by a Microtron 200 Mark III microwave generator	Electromedical Supplies, Wantage, Oxon	
Gas flow rate controller	Panchromatogram	Pye-Unicam, Cambridge	Reference outlet for flushing flask and column 1 outlet for laminar flow. Column 2 outlet closed
Chart recorder	Elektronik 194	Honeywell, Brentford, Middlesex	Typically 5 min/in. and 10 mV f.s.d.
Integrator feeding linear amplifier	5530000	Honeywell	10 mV span
G.c. integrator	TP503	Honeywell	Typically 1% threshold level, 1s response time
Syringe	1 ml disposable syringe (plastic)	Gillette, Brentford, Middlesex	

Mercury(II) nitrate solution. Dissolve mercury(II) nitrate hemihydrate (1.6630 g) in 1 l of potassium dichromate solution. Prepare calibration standards by appropriate dilution.

Procedures

Preservation of standards and sample digests. Stabilise standard solutions and sample digests by dilution with a solution of potassium dichromate (0.3 g l^{-1}) in dilute nitric acid (1.6 M) as recommended by Feldman [17]. When the solutions were stored in borosilicate glassware, this diluent prevented loss of mercury for several days at levels of 1 ppb and lower, and for several weeks at concentrations of 20 ppb and higher.

Digestion of seeds and orchard leaves. Take a known weight of the sample (2.0 g of orchard leaves or 0.5 g of barley seeds) and allow to stand overnight in concentrated nitric acid (20 ml for the orchard leaves and 10 ml for the seeds) in a stoppered flask. Reflux at 80°C on a water bath for 30 min. Dilute the resulting solution in a volumetric flask (to 50 ml for the orchard leaves and 100 ml for the seeds) using the potassium dichromate solution as diluent.

Determination of mercury. Switch on both argon gas flows. Inject aliquots (0.5 ml) of the standard and sample solutions through the rubber septum into the tin(II) chloride solution (3 ml) in the reduction call. Sweep the evolved mercury into the atom cell and record the peak area of the fluorescence signal. Inject each solution at least 5 times and calculate the mean peak area. Elution time varies as a function of the mass of mercury injected but is typically complete in 30—100 s.

RESULTS AND DISCUSSION

Optimisation of the system

Gas flow rates. Argon was chosen both for flushing the reduction cell and for laminar flow because of its low cross-section of quenching of fluorescence relative to air and nitrogen. The two flow rates were optimised by measuring the size of the fluorescence signal at 5 different carrier flow rates, each one being used with six different laminar flow rates. The results of this experiment are shown in Fig. 3. It can be seen that for a given laminar flow rate, the signal decreased as the carrier flow rate increased because the residence time of mercury atoms in the light path was reduced by the increased driving force of the carrier stream. At very low carrier flow rates (0.3 l min^{-1}

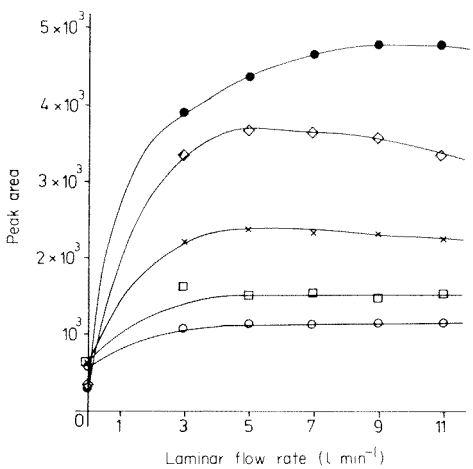

Fig. 3. Effect of argon flow rates on fluorescence intensity (arbitrary units). Carrier flow rate: (•) 0.3; (◇) 0.4; (×) 0.5; (□) 0.7; (○) 0.9 l min^{-1}.

or less), however, insufficiently vigorous flushing caused the mercury to reach the fluorescence zone too diffusely, resulting in deformed peaks and poor precision. This effect became more pronounced as the volume of solution in the reduction cell increased after each subsequent injection. Although peak area was only slightly affected, peak height and shape were markedly altered, the peak width at half-height increasing with the volume of solution in the reduction cell.

Thus, the optimum carrier flow rate was identified as 0.4 l min^{-1}, and this curve in Fig. 3 shows a maximum at a laminar flow rate of 5.0 l min^{-1}. It might be expected that the confinement of mercury in the volume irradiated by the electrodeless discharge lamp would be more efficient at higher laminar flow rates, but at certain optimum flow rates "pinching" occurred some distance above the fluorescence zone and vertical expulsion of the mercury was retarded. This further increased the residence time of the mercury in the light path and so enhanced the signals. This effect was confirmed by placing two Dreschel bottles, one containing dilute ammonia solution and the other dilute hydrochloric acid, in series in the laminar flow stream. When the evolved ammonium chloride was passed through the capillary tubes at the above optimum flow rates, the "pinching" effect was clearly visible some 3—4 cm above the top of the laminar flow device. When the ammonium chloride was passed through the reduction cell instead, a regular, vertical flow of gas past the lamp and slit was observed up to 30 mm above the top of the laminar flow device. The "pinching" effect of the laminar flow then caused the ammonium chloride to eddy slightly in the space between the lamp and slit before slowly diffusing away vertically.

Some experiments were done with 3 mm i.d. tubing to introduce the aeration gas. Better results were, however, obtained with capillary tubing because more vigorous agitation occurred and sharper, more reproducible peaks were observed.

At the optimized flow rates, it was observed that the signal size was approximately 10 times greater than with no laminar gas flow. Precision generally was slightly improved at higher laminar flow rates, typically 1—5% relative standard deviation (RSD) in the range 3—11 l min^{-1}, compared with 6—26% RSD without the laminar gas flow.

Reduction cell volume. During the initial work, the reduction flask was connected directly to the bottom of the laminar flow device, but the frequent need to empty the cell led to difficulty in realigning the optics of the system. The flask could be removed without disturbing the laminar flow device when the two were connected via 450 mm of polypropylene tubing (10 mm i.d.). This increase in dead volume did not cause any peak broadening, but when a 100-ml flask was substituted for the 25-ml flask, much broader peaks were observed and peak area decreased by approximately 20%. With the smaller flask there was obviously less dispersion of mercury vapour within the flask, and the mercury vapour travelled along the tube as a more compact zone. The 25-ml flask was therefore used for all subsequent work.

Sample size. The need for the small reduction cell placed constraints on the size of the sample used. When 0.4 l Ar min^{-1} was used for flushing, excessive frothing occurred (causing large amounts of water vapour to enter the fluorescence zone) when the total volume of solution in the cell exceeded 14 ml. Moreover, the mercury could not be purged rapidly from the large volume of solution and peak broadening occurred. When 3 ml of tin(II) chloride solution was used with injection volumes of 0.5 ml, up to 20 injections could be made before it became necessary to empty the cell. In practice the tin(II) chloride was replaced after 15—18 injections.

Injection volume precision was checked by injecting eleven 0.5-ml aliquots into the cell on a precision balance and weighing each injection. Three different syringes were used and a mean relative standard deviation of 0.6% was obtained.

Angle of fluorescence measurement. Conventionally the source—cell—detector geometry in a.f.s. has been set at 90° for reasons of convenience, despite the increased prevalence of scatter at this angle which in turn results in elevated background readings. When the 90° angle was used here, a significant background signal was obtained, and consequently smaller angles (front surface illumination) were investigated. At an angle of 45°, the background signal was much reduced, so that greater signal amplification could be used with minimal increase in noise. To accommodate this new geometry, the laminar flow device could not be situated closer than about 75 mm from the entrance slit lens of the spectrometer. However, any loss in signal arising from the increased distance between the atom cell and detector, and hence the decrease in solid angle of fluorescence detected, was compensated by the new position of the cell falling at approximately the focus of the spectrometer lens. A small, blackened collimator placed in front of the microwave cavity aperture reduced scatter and reflection in the spectrometer cavity still further.

Microwave power. A graph of integrated fluorescence signal against incident microwave power was linear from 18 W (lowest available setting) to about 48 W. At high power, noise was excessive; the highest incident power level yielding consistently acceptable signal-to-noise ratios was about 36 W.

Limit of detection and linear working range

Under the above optimised conditions, the limit of detection (that mass or concentration of mercury producing a signal equal to the blank mean plus twice the standard deviation of the blank mean) was 0.010 ng for 0.5-ml injections, i.e. 20 ng l^{-1}.

At high concentrations of mercury, little scale expansion was required and the effects of baseline drift were minimal. Good precision was obtained, typically 1—3% RSD down to concentrations as low as 3 $\mu g\ l^{-1}$. At very low concentrations of mercury, however, short-term fluctuations in the baseline coupled with high scale expansion resulted in either premature or retarded

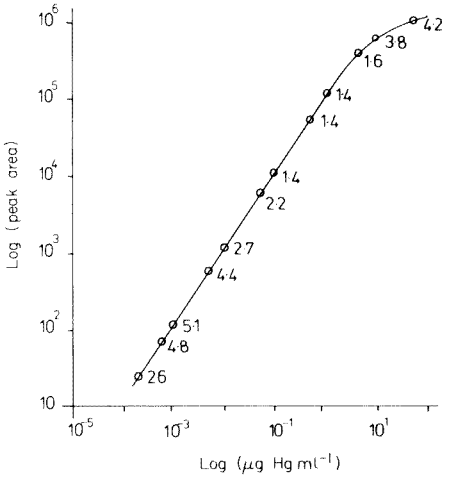

Fig. 4. Working curve for mercury at 253.7 nm obtained by plotting the logarithmic peak area (arbitrary units) against the logarithmic mercury concentration. The numbers on the curve are the RSD (%) values for 6 determinations at each point.

tripping of the integrator, and precision was degraded accordingly. If 10% is taken as the maximum tolerable RSD, the lower end of the useful working range of the method is 0.2 ng (i.e. 0.4 μg l^{-1} for 0.5-ml injections). The working curve and observed RSD values are shown in Fig. 4. It can be seen that the calibration curve was linear over approximately four orders of magnitude, from 0.2 ng (10% RSD) to 1000 ng (3% RSD).

Determination of mercury in Orchard Leaves and barley seeds

The analytical performance of the optimised system was assessed by determining mercury in Orchard Leaves (Standard Reference Material 1571, National Bureau of Standards, Washington, DC) and barley seeds which had been coated with a seed dressing containing phenylmercury(II) acetate. In each case a reagent blank was also determined and subtracted. The calibration curves were prepared from the aqueous mercury stock solution.

TABLE 2

Mercury content of Orchard Leaves and barley seed

Sample	Mean Hg found[a] (ng g^{-1})	Range (ng g^{-1})	SD of mean (ng g^{-1})	RSD (%)
SRM 1571	157[b]	153—159	1.0	0.6
Barley seed	10.74	10.55—10.98	0.07	0.6

[a]The values quoted are the means of 7 separate determinations; for each determination, 5 measurements were made on the same digest. [b]Certificate value, 155 ± 15 ng g^{-1}.

TABLE 3

Recoveries of phenylmercury(II) acetate (2.50 ng Hg g^{-1}) added to barley seeds (containing 10.74 ng Hg g^{-1})

Replicate number	1	2	3	4	5	6
Hg found (ng g^{-1})[a]	13.22	13.31	13.23	13.24	13.18	13.23
Recovery (%)	99.2	102.8	99.6	100.0	97.6	99.6
					Mean recovery =	99.8%

[a]Each reported figure is the mean of 5 measurements of the same digest.

Results for 7 replicate digestions of Orchard Leaves (see Table 2) showed both excellent agreement with the certified values and excellent precision.

The results for the determination of mercury in replicate digests of the barley seeds also show excellent precision (see Table 2). In this case, an independent mercury content figure was not available and the accuracy of the results was assessed by using recovery tests. Known amounts of phenylmercury(II) acetate were added to replicate samples of the seeds before digestion and then the mercury content determined as before. These results (Table 3) demonstrate that no mercury was lost during the digestion or analytical procedure.

Matrix effects were not expected as the mercury is separated from the matrix before measurement. The absence of such effects was confirmed by determining the mercury content of a series of solutions each containing a fixed volume of a standard solution of mercury and various known volumes of a seed digest of known mercury content. The plot of integrated fluorescence signal against mercury content of these solutions was found to be linear indicating no matrix enhancement or suppression effects.

Conclusion

The difficulty of preparing light sources offering a high signal-to-noise ratio has hindered the development of a.f.s. and largely negated the inherent advantages (increased sensitivity and extended linear dynamic range) of the technique over a.a.s. In the case of mercury it is relatively easy to prepare suitable sources and, for cold-vapour mercury determinations, a.f.s. offers additional advantages of excellent freedom from interference and the opportunity to use a windowless cell. The simply constructed apparatus and technique described above provides an elegant method for the determination of mercury in a wide range of samples. The device described can conveniently be mounted in any a.a.s. instrument and the results obtained demonstrate excellent accuracy and precision with impressive sensitivity.

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DETERMINATION OF ALUMINIUM IN BLOOD PLASMA OR SERUM BY ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY

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SUMMARY

A carbon-furnace atomic absorption method is used to determine aluminium in blood serum or plasma, diluted (1 + 2) with purified water prior to injection (20 μl) into the furnace. Procedures are described to reduce contamination during sample collection, storage and preparation of samples. A study of the interferences of inorganic ions shows that the temperature programme developed minimises these, allowing the use of aqueous standards for calibration. Ashing at 1400°C, prior to atomisation, also removes non-specific background effects, and optical correction is not required. A sample throughput of 50 duplicate analyses per day is possible and the precision (between batch) at 24 μg Al l^{-1} was 11.2% (n = 10) and at 340 μg Al l^{-1} was 6.3% (n = 18). Down to 4 μg Al l^{-1} can be determined. Reference values for a healthy population were 4.1—20 μg Al l^{-1} (mean 10.2).

Aluminium, formerly thought of as biologically inert, is now known to be toxic to patients with renal disease [1]. This is most readily established when treatment by haemodialysis uses water supplies which contain significant (over 50 μg l^{-1}) amounts of aluminium added as a flocculating agent. However, sustained oral ingestion of aluminium compounds, as phosphate-binding agents or antacids, is now also known to cause an increase in tissue levels of that element. The adverse effects of aluminium are most dramatically demonstrated in patients suffering dialysis dementia. This is a progressive series of neurological disorders leading eventually to death, with a geographical distribution corresponding to areas with high levels of aluminium in the domestic water supply, when this is used unpurified for renal dialysis [2]. Aluminium toxicity is also associated with bone disease and an unusually severe form of anaemia [3].

Instrumental methods that have been applied to the determination of aluminium in biological materials include flame atomic absorption [4, 5] and emission spectrometry [6], neutron activation [7—9], carbon-furnace

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atomic absorption spectrometry [10—16], and inductively-coupled plasma emission spectrometry [17]. The flame methods lack the required sensitivity for measurements below 1 mg l^{-1} and although neutron activation could be used to determine lower levels of aluminium, the methods developed are time-consuming and the facilities are not always readily available. Carbon-furnace atomic absorption spectrometry (c.f.a.a.s.) offers a sensitive and simple technique. Because of the unpredictable behaviour of undiluted biological fluids in the furnace when they are injected directly, and the potential of concomitant salts to interfere with the measurements, two approaches to this determination have appeared in the literature: direct injection of samples with little or no pre-treatment but attention paid to the choice of instrumental conditions, and wet ashing followed by measurements on the acid digest.

With the first approach, three problems can be encountered. Firstly, samples may not dry evenly and, in some cases, may splutter, resulting in imprecise and inaccurate results. Secondly, a residue may build up which interferes. Thirdly, aluminium may be lost as its chloride. In one of the earliest direct methods using electrothermal atomisation, Fuchs et al. [13] solved the first two problems for the analysis of serum by using a two-step ashing stage after slow drying at 100°C. Gorsky and Dietz [11], however, found that a single drying stage at 350°C for 60 s was adequate. In the method described by Pegon [14] for the determination of aluminium in cerebrospinal fluid and serum, a wetting agent (Teepol) was added to alleviate these problems, and in addition, aluminium was converted to aluminate with ammonia to avoid its loss as chloride. Triton X-100 was also found to be useful in solving the second problem, as described by Kaehny et al [18]. Alderman and Gitelman [16] used a diluent containing EDTA as well as Triton X to prevent precipitation and ammonium sulphate to prevent losses as chloride.

Using the second approach, Julshamn et al. [5] reported that although the serum matrix had been simplified after wet digestion they had to resort to standard addition to take care of inorganic matrix effects. However, digestion of the sample does solve the first two problems mentioned above. The major disadvantages associated with this approach are that it is time-consuming, the probability of sample contamination by aluminium in reagents and on vessels is greatly increased, and sensitivity is lost because of suppression of the signal by the high concentration of acid.

In deciding which approach to adopt for routine use in a clinical laboratory, three criteria were considered. These were, that the method should be simple, that sample pre-treatment should be minimal, thus reducing the sources of contamination, and that a throughput of 50—80 samples should be possible in a working day. The approach based on direct injection with careful control of instrumental conditions appeared to meet the above criteria but first the problems outlined had to be overcome. Earlier work by Sabet [19] had shown that a direct approach was feasible and his method

was applied to studies on patients on renal dialysis [3]. In this paper an improved, faster method is described.

EXPERIMENTAL

Apparatus

A Perkin-Elmer 370 spectrometer, equipped with a Perkin-Elmer HGA 76 heated graphite atomiser and AS-1 autosampler, was used. The signals from the spectrometer were recorded on a Servoscribe RE 541.20 potentiometric strip-chart recorder, which was automatically triggered during the atomisation stage. Table 1 shows the instrumental conditions used.

Reagents and standards

A stock solution of 1000 mg Al l^{-1} (aluminium nitrate, BDH standard solution for atomic absorption spectroscopy) was used to prepare standards. The reagents used in the interference studies (sodium chloride, calcium chloride, magnesium chloride, iron(III) chloride, hydrochloric acid and phosphoric acid) were of analytical-reagent grade. Water was deionised and then distilled.

Preparation of calibration standards. From the 1000 mg l^{-1} stock solution, a 10 mg Al l^{-1} intermediate stock standard was prepared daily; 0, 20, 40, 80, 120, 160 and 200 μl of this solution were pipetted into separate polystyrene vials and made up to 20 ml with distilled water. These standards correspond to 0, 10, 20, 40, 60, 80 and 100 μg Al l^{-1}, respectively.

Solutions for interference studies. A solution of 60 μg Al l^{-1} was prepared containing a given concentration of interfering ion (see Table 3). For comparison a simple aqueous standard of the same aluminium concentration was also prepared.

TABLE 1

Instrumental conditions for the determination of aluminium in serum

Wavelength (nm)	309.3	Sample size (μl)	20
Lamp current (mA)	30	Scale expansion on recorder	x 2
Spectral band-width (nm)	0.7		

	Temp. (°C)	Ramp setting[a]	Hold time (s)
Dry	110	Rate 2 (240 s)	30
Ash (1)	700	Rate 1 (7 s)	10
Ash (2)	1400	Rate 2 (240 s)	10
Atomise[b]	2700	Rate 0 (2 s)	10

[a]The rate at which the temperature increases from ambient to the maximum of about 2650°C. The time required to cover the entire temperature range is given in parentheses.
[b]Gas flow through the tube was stopped during the atomisation stage. Background correction was not essential.

Sample collection

Blood samples, obtained by standard venepuncture, were transferred into 10-ml polyethylene sample tubes containing heparin (for plasma) or empty (for serum). After being centrifuged at 2500 rpm for 15 min, the plasma or serum was transferred into 5-ml polystyrene sample tubes with tight-fitting polyethylene caps.

In the determination of reference values, blood was taken from normal persons through a plastic cannula. The cannula had been washed with blood by taking at least 30 ml of blood first for other determinations. A 10-ml portion of blood was then collected in an acid-washed plastic centrifuge tube. After being centrifuged as above, the serum was transferred to an acid-washed sample tube. The mean normal value for aluminium in serum obtained in this way was compared with the mean normal for samples collected using standard venepuncture techniques.

Analytical procedure

Transfer 200 μl of serum or plasma to an acid-washed disposable test-tube (LP3). Add 400 μl of purified water and mix thoroughly. Centrifuge at 3000 rpm for 15 min and transfer the diluted sample to an acid-washed auto-sampler cup. Arrange the cups containing standards, samples and a water blank in the auto-sampler tray, placing a standard after every five samples to act as a check for drift in calibration during the run. Set the instrument according to the conditions in Table 1. On starting the auto-sampler, a 20-μl aliquot is transferred to the furnace and the heating programme is started. Samples and standards are measured in duplicate. If the duplicates differ in peak height absorbance by more than 0.02, repeat the analysis. Plot a calibration graph of peak height against aluminium concentration .

Precautions against contamination

Test tubes and auto-sampler cups were cleaned by filling with 20% (v/v) nitric acid and leaving overnight. They were then thoroughly rinsed with distilled water before use. Pipette tips were cleaned before use by repeated pipetting of 20% (v/v) nitric acid and then distilled water.

RESULTS AND DISCUSSION

Choice of optimum conditions for the electrothermal atomiser

The drying temperature of 120°C and the temperature ramp rate 2 were chosen to ensure that the sample dried evenly and without spluttering.

To establish the optimum ashing temperature, a diluted (1 + 5) plasma sample was analysed using the conditions described in the experimental section but at various ashing temperatures. Figure 1 shows a plot of the absorbance of the background-corrected atomic absorption signal vs. ashing temperature. Three features on this plot are worth noting: below 800°C the

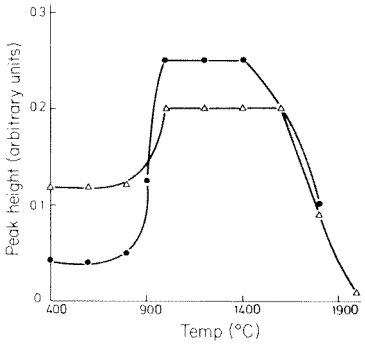

Fig. 1. Effect of ashing temperature on the aluminium peak height obtained during the atomisation stage, using the conditions given in Table 1 for different solutions: (△) a diluted (1 + 5) plasma containing ca. 340 μg Al l^{-1}; (•) a 2000 μg NaCl ml^{-1} solution containing 60 μg Al l^{-1}. Each point is the mean of duplicate determinations.

aluminium signal is depressed, between 800°C and 1000°C the aluminium peak height rises rapidly to a plateau, and at ashing temperatures above 1600°C aluminium is lost by volatilisation.

The rapid increase in the aluminium peak height between 800°C and 1000°C indicated that the depression is due to the inorganic matrix components rather than protein. Since the boiling point of sodium chloride is about 900°C a solution containing 60 μg Al l^{-1} and 2000 mg NaCl l^{-1} was prepared and examined under identical conditions. Figure 1 also shows these results. It can be seen that the characteristics of the plots are similar with the exception that aluminium is lost at an ashing temperature above 1400°C in the sodium chloride solution. These experiments demonstrate that inorganic matrix components play a significant role in determining the observed aluminium peak height. To remove this interference, an ashing temperature greater than 1000°C is required. This is in agreement with the results of Persson et al. [20] who studied the effects of hydrogen, chlorine, sulphur and nitrogen on the aluminium signal, and concluded that to remove interfering elements the ashing temperature should be high.

In an attempt to shorten the total time required, the temperature ramp Rate 1 was applied throughout the ashing stage rather than up to 700°C as indicated in the experimental section. However, this resulted in a reproducible loss of 11% of the aluminium signal (Table 2), which is probably due to co-volatilisation of aluminium with the inorganic matrix. Thus, a fast ramp rate can be used up to 700°C, but during the crucial stage at which the inorganic matrix components are being volatilised, it is essential to have a slow ramp rate. Other c.f.a.a.s. methods [10, 11, 13] have advocated the use of a slow temperature-ramp rate throughout the ashing stage or after 300°C. It was found here that this unnecessarily lengthens the procedure.

Since the aluminium peak height kept increasing with increasing atomisation temperature, the maximum temperature attainable by the Perkin-Elmer HGA 76 was used.

TABLE 2

The effect of different heating rates on the aluminium signal when ashing at temperatures above 700° C

	Rate 1[a]	Rate 2[a]
Mean peak height (cm)	6.7	7.6
Standard deviation[b]	0.18	0.22
Coefficient of variation (%)[b]	2.7	2.9

[a]For explanation, see Table 1. [b]For n = 20.

Effect of other ions of biological importance

The instrumental conditions already established were used in the investigation of the effect of various major cations and anions in biological fluids on the aluminium signal response. For this study, a solution containing 60 μg Al l^{-1} was examined with and without the addition of the possible interfering ion. The results (Table 3) show that calcium enhances the aluminium signal while iron and chloride have a depressive effect. A review of the literature on aluminium revealed contradictory evidence on the effect of calcium. While some authors [21, 22] have reported enhancements, others [12] have observed that the aluminium signal is depressed. These observations could be due to differences in the instrumental conditions chosen and the amount of calcium present. In this study, interferences were observed only at concentrations about 2.5-times greater than those normally found in human serum for calcium, 200 times for iron and 10 times for chloride. Under the experimental conditions used and at the concentrations normally present in diluted serum, these ions will not interfere in the determination.

TABLE 3

Interference effects of some important ions in blood on the determination of aluminium

Possible interfering ion	Concentration (mg l^{-1})	Peak height of 60 μg Al l^{-1} + other ion (cm)	Peak height of ion alone (cm)	Corrected peak height (cm)	Peak height of 60 μg Al l^{-1} alone (cm)	Interference[a] (%)
Ca^{2+}	250	9.6	0.4	9.2	8.0	+16
	1 000	13.8	1.2	12.6	8.4	+50
Mg^{2+}	250	14.0	6.2	7.8	8.2	0
Na^{+}	10 000	7.8	0.2	7.6	8.0	0
Fe^{3+}	200	11.2	4.0	7.2	8.0	−10
PO_4^{3-}	1 000	7.8	0.2	7.6	8.0	0
	5 000	8.2	0.4	7.8	7.6	0
Cl^{-}	3 500	8.0	0.4	7.8	8.0	0
	35 000	9.2	2.0	7.2	8.0	−10

[a](+) and (−) denote enhancement and depression of the signal, respectively. Changes of less than 5% were ignored.

Calibration procedure for determination of aluminium

For the determination of aluminium in serum and plasma, calibration with aqueous standards was found to be adequate. This was confirmed by comparing the slopes of the calibration graphs obtained by using aqueous standards and by standard addition to a plasma sample having a low aluminium concentration. Figure 2 shows that the slopes are parallel indicating the absence of any significant matrix effect. The linear range extends to about 100 $\mu g\ l^{-1}$ and samples are diluted as required to bring their aluminium concentration within the calibration range.

The use of background correction was unnecessary for the measurement of aluminium in the serum and plasma matrices. A comparison of the peak heights for a serum and an aqueous solution containing 50 μg Al l^{-1} measured with and without background correction is presented in Fig. 3. With back-

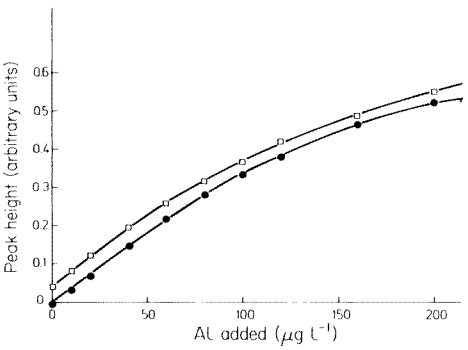

Fig. 2. Calibration graphs for aluminium in (□) diluted (1 + 1) plasma; (•) aqueous solution. The natural level of aluminium has not been subtracted from the plasma readings.

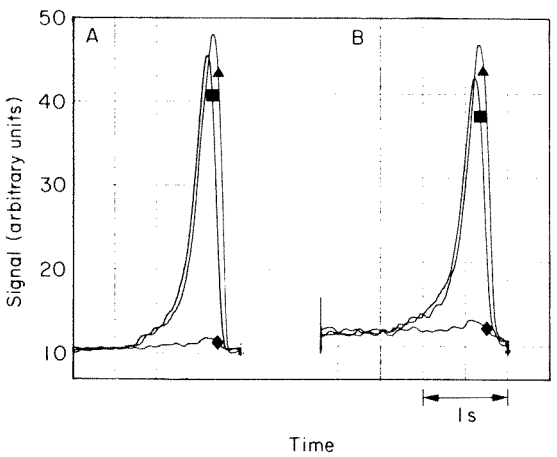

Fig. 3. Absorption signals at 309.3 nm obtained during atomisation of (▲) a diluted (1 + 1) serum sample spiked with 50 μg Al l^{-1}; (■) aqueous 50 μg Al l^{-1} solution; (♦) blank serum sample diluted (1 + 1). The signals were recorded without (A) and with (B) background correction. Full scale deflection (100) represents 0.5 absorbance.

ground correction both aluminium signals were slightly depressed but the difference remained constant.

Assessment of the analytical performance of the method

To assess the accuracy of the method, two serum samples with low aluminium concentrations were spiked with 100 and 200 μg Al l^{-1}, respectively, and the samples analysed. The results (Table 4) gave a mean recovery of 95%. In addition, repeated determinations on two serum samples by using aqueous standards gave good agreement with results obtained by standard addition (Table 5).

The precision for serum is not as good as for aqueous standards, but is adequate, both within and between batches, for clinical use. For example, 35 determinations of 340 μg Al l^{-1} in a serum after (1 + 5) dilution had a coefficient of variation of 3.9%. Between-batch coefficients of variation for

TABLE 4

Results of recovery experiments

Al in original sample (μg l^{-1})	Al added (μg l^{-1})	Al recovered (μg l^{-1})	Recovery (%)	Al in original sample (μg l^{-1})	Al added (μg l^{-1})	Al recovered (μg l^{-1})	Recovery (%)
56	100	102	102	63	200	187	94
62	100	93	93	66	200	204	102
56	100	98	98	65	200	184	92
56	100	92	92	63	200	185	93
55	100	90	90	65	200	200	100
		Mean ± 1 s.d.	95.0 ± 4.9	63	200	181	91
						Mean ± 1 s.d.	95.0 ± 4.5

TABLE 5

Comparison of results obtained for aluminium in serum by calibration with aqueous standards and by standard addition

	Mean (μg l^{-1})	No. of results	Standard deviation (μg l^{-1})	Coefficient of variation (%)
Low aluminium[a]				
Aqueous standards	63	7	3	4.2
Standard addition	62	7	5	7.7
High aluminium[b]				
Aqueous standards	294	9	11	3.7
Standard addition	306	5	7	2.4

[a]Samples diluted (1 + 1) before analysis. [b]Samples diluted (1 + 5) before analysis.

10 samples of 24 μg Al l^{-1} and 18 samples of 340 μg Al l^{-1} were 11.2 and 6.3%, respectively. The concentration of aluminium that gave a 1% absorption signal (0.0044 absorbance) was 1.2 μg l^{-1}. The detection limit (2σ) measured with a 60 μg l^{-1} aqueous standard, was 3.6 μg l^{-1}.

To obtain reliable results, frequent checks are required during the run to ensure that the calibration has not changed. This is particularly important as the tube ages. In our experience, a tube could be used for between 50 and 100 firings. Although pyrolytically-coated tubes should increase the sensitivity for refractory elements such as aluminium, in fact a 10% loss of sensitivity and poorer reproducibility were found. This may well depend on the quality of the coating of pyrolytic carbon applied during manufacture of the graphite tubes.

Normal concentrations of aluminium in serum

Values reported in the literature vary greatly [23] from mean values of 2.1 [16] to 1450 μg l^{-1} [24]. Although there remains the possibility that healthy persons in different areas could have various levels of aluminium in their blood through differences in exposure to aluminium, the greater part of this spread is probably due to contamination in handling of samples and inaccurate measurement [23]. Earlier work in the authors' laboratories gave a mean normal value of 16 μg l^{-1} (0.6 μmol l^{-1}). Results obtained by the present method give slightly lower results. When 19 blood samples were collected through a plastic cannula into acid-washed sample tubes (a technique which was shown to be necessary for reliable determinations of manganese [25]), a mean value of 9.8 μg Al l^{-1} (range: 4.1—20.0 μg l^{-1}) was obtained. This was not significantly different from the mean value of 10.2 μg l^{-1} (range: 3.2—32.4 μg l^{-1}) obtained when blood was collected from 15 subjects via a normal stainless steel needle and syringe and when untreated sample tubes were used. Thus the more elaborate procedure of using cannula sampling and acid-washed sample tubes does not seem necessary in routine use, but this should be confirmed by each laboratory proposing to undertake analysis for aluminium.

Although the present results are similar to those obtained by some other workers [23], significantly lower values (mean 2.1 μg l^{-1}, range: 0.0—7.6 μg l^{-1}) have been obtained recently by Alderman and Gitelman [16] for 14 subjects. Therefore, there remains a possibility that residual contamination may affect the accuracy of measurement of normal aluminium levels, but this becomes less significant for the measurement of serum aluminium levels of patients on renal dialysis, which range from 50 to 600 μg l^{-1}.

Conclusions

For the determination of aluminium in serum or plasma, two factors have been shown to be important. First, an ashing temperature above 1000°C is required to remove the interference effects caused by the inorganic matrix components. Thus, in this method, calcium, sodium, magnesium, iron,

phosphate and chloride do not interfere at the levels normally present in serum. Secondly, a slow ramp rate is important between 800 and 1000°C to avoid losses of aluminium by co-volatilisation.

The method described has been in routine use in the laboratories of the Glasgow Royal Infirmary for two years and is proving satisfactory for monitoring patients on renal dialysis. The main modification made in the light of routine experience was to centrifuge the diluted samples to remove sediment which caused poor reproducibility. Spurious results are still occasionally seen, requiring repeat analysis. However, our experience is different from that of Allain and Mauras [17] who found that c.f.a.a.s. gave very poor reproducibility with frequent erratic peaks, requiring 3—5 injections per sample. As a result, they changed to an inductively-coupled plasma emission method.

The method proposed here has been kept deliberately simple. It gives no problems from residue formation, and so does not require special diluents [14, 16, 18], and gives good recoveries without resorting to coating tubes with molybdenum [16].

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DETERMINATION OF LEAD IN VEGETATION BY A RAPID MICROSAMPLING-CUP ATOMIC ABSORPTION PROCEDURE WITH SOLID SAMPLE INTRODUCTION

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SUMMARY

A simple, reagent-free solid-sampling method for determining lead in vegetation is described. The dry sample is ground to a powder and 0.5 g is suspended in 10 ml of deionized water. Aliquots (20 μl) are pipetted into nickel microsampling cups, which are dried at 110°C and inserted into an air—acetylene flame. The resulting lead atomic absorption signal is time-resolved from any non-specific absorption, making further sample pretreatment unnecessary. Suitable dilutions of the suspension provide a linear range of 0.072—240 μg Pb g^{-1} of dry vegetation. The method is simple, accurate, faster than competitive methods, and provides adequate precision (typically 4.9% r.s.d.) for this application.

Lead may occur in vegetation as a result of uptake through the roots, or it may be deposited on the leaves by atmospheric fall-out. Several investigations [1—3] have shown increased lead pollution close to highways as a result of contamination from motor vehicles burning gasoline containing alkyl lead antiknock agents. A convenient means of assessing the environmental impact is by determining lead in roadside grass. Plants growing on land-fill sites or other contaminated areas may take up lead, and those interested in land reclamation schemes or intending to grow crops in such areas may need to determine plant lead content, especially if the plants are likely to be consumed by humans or animals.

Dry ashing followed by dissolution in dilute acid is the most commonly used sample pretreatment procedure for vegetation, and trace metals are then determined usually by flame atomic absorption spectrometry (a.a.s.). In order to provide improved sensitivity, however, electrothermal atomization has been used by some workers [4, 5]. Disadvantages of dry ashing are that it is time-consuming and prone to volatilization losses of elements such as lead. It has been shown [6] that such losses can be avoided, by a complex procedure involving heating in a stream of oxygen, subliming the lead metal

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in a stream of hydrogen, and dissolving the condensate in nitric acid. Wet oxidation is a less common sample destruction procedure, for example, with a mixture of nitric and sulphuric acids and hydrogen peroxide [7]. This is usually faster than dry ashing and volatilization losses are less likely, but constant surveillance is often required. Alternatively, acid digestion bombs have been used [8]. With wet oxidation procedures high blanks may be encountered from reagent contamination, and the hazardous nature of the reagents necessitates care.

In order to overcome the disadvantages of dry ashing and wet oxidation a recent trend has been towards the direct analysis of solid samples. Nichols et al. [9] determined cadmium, copper, lead and zinc directly in orchard leaves by weighing the sample into a graphite crucible which was inserted into a constant-temperature graphite furnace. The analyte and matrix gases passed through porous graphite prior to entering the light path, and so particulates which would have produced non-specific absorption were filtered out. Microsampling-cup a.a.s. though most commonly used for blood lead [10, 11] is well-suited to solid sample introduction. The technique is limited to determinations of more volatile metals, but for these it has certain advantages over electrothermal furnace techniques; firstly, the sample is vaporized in a flame which permits fewer interferences than furnaces, and secondly, any pretreatment of samples is performed "off-line", avoiding the time-consuming dry and ash cycles following injection into the typical furnace. Pachuta and Cline Love [12] determined lead in air particulates collected on a filter by placing a section of the filter directly into a micro-sampling cup, and, without pretreatment, inserting it into a flame. Precision, though adequate for this type of sample, suffered from variable combustion of the filter.

Typically, solid sample introduction requires individual micro sample aliquots to be weighed. This is a distinct disadvantage because it is slow, weighing errors can be introduced, and it may be difficult to obtain a representative sample. A microsampling-cup method which overcame these problems was applied to the direct analysis of cadmium [13] and lead [14] in biological tissues. A large sample was homogenized with water and the resulting suspension was pipetted (50 μl) into nickel cups which were merely dried prior to insertion into an air—acetylene flame. It is usual practice with micro techniques to perform replicate determinations, and in this case triplicate aliquots could be pipetted from one initial weighing. The adaptation of this technique to the determination of lead in roadside grass, vegetation from a land-fill site, brassicas and standard botanical reference materials (orchard and tomato leaves) is described in this paper.

EXPERIMENTAL

Instrumentation

An Instrumentation Laboratory Model 151 atomic absorption spectrometer, equipped with a Boling air—acetylene burner and Delves microsampling accessory, was used. Transient absorption signals were read using a Honeywell Electronik 194 chart recorder.

Preparation of samples and standards

Samples which had been collected by cutting with lead-free stainless steel scissors were oven-dried at 110°C (usually overnight). Approximately 1 g was ground in a small tungsten carbide ball mill of the type typically used for preparing samples for infrared analysis (Rotomill RM-100, Beckman-RIIC Ltd.). Grinding for 5 min at maximum speed produced a sufficiently fine powder and 0.5 g was weighed accurately into a 25-ml beaker. After adding 10.0 ml of deionized water a suspension was produced by stirring magnetically at the highest speed possible without frothing. Aliquots (20 μl) of the suspension were transferred by means of an Eppendorf micropipette into nickel microsampling cups. It was important, in order to obtain representative aliquots, to continue stirring while pipetting.

A standard stock solution (10 000 mg Pb l^{-1}) was prepared by dissolving 15.9852 g of lead nitrate (reagent grade) in 5 ml of concentrated nitric acid diluted to 1 l with deionized water. By suitable dilution, aqueous standards of 0.5, 1, 2, and 3 mg Pb l^{-1} were obtained.

Procedure

The absorption tube was brought to constant temperature by lighting the stoichiometric air—acetylene flame at least 10 min prior to analysis.

The lead content of a low-lead grass was first determined by the method of standard additions. Aliquots (20 μl) of each aqueous standard were pipetted, in triplicate, into separate cups containing the sample suspension (see above). These, together with three cups containing just the sample suspension, were dried at 110°C for 10 min and inserted into the flame. Peak absorbance at 283.3 nm was read from the recorder and the lead content of the sample was calculated from a standard additions calibration graph. For the determination of later samples, it was possible to read the lead content directly from the above standard addition graph. To do this it was merely necessary to insert cups, containing the dried suspension, in triplicate, into the flame in the same run as the above calibration samples.

RESULTS AND DISCUSSION

Examination for non-specific absorption

Electrothermal a.a.s. almost always requires the use of background correction. In the microsampling-cup method, however, the lead peak and non-

specific absorption peak were time-resolved, making background correction unnecessary [14]. A typical recorder trace for three aliquots of a grass sample is shown in Fig. 1; two resolved peaks are obtained 2 and 10 s after introduction of the cup into the flame. In order to determine the contribution from non-specific absorption, the determination was repeated at a non-absorbing line close to the lead 283.3-nm line. Manganese does not give an atomic absorption response by this technique with an air—acetylene flame, and the 279.5-nm manganese line merely gave the small signal appearing after 2 s, demonstrating that the second peak (10 s) was due entirely to lead atomic absorption. Similarly, the first peak was shown to be due to non-specific absorption by use of the background corrector. In practice, if the microsampling cup was positioned too close below the hole in the absorption tube, these two peaks overlapped slightly. They could be almost completely resolved by positioning the cup approximately 2 mm below the tube, and it was normal practice to optimize this position before measurements. It was then found that background correction was not needed.

Sample introduction

Grass was used to establish the method of sample preparation, and direct weighing of powdered grass into the microsampling cups was first inves-

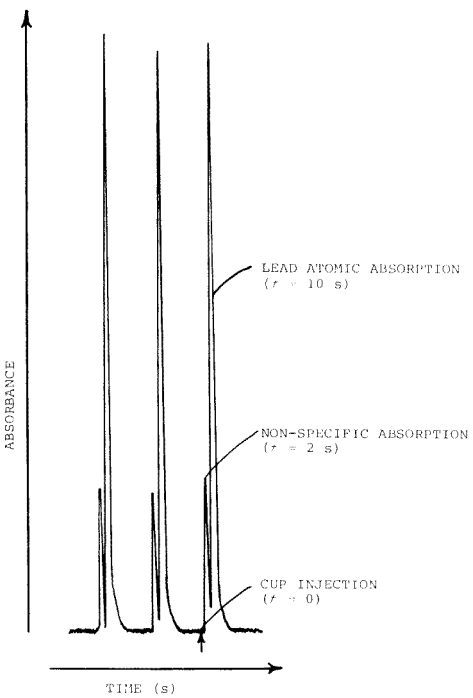

Fig. 1. Typical recorder traces for triplicate analyses of a grass sample, showing peak appearance times (t).

tigated. A graph of peak absorbance vs. sample weight gave poor correlation (r = 0.69, 18 samples, 0.45—1.85 mg), and this was attributed to the variable positioning of the sample in the cup. Pipetting a suspension ensured a uniform deposit over the bottom of the cup after drying. Thus, direct weighing was abandoned in favour of the suspension technique which was also more convenient for triplicate measurements.

It would be time-consuming to repeat the standard additions procedure for every sample, so a low-lead grass was spiked with aqueous lead standards and the lead determined by standard additions several times to establish its concentration accurately. Then, assuming that the matrix did not differ appreciably between samples, this spiked calibration curve was used for the direct determination of subsequent grass samples. Several samples were analyzed in this way and the results were verified by standard additions to each sample. It can be seen from Table 1 that several grasses gave comparable results by both methods.

Accuracy, precision and linear range

The accuracy of the method was examined by analyzing two appropriate reference materials, Orchard Leaves and Tomato Leaves (SRM 1571 and 1573, respectively, National Bureau of Standards). Although obtained in a dry powdered form, these materials were subjected to the same grinding procedure as the samples in order to produce a satisfactory suspension. Table 2 shows results obtained by standard additions and by the direct method of calibration using a spiked suspension of grass sample number 1. For the Orchard Leaves, both calibration methods give results within the certified limits. Recoveries are slightly low from the Tomato Leaves, but for much lower concentrations than are normally found in grass.

TABLE 1

Comparative lead results by direct calibration with spiked grass sample 1 and standard additions (all results are μg Pb g^{-1} of grass)

Grass sample	1	2	3	4	5	6
Direct calibration	—	46	54	121	128	243
Standard addition	38	43	53	124	125	257

TABLE 2

Analysis of certified reference materials

	Lead content (μg g^{-1})		
	Direct calibration	Standard additions	N.B.S. certified concentration
Orchard Leaves	47	45	45 ± 3
Tomato Leaves	5.6	5.4	6.3 ± 0.3

The relative standard deviation of the instrumental determination, established by pipetting 10 replicates of the aqueous suspension of grass sample 3 (53 μg Pb g^{-1}) was 4.9%. This demonstrates that the suspension method gives a satisfactorily uniform sample, and it is possible from results of previous work [13] that peak integration would improve this figure.

The detection limit (s/n = 2) was 72 pg for a 20-μl aliquot; this is equivalent to 72 ng Pb per g of vegetation if 0.5 g is slurried with 10 ml of water.

The spiked calibration graphs were linear up to 3 mg Pb l^{-1}, giving a linear range of 0.072—60 μg Pb g^{-1} of vegetation with the normal suspension. It was shown that this range could be extended by further dilution of the suspension without the need to re-run the calibration standards (spiked grass sample 1) at the new dilution. Suspensions (10 ml) containing various weights (0.125—0.625 g) of grass were taken through the analytical procedure. A graph of peak absorbance vs. weight of grass in the suspension was linear (r = 0.998) indicating that within this weight range, the ratio of grass to water would not affect the results. Hence, by preparing a suspension containing only 0.125 g of grass in 10 ml, the linear range was extended up to 240 μg Pb g^{-1} of grass. The upper working limit can of course be extended further if a curved calibration graph is acceptable, and this is how sample number 6 (Table 1) was determined.

Further applications

Initial reclamation of land-fill sites is usually done by planting grass and clover mixtures. Different species have different tolerances to lead and exhibit different uptake rates. The extent of uptake is of crucial importance if the land is to be used for grazing, and the differing tolerances are valuable in assessing future re-vegetation plans. The method described here, because of its simplicity and rapidity, would enable comprehensive low-cost surveys of such sites. In addition, the micro-sample requirements allow for separate investigation of root and foliage components. Some illustrative examples from such a survey of a quarrying-waste land-fill site are shown in Table 3. It can be seen that different species can have significantly different lead concentrations (e.g., grass and clover), although separate samples of the same species can show quite large variations in concentration. Litter (decayed vegetation) tends to have very high lead concentrations.

In the past, interest has been expressed in the deposition of lead on the leaves of consumer vegetables because of the proximity of heavy traffic [15]. As an illustration of the usefulness of the method for investigating this deposition on vegetables sold outdoors, two cabbages were analysed, one from a shop and one from an outdoor market. The lead content of the outer leaves of the shop cabbage was 1.4 μg g^{-1} (unwashed) or 1.0 μg g^{-1} (washed). Comparative figures for the outdoor cabbage were 2.7 and 2.4 μg g^{-1}, respectively. Further studies of the inner and outer leaves suggested that most of the lead in the cabbages is to be found on the outer leaves. Similar results were found for lettuces.

TABLE 3

Survey of grasses and clover from a land-fill site

Species	Lead content of individual samples (μg Pb g^{-1})	Mean
Lolium perenne (rye grass)	80, 69, 54, 68, 64, 85, 70, 65, 73, 101, 102	76
Agrostis tenuis	118	
Festuca rubra	28, 44, 77, 131	70
Agrostis stolonifera	55	
Trifolium repens (clover)	270, 217, 256	248
Litter	431, 366, 419, 476, 419	422

Conclusions

A micro-sample method is normally preferred to conventional flame a.a.s. either when higher sensitivity than that afforded by a flame is required, or when sample size is limited. In this case, a micro technique enabled solid samples to be inserted directly without prior matrix degradation. This produced an analytical method which requires no reagents, and is probably simpler and faster than previous methods for this application. The preparation of an aqueous suspension not only provided a more representative vegetation sample than direct weighing would have allowed, but the dynamic range could be extended by varying the suspension concentration. The method could be scaled down for smaller sample weights, and it should then be useful where the uptake of lead by plants has to be investigated, as different parts of the same plant can be individually analyzed for lead.

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MOLECULAR EMISSION CAVITY ANALYSIS
Part 20. Indirect Determination of Amines by Their Reaction With the Formaldehyde—Sulphite Addition Compound

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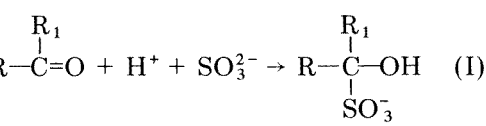
(Received 6th March 1981)

SUMMARY

Aromatic and aliphatic amines and hydrazine in the range 1—30 × 10^{-5} M are determined by measuring the S_2 emission in a carbon cavity held in a hydrogen—nitrogen flame arising from the formation of an addition compound between the amine, formaldehyde and sulphite. As little as 1.8 ng of aniline in 5 μl (4 × 10^{-6} M) can be detected.

Carbon compounds containing nitrogen give rise in flames to band emissions from NO, NH and CN, as well as from CH and C_2 [1]. These bands, particularly those from CN, have been used analytically [2—4]. The NO, NH and CN emissions can also be produced in molecular emission cavity analysis (m.e.c.a.) [5] but are not particularly sensitive. Greater sensitivity for determinations of amines and amino acids can be obtained indirectly by measuring S_2 emissions, after reaction with carbon disulphide [6]. Since the reaction of many amines with carbon disulphide is rapid and the dithiocarbamic acid produced is unstable, the reaction was carried out directly in the cavity for 1 min, the excess of carbon disulphide was evaporated, and the dithiocarbamic acid was determined from its S_2 emission. This method had a limited useful range of ca. 0.6—3 μg in 5 μl and a detection limit of ca. 0.25 μg in 5 μl for the lower aliphatic primary and secondary amines. Because the equivalent reaction of amino acids was much slower, the reaction was carried out in alkaline conditions in a closed vessel for 3 h before measurement was made. This resulted in higher sensitivity, because reagent was not lost during the reaction. The useful range was generally 10—300 ng of amino acid in 5 μl, with detection limits of 2 or 3 ng in 5 μl.

It is well known that carbonyl compounds react with sulphite to form addition compounds [7, 8]



$$R-\underset{\displaystyle R_1}{\underset{|}{C}}=O + H^+ + SO_3^{2-} \rightarrow R-\underset{\displaystyle R_1}{\underset{|}{\underset{\displaystyle SO_3^-}{\underset{|}{C}}}}-OH \quad (I)$$

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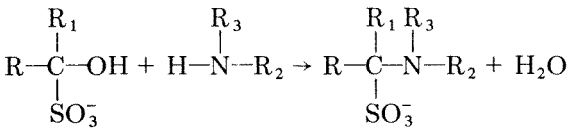
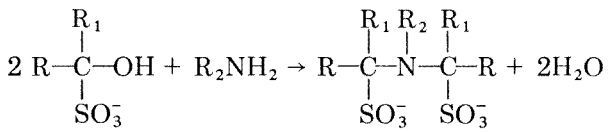
The indirect determination of nanogram amounts of carbonyl compounds by m.e.c.a. has been based on this reaction [9, 10]. The addition compound (I) has been reported to react with amines [8, 11—13]. For example, secondary amines react



The addition compound commonly used for the reaction is that of formaldehyde, although in a few cases the use of some other aldehydes has been reported [8]. The addition compounds of ketones have not been used except for that of acetone [8, 14]. Ammonia, aliphatic, aromatic and heterocyclic primary or secondary amines participate in the reaction. If a primary amine is used, a disulphonate is formed



Hydrazines [8, 15] and hydrazides [8, 16] react in the same manner.

The reaction is stated [17] to be conducted easily by mixing and sometimes heating the amine, aldehyde and sulphite, usually in aqueous solution.

Preliminary experiments confirmed the facility of the reaction, and showed that the amine—sulphite compound gave an S_2 emission peak in the m.e.c.a. cavity earlier than the sulphite addition compound itself. This paper describes the development of a method for the determination of amines at the $\mu g\ ml^{-1}$ level, based on the above observations.

EXPERIMENTAL

Reagents

All water used was double-distilled, and reagents were of analytical grade. Stock sodium sulphite solution (0.06 M, 2000 μg S ml^{-1}) was prepared by dissolving 3.9396 g of $Na_2SO_3 \cdot 7H_2O$ in water and diluting to 250 ml with water. Formaldehyde solution (0.75 M) was prepared by diluting 1.31 ml of 37% formaldehyde solution to 25 ml with water. Amine stock solutions (0.25 M) were prepared in water.

Apparatus

A prototype MECA 22 spectrophotometer (Anacon Inc., Houston, Texas) was used in conjunction with an Oxford 3000 potentiometric recorder (Oxford Electronic Instruments, Oxford, U.K.) as described previously [18]. A 2.5-mm slit (≡42.5 nm) was used. Carbon cavities (4 mm deep, 4 mm diameter) supplied with an aluminium base (12 mm cube) were used through-

out. They were heated for 15 s in the hydrogen—nitrogen—air flame after each measurement to remove any organic deposit. The cavity was taken from the flame and cooled with a cold air blower for 1 min before the next sample was injected.

Recommended procedure for amines and hydrazine

Add 5 ml of 0.015 M sodium sulphite and 5 ml of 0.015 M formaldehyde to a series of 25-ml volumetric flasks, and leave to react for 15 min. Add $\leqslant$11 ml of amine solution (see below for applicable concentration ranges) to each flask (e.g., 1—11 ml of 2.5×10^{-3} M diethylamine for calibration) followed by 3 ml of 1 M phosphoric acid. Allow to stand for 1 h when aromatic amines are to be determined; this is not necessary for aliphatic amines and hydrazine.

Clean an empty cavity by heating for 15 s in a relatively hot flame (1.8 l H_2 min^{-1}, 4.2 l N_2 min^{-1}, 3.5 l air min^{-1}), cool with a cold air blower for 1 min, and turn off the air supply to the flame. Inject 5 μl of solution into the cavity and evaporate the excess of sulphur dioxide for 1.5 min with a cold air blower. Rotate the cavity into the nitrogen—hydrogen diffusion flame (1.8 l H_2 min^{-1}, 4.2 l N_2 min^{-1}) and record the S_2 emission at 384 nm as a function of time. Measure the height of the first peak.

PRELIMINARY INVESTIGATIONS

Formaldehyde, acetaldehyde and acetone have been determined by m.e.c.a. by addition of excess of aqueous sodium sulphite in the presence of 0.06 M phosphoric acid [9]. Formaldehyde was chosen for the present investigation because a good yield of aldehyde—sulphite—amine addition compound is reported [8].

When 5 μl of a solution originally 6×10^{-3} M in sodium sulphite, 1×10^{-3} M in formaldehyde and 0.1 M in phosphoric acid was injected into a carbon cavity and the excess of sulphite was evaporated as sulphur dioxide using a cold air blower, only one peak, at t_m = 5.0 s (t_m is the time elapsed between inserting the cavity into the flame and achieving maximum intensity), which was due to the formaldehyde—sulphite addition compound, was obtained upon subsequent introduction of the cavity into the flame (1.8 l H_2 min^{-1}, 4.0 N_2 min^{-1}). The t_m value was much smaller than that reported when a silica-lined stainless-steel cavity was used (18 s), mainly because of the slower heat-up rate of the silica lining, causing a delay in the appearance of the bound sulphite peak [19].

When 5 μl of 10^{-3} M diethylamine was introduced into the same flame, no emission response was obtained. However, when diethylamine was present in the above solution containing formaldehyde and sulphite, two peaks (t_m = 1.9 and 5.0 s) instead of one were obtained after solvent and sulphur dioxide evaporation. The second peak was due to the excess of formaldehyde—sulphite addition compound while the first was due to the diethylamine—

formaldehyde—sulphite compound. The height of the first peak increased with the amount of amine and at the same time the height of the second peak decreased. A similar comparison of amine-sulphite solutions with and without formaldehyde confirmed that the peak at 1.9 s was due to the triple addition compound and not to any compound formed between sulphite and the amine. Similar results were obtained for anthranilic acid.

Optimization of experimental conditions for determination of diethylamine

Order of addition of reagents. The order of addition of reagents can be important for obtaining the highest yield of product and may affect the rate of product formation. For example, phosphoric acid (necessary to remove cationic depressive effects on the m.e.c.a. measurement [9, 19]) delays the formation of the formaldehyde—sulphite compound by decreasing the sulphite concentration, and must be added last, when the reaction is complete.

Experiments showed that addition of diethylamine before phosphoric acid to a formaldehyde—sulphite solution that had been allowed to react for 15 min (see below) gave a much greater peak height (16 mV) than when the amine was added immediately after the acid (2 mV). This also illustrates the very rapid reaction of the amine with the addition compound when the acid is absent.

Reaction time. The reaction of formaldehyde and sulphite is complete after 10—15 min at room temperature [9, 20]. Therefore, the mixture of formaldehyde and sulphite was left for 15 min before addition of diethylamine, and the effect of subsequent reaction time on the formation of the diethylamine addition compound investigated. A 1-ml portion of 5×10^{-3} M diethylamine was allowed to react for various times before addition of the phosphoric acid; otherwise the recommended procedure was followed and the emissions were measured immediately after addition of phosphoric acid and dilution to 25 ml. The reaction was complete in <5 min and the intensity from the triple addition compound remained constant for at least a further 92 min. It was also found that the time elapsed after addition of phosphoric acid had no effect, up to at least 90 min, if the reaction was complete before addition of the acid.

Reagent concentrations. An appreciable excess of formaldehyde—sulphite addition compound was needed to give maximum intensity from the amine addition compound, but above a certain concentration (18×10^{-4} M for 2×10^{-4} M diethylamine in the final solution) the intensity was independent of the formaldehyde—sulphite concentration, when the recommended procedure was followed in other respects.

Similarly, increasing the phosphoric acid concentration caused a steady increase in intensity up to 0.04 M in the final solution, above which the intensity remained nearly constant until it began to decrease above 0.2 M (Table 1). Similar behaviour occurs with the formaldehyde—sulphite addition compound [9] and other sulphur compounds [21, 22]. The enhancement is mainly caused by removal of the depressive effects of sodium ions [19].

TABLE 1

Effect of phosphoric acid concentration on the emission peak height from the diethylamine addition compound (2×10^{-4} M diethylamine[a])

H_3PO_4 (M)[a]	0.008	0.02	0.04	0.08	0.12	0.20	0.28
Emission intensity (mV)	7.5	13.5	20.0	20.5	21.5	21.5	15.0

[a]Concentration in final solution.

Flame composition. In order to obtain maximum emission intensity from the amine compound and also to resolve its peak from that of the excess of formaldehyde—sulphite compound, the effects of varying the flame conditions were measured, by changing one gas flow whilst the others were held constant.

The hydrogen flow rate was changed while the nitrogen flow was kept constant at 4.2 l min^{-1}. No air was introduced at this stage. It was found that the smallest possible hydrogen flow rate (1.8 l min^{-1}) gave maximum intensity. Below this flow rate the flame was not stable and was hard to ignite. The optimum nitrogen flow was 4.2 l min^{-1} for a 1.8 l min^{-1} hydrogen flow.

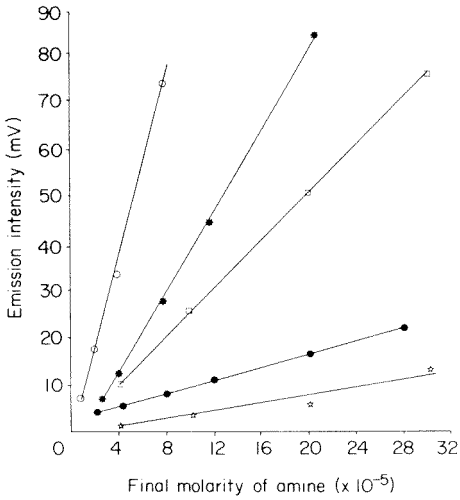
When air was added to this optional hydrogen—nitrogen diffusion flame, the higher temperature of the flame caused both peaks to appear very rapidly and to be rather irregular. Also the flame background increased. As the amount of air was increased, the intensity arising from the diethylamine compound decreased, so that at an air flow rate of 2.6 l min^{-1}, no emission was obtained from 5 μl of a formaldehyde—sulphite solution which was 2×10^{-4} M in diethylamine. Therefore, it was decided to use a hydrogen—nitrogen diffusion flame, in order to obtain maximum emission intensity and achieve good resolution.

RESULTS

Aliphatic amines

A linear calibration graph for diethylamine ($0.4—8 \times 10^{-4}$ M in the final solution) obtained under the recommended conditions is partly shown in Fig. 1. The linear plot of log (intensity) vs. log (concentration) has a slope of 0.8. A similar slope was obtained previously for sulphite—carbonyl addition compounds [10] and indicates that the rate-determining step in the formation of the S_2-emitting species is not the interaction of two sulphur-containing species in these instances [19]. The t_m value for the diethylamine peak was 1.9 s (Fig. 2). The relevant analytical parameters are summarized in Table 2.

When the method was applied to n-propylamine, its peak (t_m = 3.5 s) was superimposed on the formaldehyde—sulphite peak (Fig. 2). Attempts to cool



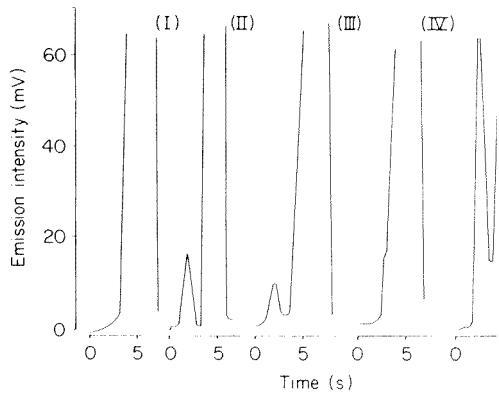


Fig. 1. Calibration graphs for: (○) aniline; (*) anthranilic acid; (□) hydrazine; (•) diethylamine; (☆) di-n-butylamine.

Fig. 2. Typical emission—time responses from: (I) blank; (II) diethylamine; (III) di-n-butylamine; (IV) n-propylamine; (V) anthranilic acid (all 2×10^{-4} M) in a flame of 1.8 l H_2 min^{-1}, 4.2 l N_2 min^{-1}.

TABLE 2

Analytical parameters for the determination of some amines

Compound	(t_m) (s)	R.s.d.[a] (%)	Log—log slope	Sensitivity (mV ng^{-1})	Detection limit[b] (in final solution)	
					(ng/5 μl)	(M)
Diethylamine	1.9	5.8	0.8	0.20	7.3	2×10^{-5}
Di-n-butylamine	1.7	7.0	1.2	0.70	2.6	4×10^{-5}
Anthranilic acid	1.9	2.1	1.1	0.60	4.0	6×10^{-6}
Aniline	1.9	4.2	1.0	2.0	1.8	4×10^{-6}
Hydrazine	1.9	4.4	1.0	1.0	5.0	2×10^{-5}

[a]Relative standard deviation for 7 injections of 4×10^{-4} M diethylamine, di-n-butylamine or hydrazine, 1.2×10^{-4} M anthranilic acid or 5.2×10^{-5} M aniline. [b]Concentration giving intensity twice the standard deviation of the blank.

the flame by increasing the nitrogen flow rate were not successful in separating the two peaks.

The determination of di-n-butylamine was successful (Table 2). This amine gave a peak with t_m = 1.7 s (Fig. 2). The calibration graph is shown in Fig. 1. The log—log plot has a slope of 1.2. Di-*iso*-butylamine gave no peak, so that apparently this amine does not react with the formaldehyde—sulphite addition compound under the above conditions. This may be

attributed to the steric hindrance of the bulky isobutyl group, since the isomeric di-n-butylamine reacted readily.

Aromatic amines

Anthranilic acid was initially investigated. When 1 ml of 5×10^{-3} M anthranilic acid was subjected to the recommended procedure for aliphatic amines, it gave rise to a peak of t_m = 1.9 s (Fig. 2). However, unlike the aliphatic amines, the peak height increased with time after addition of phosphoric acid, appreciably for 15 min, and slowly for another 45 min (Fig. 3), after which it remained constant for at least 17 h. Thus aromatic amines should be allowed a 1-h reaction time to ensure greatest sensitivity. Addition of phosphoric acid otherwise had the same effect as for aliphatic amines. The optimum final concentration for 3 ml of 1×10^{-3} M anthranilic acid was again 0.12 M.

Linear calibration graphs (Fig. 1) were obtained for anthranilic acid and aniline on the basis of the above procedure. The relevant analytical parameters are summarized in Table 2. Aniline gave the greatest sensitivity of all the amines tested.

Hydrazine

Hydrazines are reported to react with the formaldehyde—sulphite compound [8]. Hydrazine gave a linear calibration graph (Fig. 1) when subjected to the procedure for aliphatic amines. The relevant analytical parameters are summarized in Table 2.

DISCUSSION

A method for determination of nanogram amounts of some aliphatic and aromatic amines and hydrazine has been devised, based on the S_2 emission from their sulphite—formaldehyde compound in a m.e.c.a. cavity using a

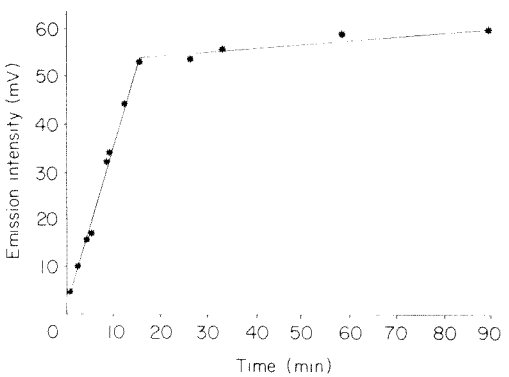
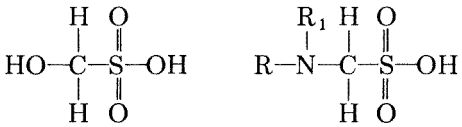


Fig. 3. Effect of time after addition of phosphoric acid on the intensity from 1.6×10^{-4} M anthranilic acid.

hydrogen—nitrogen flame. The peaks from these compounds appear before the formaldehyde—sulphite peak. This may be explained as follows. The t_m value depends either on the boiling point of the analyte or on the temperature of decomposition of the analyte to give volatile sulphur compounds. The latter mechanism has been shown [10] to operate for several organic compounds. If it is assumed that the emissions obtained from the formaldehyde—sulphite (II) and amine—formaldehyde—sulphite (III) compounds result from release



(II)

(III)

of sulphite, and subsequent vaporization of sulphur dioxide, the presence of the additional electronegative oxygen in (II) will cause the C—S bond in this compound to be stronger than the C—S bond in (III) and therefore a higher temperature is needed to break this bond in compound (II).

The t_m values of the amine derivatives were similar. Thus, attempts to separate mixtures of different amines were not successful. However, all the amines investigated showed different sensitivities. This may be due partly to the fact that a primary amine can form a disulphonate while a secondary amine forms only a monosulphonate. Anthranilic acid gave less sensitivity than aniline, possibly because the presence of the electron-withdrawing carboxyl group, which decreases the reactivity of the amino group toward nucleophilic attack, causing the reaction to be less complete than that of aniline. Also, the possibility of steric hindrance resulting from the presence of the *o*-carboxyl group cannot be ruled out. The effect of steric hindrance in decreasing the reactivity of the amino group was more obvious in the case of di-*iso*-butylamine.

An important advantage of the present method over the use of carbon disulphide for the determination of amines [5, 6] is that it can readily be applied for the determination of aromatic as well as aliphatic amines. Also, lower concentrations of amines can be determined. For example, as little as 7.3 ng of diethylamine and 26 ng of di-n-butylamine can be determined, in a 5-μl injection, compared to the detection limits of 25 ng of diethylamine and 250 ng of di-n-butylamine obtained by the previous m.e.c.a. method [6].

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STABLE FREE-RADICAL COMPLEXING REAGENTS IN APPLICATIONS OF ELECTRON SPIN RESONANCE TO THE DETERMINATION OF METALS
Part 2. Spin-Labelled Iminooxime [1]

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SUMMARY

Possibilities for the determination of metals by means of the intensity of the e.s.r. signal of the chelate-forming reagent, spin-labelled iminomonoxime — 4-oximinomethyl-2,2,5,5-tetramethyl-3-imidazoline-1-oxyl, have been studied. The dissociation constant of the oxime group (pK_a^T = 9.36 ± 0.08) and the reagent partition constant in the chloroform—water system (log K_D = 0.80 ± 0.11) are reported. The reagent extracts copper, cobalt and nickel into chloroform. Copper is extracted as its CuA_2 chelate (log K_D = 0.91 ± 0.03; log K_{ex} = —3.35 ± 0.09; log β_2 = 15.8 ± 0.2). Several properties of the spin-labelled and conventional oximes are compared. It is confirmed that a radical-containing substituent produces a strong electron-acceptor effect. Unusual extractive and e.s.r.-spectroscopic behaviour of cobalt is indicated; an adduct of the spin-labelled chelate with atmospheric oxygen seems to be formed. Methods for the determination of cobalt and nickel based on the extraction with spin-labelled oxime into chloroform and subsequent separation of the excess of reagent on a chromatographic column are described. The detection limits are 3×10^{-7} M for cobalt and 10^{-6} M for nickel.

The progress of synthetic organic chemistry has made it possible to obtain reagents whose molecules contain a chelate-forming group capable of bonding metals and a stable unpaired electron. Of special importance are the reagents based on nitroxyl radicals that retain the unpaired electron for a practically indefinite period of time. These compounds make it possible to extend substantially the possibilities of electron spin resonance as a method of determining metals, raising its sensitivity and broadening the range of the elements determined [2]. In earlier work [1, 2], the characteristic features of such applications were examined in detail, and a review of literature on related problems was presented.

In a continuation of the investigation of analytical applications of these compounds [1—6], the analytical properties of the nitroxyl-containing compound, iminomonoxime — 4-oximinomethyl-2,2,5,5-tetramethyl-3-imidazoline-1-oxyl (I, designated below as temioxime) [7] have been studied.

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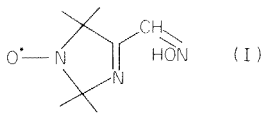


(I)

Temioxime is capable of bonding metal ions by coordination to the iminooxy nitrogen and the heterocyclic nitrogen with the formation of a five-membered ring. Some of these chelates were synthesized in aqueous medium and investigated by physical methods [8]. An extraction—radiospectrometric method for the determination of palladium with this reagent was reported [4].

The aim of the work described here was to investigate more comprehensively the analytically important properties of temioxime and to assess its possibilities as a reagent for other metals. This systematic study also provided an opportunity to check the general conclusions about the effect exerted by the spin label on the properties of an extractive reagent that were drawn in the study of spin-labelled β-diketones [1].

EXPERIMENTAL

Reagent

Temioxime synthesized by one of us (L.B.V.) at the Novosibirsk Institute of Organic Chemistry was purified chromatographically on a silica gel column (LS 5/40 μm; Lachema, Brno) using a mixture of ethyl acetate with chloroform (2 + 1) as the mobile phase. The purity of the compound was checked by occasional chromatography on plates with the same grade of silica gel using various organic solvents. Storage of the reagent for one year did not cause any significant decomposition. Temioxime solutions were prepared daily by dissolving an accurately weighed portion of the purified reagent in chloroform. The solutions and the solid reagent were stored in dark vessels in a refrigerator.

Extraction of metals

The extractions of copper, cobalt, nickel, zinc and manganese were investigated by shaking aqueous solutions of the metal with chloroform solutions of the reagent in ground-glass stoppered tubes. Distribution ratios for copper, cobalt, zinc and manganese were determined radiometrically (^{60}Co, ^{64}Cu, ^{54}Mn, ^{65}Zn). The extraction efficiency for nickel was characterized by the decrease of the radioactivity (^{63}Ni) in the aqueous phase in the course of extraction, using a ZhS-7 liquid scintillator.

Chromatography

The components of extracts were separated by liquid adsorption chromatography on columns of various sizes containing silica gel LS 5/40 μm. Separation conditions were established by chromatography on a thin layer (0.5 mm) of the same sorbent on 9 × 12-cm glass plates. Chromatograms

were detected with iodine vapour as well as with an ethanolic solution of dimethylglyoxime (detection of nickel-containing spots). Various organic solvents were used as mobile phases. All chromatographic experiments were conducted at room temperature.

Instrumentation

E.s.r. spectra were recorded with an RE-1306 radiospectrometer at room temperature using ampoules 1 mm in diameter. The g values were determined relative to diphenylpicrylhydrazyl; magnetic field sweep was checked by manganese(II) spectra in magnesium oxide powder. The same sample was used as an adjacent concentration standard.

Absorption spectra of reagent solutions in the visible and ultraviolet regions were recorded with an SF-16 spectrophotometer with 1-cm cuvettes. pH values were measured with a glass electrode using a pH-673 potentiometer.

In all experiments the reagents used were of "Pure for Analysis" or "Chemically Pure" grade. Chloroform was purified by six-fold scrubbing with an equal volume of distilled water followed by drying over calcium chloride and distillation.

Procedure for determination of cobalt

Place 2 ml of cobalt solution (4×10^{-7}—1×10^{-5} M) in borate medium (pH 8.5) into a tube with a ground-glass stopper, add 2 ml of a 10^{-3} M solution of temioxime in chloroform and shake the tube for 5 min. After phase separation, transfer the organic phase to a column with dry LS 5/40 μm silica gel compacted as much as possible (bed height 75 mm; diameter 6 mm). Pass a (1 + 2) mixture of chloroform and ethyl acetate through the column at a rate of 0.5 ml min^{-1} under pressure of nitrogen from a gas-bag. It is convenient to use long graduated glass columns so that 11 ml of eluent can be added in a single portion and the progress of the elution can be checked easily. Control the eluent consumption rate by the volume markings on the tube. (Repeated tests showed that this system provided good reproducibility.) Discard the first 7.5 ml of eluate. Collect the subsequent 3 ml; after stirring, place this solution in an ampoule (1 mm diameter) and record the e.s.r. spectrum at a modulation amplitude of 1.0×1.0 and a h.f. oscillations attenuation of 7 dB. Take the ratio between the amplitude of the complex spectrum derivative and the amplitude of the adjacent standard spectrum derivative (see below) as the measure of signal intensity. For small cobalt contents, evaporate the cobalt-containing fraction of the eluate in a stream of warm air and dissolve the dry residue in 3 ml of chloroform. Determine the cobalt concentration from a previously plotted calibration graph.

Procedure for determination of nickel

Place aqueous solutions into a tube with a ground-glass stopper so that

the concentrations in the final volume of 2 ml are 0.1 M sodium perchlorate, and 0.01 M sodium acetate, 0.001 M sodium hydroxide and (1—20) × 10^{-6} M nickel. Add 2 ml of 0.01 M solution of temioxime in chloroform and shake the tube for 40 min. Separate the organic phase, place 2 ml of the same temioxime solution in the tube and again shake for 40 min. Place the combined organic phases in a sintered glass filter funnel covered with a 2-mm layer of LS 5/40 μm silica gel protected with a paper filter. Wash the silica gel with 30 ml of ethyl acetate—chloroform mixture (2 + 1) and then pass 30 ml of ethanol through it. Evaporate the ethanol solution in a stream of warm air and dissolve the dry residue in 2 ml of chloroform. Fill an ampoule (1-mm diameter) with the solution and place it in the resonator of the spectrometer. Record the e.s.r. spectrum at a modulation amplitude of 0.7 × 1.0 and a h.f. oscillations attenuation of 7 dB. Take the ratio between the mean amplitude of the first derivative (for three spectral lines) and the mean amplitude of the first derivative of the adjacent standard lines (see below) as the signal value. Determine the nickel concentration from a previously plotted calibration graph.

RESULTS AND DISCUSSION

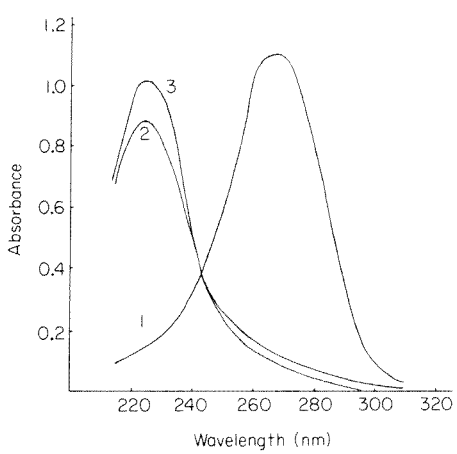
Reagent behaviour

Acid—base properties. These properties were studied spectrophotometrically. In alkaline media, the u.v. spectrum of the reagent has an intensive band with a maximum at 267 nm (Fig. 1); its location is identical with that of the bands of dissociated forms of other oximes [9]. From the dependence of the absorbance of the solution at 267 nm on pH, the dissociation constant of the oxime group [10] (21°C, 1 × 10^{-4} M temioxime, l = 1 cm, n = 7, α = 0.05) was found to be pK_a^M = 9.25 ± 0.08 at ionic strength 0.1 ($NaClO_4$) or pK_a^T = 9.36 ± 0.08.

In addition to this acid—base equilibrium for temioxime, protonation of the nitrogren atom in position 3 of the heterocycle as well as protonation of the nitroxyl group can be assumed. However, it proved impossible to investigate these processes: they seemed to take place at pH values lower than 2, where absorbances were unstable with time.

When the spin-labelled β-diketone was investigated [1], it was concluded that the tetramethylimidazolineoxyl substituent was very electronegative; according to the data obtained, the electronegativity is commensurable with that of the trifluoromethyl group. Comparison of temioxime with other monoximes with respect to the dissociation constant confirms this conclusion. Indeed temioxime has much stronger acidic properties than, e.g., salicylaldoxime (pK_a = 12.1), and its acidity is close to that of monoximes containing highly electronegative acyl groups (diacetylmonoxime 9.53; α-furylmonoxime 8.67; α-benzylmonoxime 8.60).

Reagent distribution in the chloroform—water system. Figure 2 shows the dependence of the temioxime distribution ratio (D) on pH in the chloroform—



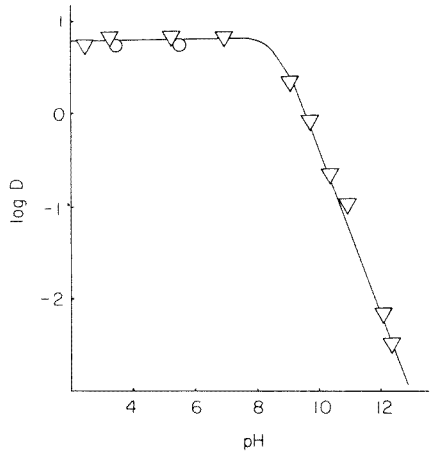


Fig. 1. Absorption spectra of aqueous solutions of temioxime (5×10^{-4} M): (1) 0.1 M NaOH (2) 0.01 M acetate buffer solution (pH 5.3); (3) 0.1 M HCl (initial moment). 0.1 M $NaClO_4$; l = 1 cm.

Fig. 2. Dependence of the temioxime distribution ratio on pH in the water—chloroform system. Concentration: (○) 10^{-3} M; (▽) 10^{-4} M; μ = 0.1 ($NaClO_4$).

water system (the data were obtained by the e.s.r. method). Temioxime is predominantly in the organic phase up to pH 10. The pH value at the inflection on the curve, as well as the slope of the descending part, indicate that the decrease in the distribution ratio at high pH values is caused by reagent dissociation. The coincidence of the distribution ratios obtained at initial temioxime concentrations of 10^{-3} and 10^{-4} M indicates the absence of association of temioxime molecules in the organic phase, at least up to the concentration of 10^{-3} M. This is in agreement with the notion of reduced electron density at the nitrogen atom of the oxime group caused by the effect of a spin-labelled substituent.

From the values of distribution ratios on the descending part of the curve (with the dissociation constant taken into account) [11], as well as from the position of the plateau in the low pH region, the temioxime partition constant in this system can be calculated: $\log K_D = 0.80 \pm 0.11$ (n = 14; α = 0.05; 22°C; μ = 0.1 ($NaClO_4$)).

In the series of monoximes, as well as in that of β-diketones, despite the great variety of substituents, the logarithm of the reagent partition constant tends to change symbathically with the size of the molecule of the distributed compound [9]: methylethylketoxime —0.49; diacetylmonoxime —0.02; phenylglyoxal aldoxime 0.90; α-furylmonoxime 1.16; 1-nitroso-2-naphthol 2.97. It is impossible to plot the exact dependence of $\log K_D$ on the molar volume of the reagent because of the absence of molar volume values. However, the closeness of the $\log K_D$ values for temioxime and for phenyl-

glyoxal aldoxime confirms the conclusion on the closeness of molar volume increments for tetramethylimidazolineoxyl and phenyl made when β-diketones were examined.

Extraction of metals

The extracting capacity of temioxime was tested for five metals: copper, cobalt, nickel, zinc and manganese. The last two were not extracted into chloroform under any conditions, including the presence of strong donor-active compounds and large poorly hydrated ions.

Copper. Copper(II) was extracted to a considerable degree into chloroform over a rather broad pH range (Fig. 3). The best extraction was achieved at pH 7—10, but even under these conditions and with a 500-fold excess of reagent, not more than 90% of the metal was extracted. The dependence of copper distribution ratio on temioxime concentration (Fig. 4) shows that it is impossible to improve radically the extraction by a simple increase in reagent concentration. The distribution ratio was not increased by the addition of tributylphosphate.

The slopes of the ascending parts of the plots in Figs. 3 and 4, which are close to 2, indicate that the extraction reaction at pH below 9 can be described by

$$Cu^{2+} + 2HA_{(o)} \rightleftharpoons CuA_{2(o)} + 2H^+$$

where HA is the temioxime molecule.

Mathematical treatment of the experimental data, taking into account competitive complexing with acetate ions in the aqueous phase [11] (pK_1 = 1.67; pK_2 = 0.98; pK_3 = 0.42; pK_4 = —0.19 [12]), made it possible to obtain the following values of the constants: log K_{ex} = —3.35 ± 0.09 (n = 5, α = 0.05); log $K_D(CuA_2)$ = 0.91 ± 0.03 (n = 14; α = 0.05). Substitution of these

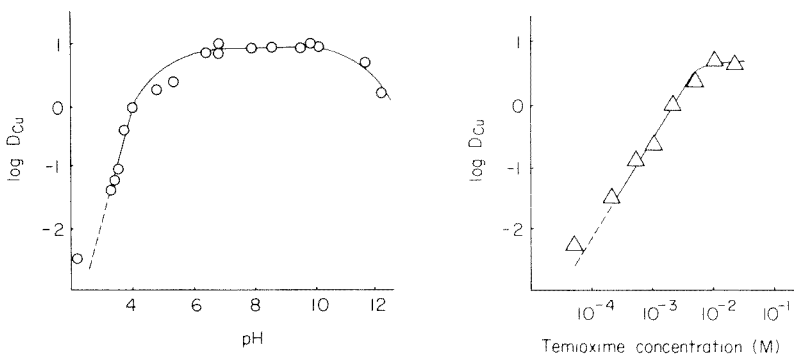


Fig. 3. Dependence of copper distribution ratio on pH; 10^{-5} M copper(II); 5×10^{-3} M temioxime; 0.01 M sodium acetate; μ = 0.1 ($NaClO_4$).

Fig. 4. Dependence of copper distribution ratio on temioxime concentration; 10^{-5} M copper; pH 6.8 (0.01 M sodium phosphate); μ = 0.1 ($NaClO_4$).

values in the equation $\beta_2 = K_{ex}K_D^2(HA)/K_a^2K_D(CuA_2)$ [11] resulted in a value for the logarithm of the total stability constant for the extracted complex in the aqueous medium: $\log \beta_2 = 15.8 \pm 0.2$. This value can be compared with the stability constants of conventional copper(II) oximates in Table 1. Copper(II) temioximate is inferior in stability to dioximates but is close to carbonyloximates. Temioxime is also similar to carbonyloximes in the dissociation constant of the oxime group. This confirms the conclusion (made quantitatively in studying spin-labelled β-diketones) that the electron-acceptor effect of the radical-containing substituent not only decreases the pK_a of the reagent but also causes a corresponding decrease in the stability of the complexes with metals, which, in turn, changes the extractive efficiency.

A serious obstacle for the analytical application of this extraction system lies in frequent and unreproducible disturbances of material balance relative to copper that cannot be avoided by introducing weak complexing reagents.

Cobalt. The dependence of the cobalt distribution ratio on pH (Fig. 5) is characterized by a sharp increase in extraction at pH 7.5: over a range of less than 0.2 pH units the distribution ratio increases by more than two orders of magnitude. The clearly defined plateau observed immediately after the rise is not wide, but the distribution ratios are large: they make it possible to extract in one step more than 97% of cobalt (with a 100-fold excess of reagent and equal phase volumes). At pH values higher than 9.5, the extraction becomes impossible because of hydrolysis.

The dependence of the cobalt distribution ratio on temioxime concentration (Fig. 6) shows that the extraction efficiency cannot be improved by raising the temioxime concentration above a certain proportion; distribution ratios on the plateaux of both plots indicate that the partition constant of the extracted complex is $\log K_D = 1.75 \pm 0.05$ ($n = 10$; $\alpha = 0.05$). The slope of the ascending part of the plot is 2, showing a 2:1 molar ratio between the reagent and the metal in the extracted complex. The cobalt distribution ratios under the optimal conditions are not improved by addition of tributylphosphate (TBP): at small and medium concentrations, TBP had no effect, whereas at 0.1 M concentrations the extraction became worse.

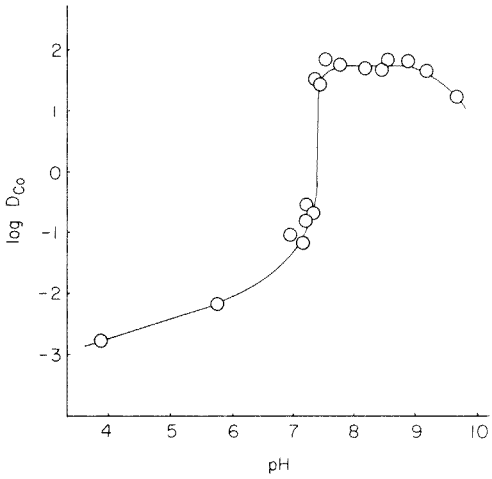
The cobalt extraction at pH 8.5 with a 100-fold excess of reagent is not

TABLE 1

Stability constants of copper oximates in aqueous media (25°C) [9]

Reagent	Conditions	$\log \beta_2$	Reagent	Conditions	$\log \beta_2$
Phenylglyoxal aldoxime	0.1 M $NaClO_4$	10.70[a]	Nitroso-R-salt	0.1 M KCl	15.0(
Diacetylmonoxime	,,	15.91[a]	Nitroso-H-salt	$\mu \rightarrow 0$	13.0(
α-Benzylmonoxime	,,	13.70[a]	Dimethylglyoxime	$\mu \rightarrow 0.1$	18.5(
α-Furylmonoxime	,,	9.46[a]	Methylethylglyoxime	0.1 M $NaClO_4$	19.67
1-Nitroso-2-naphthol	,,	14.40[a]	1,2-Cyclohexanedionedioxime	0.2 M $NaNO_3$	20.3[
2-Nitroso-1-naphthol	,,	15.60[a]			

[a]Determined by an extraction method. [b]Determined potentiometrically.



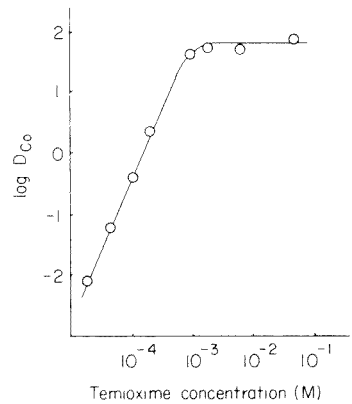


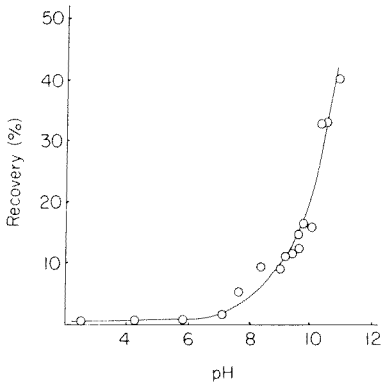
Fig. 5. Dependence of cobalt distribution ratio on pH; 10^{-5} M cobalt; 10^{-3} M temioxime; μ = 0.1 ($NaClO_4$).

Fig. 6. Dependence of cobalt distribution ratio on temioxime concentration; pH 8.4; 10^{-5} M cobalt; μ = 0.1 ($NaClO_4$); 5 × 10^{-3} M sodium tetraborate.

affected by very large amounts of borate or even tartrate ions. This permits reliable standardization of extraction pH by using, e.g., a borate buffer. The following conditions can thus be regarded as optimal for cobalt extraction with temioxime into chloroform: pH 8.5 (10^{-3} M borate solution), 100-fold excess of reagent at ionic strength 0.1 (sodium perchlorate). Under these conditions, equilibrium is established in less than 5 min of shaking.

Nickel. Radiometric investigation of nickel extraction was hampered by the extremely low radiation energy of the ^{63}Ni radioisotope; liquid scintillation counting was therefore used. The extraction of nickel into chloroform starts only at relatively high pH values (Fig. 7). Since in these conditions (especially above pH 9) there is noticeable hydrolysis, the extraction was conducted in the presence of acetate ions. This made it possible, without any appreciable reduction of recovery, to avoid nickel losses even at pH 11. Extraction efficiency increases with increasing pH, but (if a 100-fold excess of reagent is used) even at pH 10.8, recoveries do not exceed 50%. A 500-fold excess of reagent improves the efficiency (Fig. 8), but larger excesses give no benefit. Nickel recovery is somewhat increased when more acetate is added. Donor-active compounds (pyridine, TBP) do not affect the extraction efficiency; a slight increase was observed when 1 M levels of additives were used, but phase separation was then poor. The extraction was not rapid: with 2 × 10^{-6} M nickel and 10^{-3} M temioxime under the conditions used for Fig. 8, the system reached true thermodynamic equilibrium on shaking for 30 min, the recovery being 75%.

The optimal conditions of nickel extraction are: pH 10.8, at least a 500-



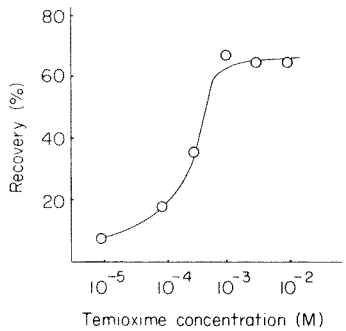


Fig. 7. Dependence of nickel extraction on pH; 10^{-4} M nickel; 0.01 M temioxime; 0.01 M sodium acetate; μ = 0.1 ($NaClO_4$).

Fig. 8. Dependence of nickel extraction on temioxime concentration; pH 10.8; 2×10^{-7} M nickel; 10^{-4} M sodium acetate; μ = 0.1 ($NaClO_4$).

fold excess of temioxime, a 500-fold (relative to nickel) excess of acetate ions and 0.1 M sodium perchlorate. About 75% of the nickel is then recovered in one extraction. After a second extraction, about 95% of the metal is recovered, which is acceptable for a relative procedure to be worked out.

Development of an extraction—radiospectrometric method for metal determinations

The necessary prerequisites for the development of methods of metal determinations with the help of a spin-labelled reagent are the completeness and the reproducibility of extraction. Of the three extraction systems investigated, those for cobalt and nickel were suitable for further study. Another important condition is the possibility of separating the signal of the spin-labelled complex of the determined metal from the reagent signal present in the excess [2]. The enormous excess of reagent in the extract excludes the possibility of such separations by purely radiospectrometric means. Comparison of the pH dependences of the distribution ratios for the cobalt chelate and temioxime itself shows that there are no extraction conditions suitable for their separation. Moreover, the excess of reagent could not be removed efficiently by scrubbing the extracts with alkaline solution. The chromatographic separation of the components of the cobalt- and nickel-containing extracts was therefore examined.

Cobalt. Chromatography of a cobalt-containing extract in a thin silica-gel layer showed two new components in addition to the excess of temioxime. Thus, when ethyl acetate was used as the mobile phase, the temioxime spot was characterized by R_f = 0.74, and the two new components by R_f = 0.50 and R_f = 0.02. Radiometric investigation proved that both these components contained cobalt, the ratio between their amounts depending on the extraction

conditions. When an aqueous 10^{-3} M cobalt solution (pH 8.5, borate solution) was shaken with 10^{-3} M temioxime solution in chloroform, the radioactivity ratio between the less and more mobile components was 3:7. However, when cobalt was extracted from 10^{-6}—10^{-5} M solutions with a 10^{-3} M reagent solution in chloroform, the fraction of component remaining at the start was approximately constant, amounting to about 3% of the total amount of metal.

These cobalt-containing components were isolated from the extract on a silica gel column by consecutive use of ethyl acetate (separate elution of temioxime and the more mobile cobalt-containing component) and then acetone (elution of the second component). E.s.r. spectra of these complexes are shown in Fig. 9. The e.s.r. spectrum of the less mobile component is the quintet usual for a nitroxyl-containing complex of a diamagnetic ion with exchange interaction of nitroxyls, and coincides with that described for the CoA_3 complex (A is temioxime anion) obtained in the aqueous phase [8]. The spectrum of the second component (the main one in the conditions of quantitative extraction) has no analogues among the described spectra of nitroxyl-containing metal complexes.

This spectrum could have been assigned to the spin-labelled CoA_2 complex of paramagnetic cobalt(II) with exchange interaction between the electrons of nitroxyls and the metal ion. It is, however, difficult then to explain why the complex should be obtained in greater amounts at lower cobalt concen-

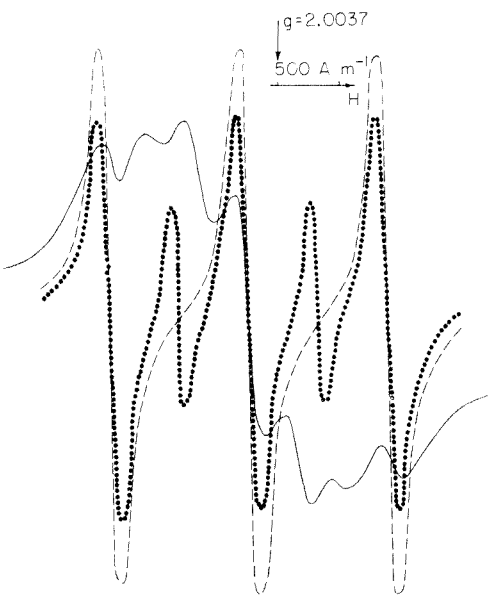


Fig. 9. E.s.r. spectra of extract components after the extraction of cobalt with temioxime: (-----) temioxime; (——) the main cobalt-containing compound; (·····) admixed (low-mobility) cobalt complex.

trations in the aqueous solution; the abrupt rise in the distribution ratio at pH 7.5, and the lack of dependence of the extraction efficiency on donor additives, cannot be explained. These contradictions can be removed if it is assumed that the extracted compound is an adduct of the chelate with atmospheric oxygen, $CoA_2O_2^-$, where cobalt(III) is present with oxygen as the O_2^- anion because of electron density delocalization [13]. This assumption is in agreement with the unusually abrupt change in the distribution ratio with increasing pH, the slope of the log $D_{Co} = f(C_{HA})$ dependence, the dependence of the percentage of this compound on the overall cobalt concentration, and the unusual e.s.r. spectrum. Moreover, this corresponds to the behaviour of cobalt in other similar systems, particularly dioxime systems [9, 14].

It is thus clear that under the conditions of quantitative extraction, the complex which has an e.s.r. spectrum with a quintet is formed only in a relatively small constant proportional amount. This compound is readily separated from the main compound on a chromatographic column. All this makes it possible to develop a method for cobalt determinations based on spectrometry of the second compound only.

Attempts to determine cobalt from the signal of this compound against the background of reagent excess were unsuccessful, although the spectrum contained portions where free temioxime absorbed insignificantly. The problem was that complete separation of the signal from the reagent in 100—1000-fold excess required the use of extremely small amplitudes of magnetic field strength modulation; such amplitudes result in a sharp decline of the sensitivity of the e.s.r. method as a whole.

Tests of various mobile phases for the chromatography of the extracts showed that the best separation of the main complex, the reagent excess and the admixed complex was accomplished with a chloroform—ethyl acetate mixture (1 + 2). The R_f values were 0.63 (reagent), 0.21 (main complex) and 0.02 (admixed complex) and the spots were very compact.

The extract was separated for e.s.r. spectrometry on a LS 5/40 μm silica gel column (75 mm × 6 mm). The column was dry-filled, with the silica gel being compacted. To speed up the separation, a nitrogen pressure of about 700 hPa was applied at the top of the column. The optimal elution rate was 0.5 ml min^{-1}. The elution curve obtained (Fig. 10) shows complete removal of the reagent in 7.5 ml of the mobile phase, and elution of the cobalt complex in the next 3 ml. The cobalt-containing fraction of the eluate can be used directly to record the e.s.r. spectrum, but the detection limit can be decreased if the ethyl acetate–chloroform mixture is replaced by pure chloroform after evaporation of the eluate to dryness.

The effects of different conditions of e.s.r. spectra recording on the detection limit and the precision of cobalt determinations were investigated. The best results were obtained when the 1-mm ampoule was completely filled with solution and submerged in the resonator as far as possible, and when its position was strictly fixed relative to both depth and vertical

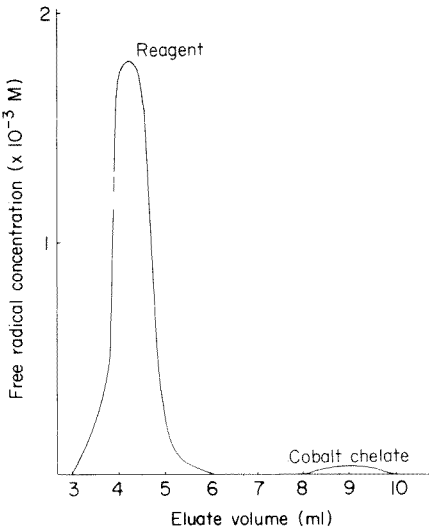


Fig. 10. Elution curve for the separation of cobalt-containing extract.

orientation. The amplitude of the signal from the complex increases monotonously with increasing amplitude of the magnetic field strength high-frequency modulation and decreasing attenuation of the high-frequency oscillations. However, with a fairly open attenuator the noise of the automatic frequency adjustment also increases, and the signal/noise ratio passes through a maximum at an attenuation of 7 dB. The optimal parameters for recording the spectra of the cobalt-containing complex on the RE-1306 radiospectrometer included a 7 dB attenuation of h.f. oscillations, and a modulation amplitude setting of 1.0 × 1.0; the time constant and magnetic field sweep rate were chosen depending on the range of cobalt concentrations, but the time of magnetic field sweep from the maximum to the minimum of the first derivative curve was always 8—10 times higher than the time constant.

Under these conditions, the complex e.s.r. spectrum of the compound blended into a broad singlet. In the 3×10^{-7}—1×10^{-5} M range, the line width did not depend on concentration, and the amplitude of the first derivative was directly proportional to the initial cobalt concentration in the aqueous phase, so that this amplitude served well as a measure of signal intensity. To exclude errors associated with uncontrollable variations in instrumental operation, the signal from the complex was correlated with that of the adjacent standard, manganese(II) in magnesium oxide.

The limit of detection for cobalt on the RE-1306 e.s.r. spectrometer without solvent substitution was 2×10^{-6} M (with respect to concentration in the initial solution prior to extraction). If chloroform is substituted in the final step, the detection limit can be reduced to (3—4) × 10^{-7} M. The

calibration plot for cobalt determination is described by the straight-line equation

$$\bar{A}/\bar{A}_{stand} = (0.52 \pm 0.08) \times 10^6 \times C_{Co} + (0.24 \pm 0.54) \quad (n = 5, \alpha = 0.05)$$

The accuracy and precision of the method are characterized by the data presented in Table 2; these results were obtained by the faster procedure without solvent substitution. The mean relative error for the determination of 10^{-5} M cobalt from one recording of the spectrum was 6.2%, whereas the error from three parallel recordings was 3.7%. Cobalt determinations were not affected by the presence of tenfold amounts of nickel and insignificantly affected by copper in the same amounts, but iron(III) and lead interfered seriously. Determinations take 30 min each without solvent substitution and 40 min with solvent substitution.

Nickel. Chromatographic investigation of a nickel-containing extract in a thin silica-gel layer showed that the nickel complex is sorbed very securely. The chloroform—ethyl acetate (1 + 2) eluent did not elute the chelate at all, but the excess of reagent moved as a very compact spot ($R_f = 0.63$), as shown above. Even such a polar solvent as ethanol eluted the complex only slightly ($R_f = 0.2$). On this basis, it was decided to use a sintered glass filter funnel to

TABLE 2

Results of determining 10^{-5} M cobalt with temioxime by the extraction—e.s.r. method

No. of parallel tests	Co found from the result of single recording ($\times 10^{-5}$ M)	Relative error of determination from single recording (%)	Co found from the mean amplitude of three recordings ($\times 10^{-5}$ M)	Relative error of determination from the mean amplitude (%)
1	0.95	5		
	0.99	1	0.94	6
	0.86	14		
2	1.09	9		
	1.03	3	1.01	1
	0.96	4		
3	1.06	6		
	0.91	9	1.05	5
	1.10	10		
4	1.16	16		
	0.97	3	1.04	4
	0.99	1		
5	0.99	1		
	1.03	3	1.01	1
	1.08	8		
6	0.98	2		
	0.90	10	0.95	5
	0.94	6		

which a 2-mm layer of silica gel was applied. After the content of the extract had been sorbed by this filter, the excess of reagent was removed by passing through it 30 ml of the chloroform—ethyl acetate mixture (1 + 2). The complex remaining on silica gel was then washed out with 30 ml of ethanol, the ethanol was evaporated in a stream of warm air, and the dry residue was dissolved in chloroform for e.s.r. spectrometry.

The spectrum of the nickel-containing complex was a triplet of equally intense lines whose parameters practically coincided with those of the reagent spectrum (g = 2.0057 ± 0.0002; a_N = 1150 A m^{-1}; ΔH_{pp} = 220 m^{-1}). The optimal conditions for the recording of this signal were similar to those for the cobalt determinations, except for a modulation amplitude setting of 0.7 × 1.0. The calibration plot for nickel determinations by this method is described by the straight-line equation

$$\bar{A}/\bar{A}_{stand} = (0.29 \pm 0.12) \times 10^6 \times C_{Ni} + (0.82 \pm 1.18) \quad (n = 5, \alpha = 0.05)$$

The limit of detection for nickel was 10^{-6} M. The mean relative error for the determination of 1.5×10^{-5} M nickel was 15%. The error of e.s.r. spectrometry itself for concentrations of 2×10^{-6} M amounts to 4%, thus the main sources of error are the operations preceding the final measurements. The most probable source of error seems to be incomplete dissolution of the residue in chloroform after the removal of ethanol.

Nickel can thus be determined with the help of temioxime, but this method is inferior to the other extraction—e.s.r. methods in a number of characteristics (rate of determination, detection limit and precision).

CONCLUSIONS

This study of the analytically important properties of the new spin-labelled reagent temioxime, has shown that temioxime differs essentially from the previously studied spin-labelled reagents in its high stability, which is comparable with that of conventional organic reagents. Suspicion that low stability is a property inherent in all radical-containing reagents is thus disproved. The investigation has confirmed that spin-labelled reagents can effectively extract metals into organic solvents. The evidence obtained confirms the statement that a spin-labelled substituent based on imidazoline has a strong electron-acceptor effect. This results in an increase in the dissociation constant of the chelate-forming group, with a corresponding change in the stability of the complexes with metals, and so influences the completeness and the regularities of metal extraction. In other respects radical-containing reagents seem to behave as their more usual analogues.

In the case of temioxime, it again proved impossible to obtain situations where one could, without a significant loss in sensitivity, determine the signal intensity from the complex against the reagent background signal. In using e.s.r. spectrometers of 3.2-cm range, separation of the excess of reagent seems to be a prerequisite for the development of highly sensitive analytical

methods based on nitroxyl-containing reagents. Investigation of temioxime has demonstrated the possibilities of chromatography as a rapid convenient method of extract separation.

Finally, with cobalt and nickel as examples, it has been shown practically that application of the e.s.r. method via the signals of spin-labelled organic radicals provides an advantage in sensitivity even for metals that can be determined by their own paramagnetism [15, 16].

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FLUORINATED β-DIKETONES IN THE DEVELOPMENT OF A GAS CHROMATOGRAPHIC PROCEDURE FOR THE DETERMINATION OF VANADIUM

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SUMMARY

Volatile, fluorinated β-diketonates of vanadium(III) have been examined for their suitability in determining vanadium by gas chromatography. Substituents in the β-diketones influence the air sensitivity of the vanadium(III) chelates, the extent of irreversible adsorption in the chromatographic column, and the ease with which excess of ligand can be separated from the derivative after solvent extraction. Derivatization involves reduction of vanadium to vanadium(III) with sodium dithionite in aqueous solution followed by chelation and extraction of the vanadium(III) chelates. A method for quantifying trace concentrations of vanadium in petroleum oils with 1,1,1,2,2-pentafluoro-6,6-dimethylheptane-3,5-dione as reagent is presented.

The determination of metal ions at trace levels by gas chromatography (g.c.) has found limited application so far because of the on-column physical and chemical behaviour of many chelate derivatives. Sensitive methods have been developed mainly for beryllium(II) [1—9], aluminium(III) [10—14] and chromium(III) [10, 14, 15—21], and to a lesser extent cobalt(III) [22], copper(II) [23, 24], nickel(II) [23—25] and palladium(II) [23]. Several other ions such as rhodium(III), ruthenium(III) and iridium(III) may also be amenable to a g.c. procedure but many ions of interest, including those of the alkali, alkaline-earth and lanthanide metals, iron, manganese, zinc, cadmium, lead and tin, have not been satisfactorily determined by this technique.

It has been shown that vanadium can be detected at trace levels either as vanadium(III) β-diketonates [26, 27] or oxovanadium(IV) chelates of tetradentate β-keto-enamines [28].

An investigation of various fluorinated β-diketones to ascertain their suitability for the determination of vanadium at trace levels is reported in this paper. Vanadium(III) derivatives of 1,1,1-trifluoropentane-2,4-dione (HTfa), 1,1,1,5,5,5-hexafluoropentane-2,4-dione (HHfa), 1,1,1-trifluoro-5-methylhexane-2,4-dione (HTbm), 1,1,1-trifluoro-5,5-dimethylhexane-3,5-

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dione (HTpm), 1,1,1,2,2-pentafluoro-6,6-dimethylheptane-3,5-dione (HPpm), and 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyloctane-4,6-dione (HHpm) are compared in terms of air stability, g.c. behaviour and ease of separation from excess of the β-diketone reagent. The most suitable derivative, $V(Ppm)_3$, is examined for the determination of vanadium in petroleum oils.

EXPERIMENTAL

Synthesis, reagents and solutions

The β-diketones and their vanadium(III) chelates were prepared and purified as previously described [29, 30].

Solvents were of analytical-reagent grade, distilled before use. Other reagents were of analytical or reagent grade. Stock solutions of interfering ions (500 $\mu g\ ml^{-1}$) were prepared by dissolving the appropriate quantity of metal chloride or acetate in distilled water. Solutions of the β-diketones (4% w/v) were prepared in methanol. An acetate buffer was prepared by dissolving sodium acetate (8.2 g) and glacial acetic acid (6.0 g) in distilled water (100 ml).

A vanadium stock solution (1.0 mg ml^{-1}) was made by dissolving pure ammonium metavanadate (574 mg) in nitric acid (15 M, 65 ml) and diluting to 250 ml with distilled water. A working standard (100 $\mu g\ ml^{-1}$) was obtained by making a tenfold dilution of the stock solution in water. To assess their air sensitivity, vanadium(III) chelates of pentane-2,4-dione (HAcac), HHfa, HTfa, HTbm, HPpm and HHpm were dissolved in air-saturated chloroform (1.0×10^{-4} M) and examined (using a Carl Zeiss PMQ II spectrophotometer) in 1-cm stoppered, quartz cuvettes. From the absorption spectra recorded in the range 320—440 nm at intervals of 0, 2, 4, 6, 8 and 24 h, the rate of oxidation of each chelate was determined by measuring the change in the maximum absorbance value for the band at 350—360 nm.

Gas chromatography

A Hewlett-Packard model 5750 gas chromatograph equipped with a flame-ionization detector was used. The column was a borosilicate glass coil (3 ft × 0.25 in. o.d.) packed with 3% (w/w) SE-30 on Chromosorb 750 (80—100 mesh). High-purity, dry nitrogen (40 ml min^{-1}) was employed as carrier gas. Although column temperatures were varied (see Figs. 1 and 4), injection port and detector temperatures were maintained at 210°C. Before use, the column was conditioned at 220°C for 12 h, silanized with 200 μl of HMDS and 200 μl of BSTFA, and then re-conditioned for a further 12 h.

Procedure for the extraction of vanadium

Vanadium standard (100 $\mu g\ ml^{-1}$, 5.0 ml) was transferred to a 50-ml beaker and evaporated to dryness on a steam-bath. Acetate buffer (2 ml) and sodium dithionite (0.2 g) were added, and the solution was re-heated for 5 min to reduce the vanadium. Reagent β-diketone solution (4 ml) was added

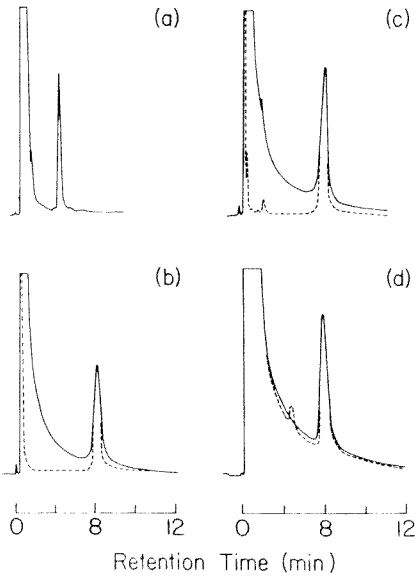


Fig. 1. Chromatograms of the extracted vanadium(III) derivatives of (a) HHfa, (b) HTfa, (c) HTbm and (d) HTpm. Broken lines show the effect of washing the extracts with saturated, aqueous sodium hydrogencarbonate (b, c) or sodium hydroxide (d). Column temperatures 40, 120, 120 and 130°C, respectively. Volume injected, 1 μl.

and the solution heated for a further 5 min on the steam-bath. The solution was cooled, diluted to 10 ml with distilled water, transferred to a 25-ml separating funnel and shaken with carbon disulphide for 2 min. The extract was finally washed with sodium hydroxide solution (1 M) to remove excess of β-diketone and examined by g.c. Both extraction and subsequent chromatography were completed with minimum delay (≯ 1 h).

In the interference studies, vanadium solution (100 μg ml^{-1}, 2.0 ml) was mixed with a solution of the test ion (0.4 ml). The above procedure was repeated, except that the carbon disulphide (5.0 ml) contained n-octadecane (0.04% w/v) as internal standard.

Analysis of petroleum oils

Approximately 10 g of oil (containing 100—300 μg V) were dry-ashed as in the standard method IP-285 [31]. The ash was dissolved in nitric acid (5 M, 2 ml), and then treated as above. Standards were prepared similarly by extracting 0, 1.0, 2.0, 3.0, 4.0 and 5.0 ml of the working standard (100 μg V ml^{-1}). Aliquots (1 μl) of extracts were injected onto the column, and vanadium concentrations determined from a plot of peak height ratios (chelate/internal standard) vs. vanadium concentrations.

For comparison, vanadium in the oils was determined colorimetrically by the standard [31] method based on formation of the tungstophosphate species.

RESULTS AND DISCUSSION

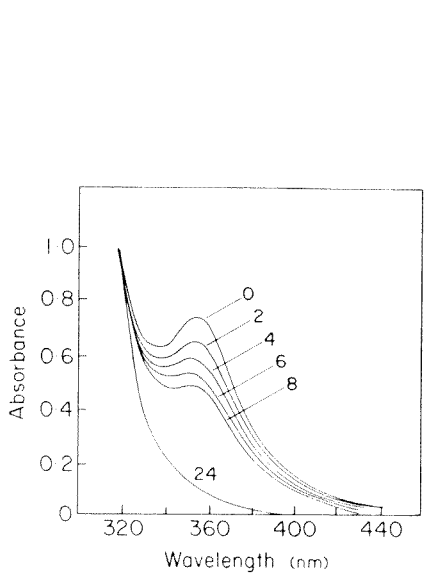
Reduction, derivatization and extraction of vanadium

Reduction of vanadium(IV) and (V) to the trivalent form was accomplished readily with sodium dithionite [30] in aqueous solution. Vanadium(III) was derivatized, extracted and examined by g.c. as described above. Chromatograms obtained for extracts of several β-diketonates (Fig. 1) show that tailing from the excess of ligand can interfere with measurement of chelate peaks.

Several procedures were tested for "back-extracting" the ligand. Washing carbon disulphide extracts with dilute sodium hydroxide (0.2 M) was suitable only for removing excess of HHfa, HTfa and HTbm, but some decomposition of the chelates occurred, as was evident from bleaching of the brown extracts. This treatment was, however, ineffective for HTpm, HPpm and HHpm, as were aqueous solutions (0.2 M) of sodium carbonate and ammonia. In contrast, sodium hydrogencarbonate was effective in removing HTfa and HTbm without decomposing the chelates, yet this mild base decomposed $V(Hfa)_3$. Further attempts to extract HTpm, HPpm and HHpm with more concentrated sodium hydroxide (2 M) were unsuccessful although this solution did not decompose the vanadium(III) derivatives. Removal of these ligands as water-soluble cyanohydrins or Schiff bases (by reaction with aqueous potassium cyanide or sodium glutamate, respectively) was equally unsuccessful. Finally in this regard, it is interesting that previous investigations [22, 32] have reported dilute hydroxide (0.1 M) to be suitable for removing excess HHpm from $Co(Hpm)_3$ and $V(Hpm)_3$ extracts in benzene. Although suitable for an electron-capture detector, this solvent is totally unsatisfactory for flame-ionization detection because of the pronounced solvent tail.

Stability of the vanadium(III) chelates in solution

The fact that vanadium(III) β-diketonates are air-sensitive [30] suggests that some difficulty may be encountered in their use for analytical purposes, particularly in trace analyses. Accordingly, the rate of oxidation of each chelate in air-saturated chloroform, as a representative solvent, was determined by measuring the change in absorbance of the band at 350—360 nm. [From the published [33] value of the Bunsen coefficient (0.205 at 16.3°C) with the application of Henry's Law and the ideal gas equation, the concentration of oxygen in such chloroform, is estimated to be 1.8×10^{-4} M.] A set of spectra is shown for $V(Ppm)_3$ in Fig. 2 and the sensitivity of five fluorinated chelates to air (compared with the HAcac chelate) is summarized in Fig. 3. These results confirm the finding [30] that the fluorinated chelates are more stable than the acetylacetonate, and that this stability increases with the degree of fluorination. Of the fluorinated chelates, $V(Ppm)_3$ and $V(Hpm)_3$ are the most stable, but all chelates were completely oxidized within 24 h, presumably to the corresponding oxovanadium(IV) chelates.



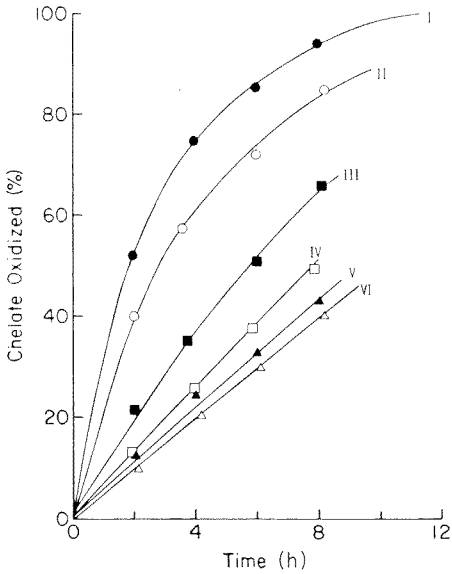


Fig. 2. Spectra of 10^{-4} M solution of $V(Ppm)_3$ in chloroform after standing for 0, 2, 4, 6, 8 and 24 h, showing changes caused by oxidation of the chelate.

Fig. 3. Relative rates of oxidation of the vanadium(III) derivatives of HAcac (I), HTfa (II), HTbm (III), HTpm (IV), HPpm (V) and HHpm (VI) in air-saturated chloroform at 21°C.

For the procedure given, this difficulty was overcome by storing the extracts under aqueous sodium dithionite prior to chromatography.

Another problem associated with the sensitivity of the vanadium(III) β-diketonates to air is their oxidation by traces of oxygen in the carrier gas within the chromatographic column. At the column temperatures employed (100—200°C), reaction of the vanadium(III) chelates with oxygen is rapid [29]. Calculation shows that a chelate having a retention time of 30 min may react with approximately 4 μg of oxygen before reaching the detector in a carrier gas containing 1 ppm (v/v) oxygen at 100 ml min^{-1}. Clearly, in such circumstances if submicrogram quantities are to be eluted, further purification of the carrier gas is essential.

Chromatographic behaviour

As is well established, retention of metal β-diketonates in g.c. columns depends foremost on the metal ion but also on the β-diketone substituents and the chromatographic conditions. When compared with other elements, vanadium(III) chelates appear less satisfactory for g.c. than those of beryllium(II), aluminium(III) and chromium(III) but superior to those of copper(II), scandium(III), iron(III), manganese(III) and other labile or coordinately unsaturated chelates. In overall behaviour, the vanadium(III) ion is comparable to cobalt(III); both require reagents such as HPpm or HHpm and relatively inert chromatographic supports (viz. Chromosorb 750 or Gas Chrom Q) for quantitative measurements at submicrogram levels.

Interference of common metal ions in the determination of vanadium with HPpm

At least three types of interference can arise in the g.c. determination of a particular metal chelate: (i) overlap of the peak with those of the solvent, organic components or pyrolysis products; (ii) overlap of the peak with those of other chelates; and (iii) interferences in the formation or extraction of the desired chelate. Of these, (i) is not relevant, here.

Interferences of type (ii) were judged from the resolution of a mixture of the chelates of Al(III), Sc(III), Cr(III), Co(III) and Cu(II), when injected onto the column. Whereas the Al(III) and Cu(II) chelates were completely separated from $V(Ppm)_3$, those of Cr(III), Sc(III) and Co(III) were incompletely separated and could, therefore, interfere if co-extracted with V(III). Interference need not occur in all such cases of course. For example, cobalt will not interfere because Co(II), and not Co(III), exists in the reducing environment necessary to form V(III).

Interference in the recovery of vanadium, and distinction between interference of types (ii) and (iii) were ascertained from the effect of various ions on the response for the vanadium derivative. This was determined by measuring the change in the peak-height ratio of the extracted $V(Ppm)_3$ (relative to n-octadecane), after the addition of each ion. Of the eight ions tested (see Table 1), only chromium(III) produced a significant level of unexpectedly negative interference. This may be due to interference in the reduction of vanadium to the trivalent state or formation of an involatile binuclear species containing Cr(III) and V(III). Ions such as Mn(II), Fe(II) and Ti(IV) gave coloured extracts but did not interfere.

Analysis of petroleum oils for vanadium with HPpm

The derivatization and extraction procedures were applied to vanadium standards and to samples of authentic petroleum products. A typical chromatogram for the extract of one of the standard solutions is shown in

TABLE 1

Interference of common metal ions in the g.c. determination of vanadium as $V(Ppm)_3$

Interfering ion	Peak height ratio[a] (%)	Error (%)	Colour of extract	Interfering ion	Peak height ratio[a] (%)	Error (%)	Colour of extract
O	58.3[b]	—	Amber	Zn(II)	62.7	+7.5	Amber
Mn(II)	58.0	0	Bright yellow	Cr(III)	23.3	−60[c]	Yellow—Green
Co(II)	54.8	−6	Amber	Fe(II)	57.0	−2	Yellow—Orange
Ni(II)	55.6	−4	Amber	Ti(IV)	51.2	−12	Yellow
Cu(II)	51.3	−12	Amber				

[a]Unless stated otherwise, values are means of duplicate results, expressed as a percentage of the n-octadecane peak. [b]This value is the mean of 7 results (r.s.d. 11.2%). [c]A second duplicate determination (−63%) confirmed this effect.

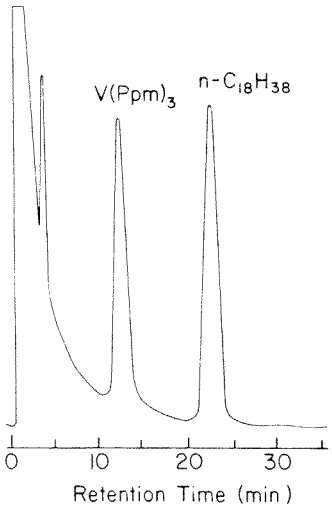


Fig. 4. Chromatogram showing peaks of the extracted vanadium derivative $V(Ppm)_3$ and internal standard (n-octadecane). The peak for the derivative corresponds to about 40 ng of vanadium. Column temperature, 150°C. Volume injected, 1 μl.

Fig. 4; chromatograms for the sample extracts were similar in form, and free from peaks attributable to other metal ions. Repeated injection onto the column of the extract for the least concentrated vanadium standard (20 μg ml^{-1}) gave a constant area response ratio, indicating negligible column loading effects. A linear calibration curve for the range 0–100 μg V ml^{-1} of extract was also obtained (peak height ratio range (0—120%)).

The vanadium content of four oils, determined by the calibration method, are shown in Table 2, together with the results obtained by a standard [31] colorimetric method. If the values obtained by the latter method are correct, the new g.c. method gives results which are significantly lower (by 16—37%) than the actual concentrations. Other metal ions in the oils, having an effect similar to that of Cr(III), are probably responsible for these low values.

TABLE 2

Comparison of the vanadium concentrations found in petroleum oils by g.c. and the standard colorimetric method IP285

Sample	Vanadium concentration (μg g^{-1})	
	G.c. method[a]	IP285[b]
Heavy arab crude	17.3	20.5
Fuel oil I	16.0	24.2
Fuel oil II	15.2	24.2
Fuel oil III	20.2	31.1

[a]Results are means of two determinations and duplicate injections. [b]Results are means of 2—3 determinations.

Similar discrepancies were not reported [32] in the determination of vanadium [as $V(Hpm)_3$] in polymers and vanadium-containing catalysts although low concentrations of interfering ions in these samples seem possible. The introduction of less convenient procedures (such as standard addition, suitable masking reactions for interfering ions, or a prior separation of vanadium or interference) would be necessary to solve this problem. Such possibilities were not examined because of the advantages inherent in the use of tetradentate Schiff bases [34] for the determination of vanadium in petroleum oils. Nevertheless, in some applications where vanadium can exist partly in the trivalent form (as in catalysts and polymers [32], oxides and glasses [35], or biological materials [36], the above procedure may be of value. Reference has been made elsewhere [27] to the g.c. separation of V(III) and V(IV) as β-diketonates.

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DETERMINATION OF VANADIUM IN PETROLEUM CRUDES AND FUEL OILS BY GAS CHROMATOGRAPHY

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SUMMARY

A method is described for determining levels of vanadium as low as 0.1 $\mu g\ g^{-1}$ in petroleum oils by gas chromatography with flame-ionization detection. The method involves decomposition of the oils, derivatization and solvent extraction of vanadium as the chelate, [2,2,2′,2′-tetramethyl-5,5′-(ethane-1,2-diyldiimino)bis(hex-4-en-3-onato)-(2-)]oxovanadium(IV), followed by gas chromatography on a non-polar (SE-30) column. The procedure is applied to a variety of samples, including standard reference and fuel oils used in an interlaboratory study. The results are compared with those of emission spectrography and two standard methods. The new procedure can be extended to the selective, simultaneous detection of copper, nickel and vanadium in such samples.

Numerous techniques have been applied to the determination of vanadium in petroleum oils [1—3], but the current standard methods are based on colorimetry [4, 5], atomic absorption spectrometry (a.a.s.), [6, 7] and emission spectrography [8]. Colorimetric methods [4, 5] for determining vanadium concentrations greater than 10 $\mu g\ g^{-1}$ in oil depend on the formation of a yellow tungstophosphate complex, and although reliable, are time-consuming and lack adequate sensitivity. More sensitive, but equally time-consuming methods for the range 0.1—1.0 $\mu g\ g^{-1}$ use haematoxylin [9] or 3,3′-dimethylnaphthidine [10].

Rapid methods requiring only dilution of the oil, use a.a.s. with either a nitrous oxide—acetylene flame [11, 12] or furnace for atomization [13—15]. Similar, more sensitive methods [16] employ emission spectrometry with an inductively-coupled plasma. Of the standard methods, those employing a.a.s. with flame atomization are generally not suitable below the 10 $\mu g\ g^{-1}$ level. Furthermore, their accuracy has been questioned [11, 17, 18] because absorbance values obtained with a given standard solution vary with the type of organometallic vanadium compound. With furnace atomization, concentrations in the range 0.5—20 $\mu g\ g^{-1}$ can be determined directly in oils

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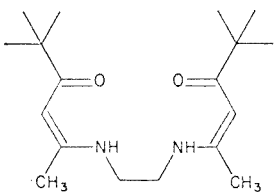
without critical dependence on the organometallic standard used [15]. However, difficulties encountered with this technique include matrix interference [19, 20], spectral interference from the furnace, memory effects and rapid furnace deterioration [21].

Since petroleum oils may contain [22] less than 0.01 μg V g^{-1}, it is desirable that rapid and more sensitive methods be developed. In this paper, a novel method is reported for the determination of vanadium in petroleum oils employing gas chromatography (g.c.). The method involves decomposition of the sample followed by reaction with the Schiff base 2,2,2′,2′-tetramethyl-5,5′-(ethane-1,2-diyldiimino)bis(hex-4-en-3-one), I, to give the volatile chelate, II. The reagent, I, which has been used previously [23] for



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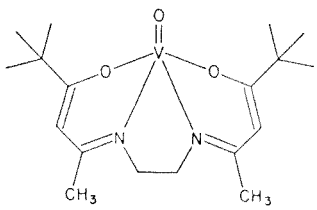

II

the determination of copper and nickel is easily prepared from commercial reagents.

Earlier reports [24, 25] of the determination of vanadium by g.c. involve the formation of fluorinated vanadium(III) β-diketonates which are air-sensitive and tend to oxidize in solution. The Schiff base chelate II, however, appears more suitable for analytical purposes [26].

EXPERIMENTAL

Properties of the ligand

Ligand I was prepared by the condensation of 2,2-dimethylhexane-3,5-dione and 1,2-diaminoethane in ethanol [27] and purified by recrystallization from petroleum spirit (b.p. 100–120°C) giving white needles m.p. 115°C (lit. [28] 117–118°C). (Found 70.6% C, 10.3% H, 9.0% N; calc. for $C_{18}H_{32}N_2O_2$, 70.1% C, 10.4% H, 9.1% N). N.m.r.: ($CDCl_3$) δ 1.10 (Bu^t), 1.92 (CH_3), 3.41 (CH_2), 5.14 (ring CH=), 11.08 (ring NH, exchangeable with D_2O); proton counts were consistent with the structure.

Reagents and solutions

Unless stated otherwise, solvents and reagents were of analytical grade, and were used without further treatment. Details given below are additional to those specified by the standard methods or in the procedures.

Methanolic acetate buffer. Ammonium acetate (10.7 g) and glacial acetic acid (6.0 g) were dissolved in 100 ml of methanol.

Internal standard solution. 0.0015% n-octacosane (w/v) in carbon disulphide was prepared by dilution of stock 0.15% n-octacosane in carbon disulphide.

Solutions for interference studies. Stock solutions (500 $\mu g\ ml^{-1}$) of thirteen metal ions (see Table 2) were prepared by dissolving an appropriate salt of each metal ion in distilled water.

Preparation of vanadium standards

For emission spectrography. Vanadium pentoxide (0.180 g; Merck, extra pure) was ground with lithium carbonate (4.82 g; BDH) to a homogeneous powder. Consecutive, ten-fold dilutions of this standard (1.00% w/w) with lithium carbonate gave additional standards of 0.100%, 0.010% and 0.0010% vanadium.

For colorimetry, g.c. and a.a.s. A stock solution (1000 $\mu g\ V\ ml^{-1}$) was obtained by dissolving ammonium metavanadate (0.574 g; Merck, p.a.) in concentrated nitric acid (65 ml), and diluting to 250 ml with distilled water. Working standards (100 and 10 $\mu g\ ml^{-1}$) were prepared freshly before use from the stock solution by making successive ten-fold dilutions in water.

For the standard method IP288 [6], a stock solution (500 $\mu g\ V\ ml^{-1}$) was prepared by dissolving bis(1-phenylbutane-1,3-dionato)oxovanadium(IV) in isopropanol—white spirit (1:10 by volume). For this purpose, the chelate was prepared and purified as previously described [3]. Its vanadium content was determined gravimetrically [29] and by a.a.s. after decomposition of the chelate. (Found, 61.1% C, 5.2% H, 13.0% V; calc. for $C_{20}H_{18}O_5V$, 61.7% C, 4.6% H, 13.1% V.)

Instrumentation

Emission spectrography. Emission spectra were obtained on a Baird GX4 Spectrograph with d.c. arc excitation and recorded on Ilford No. 30 photographic plates in the range 232 321 nm. Densities of selected emission lines were determined with a microdensitometer (Optica Milano 651). Details of sample excitation, wavelength calibration and plate development are recorded elsewhere [3].

Colorimetry. Vanadium was measured as the tungstophosphate at 405 nm in 4-cm quartz cells using a Carl Zeiss PMQ II spectrophotometer.

Atomic absorption spectrometry. A Varian-Techtron instrument (model AA6) was used with a nitrous oxide—acetylene flame and background corrector (model BC-6).

Gas chromatography. A Hewlett-Packard F and M Model 5750B gas chromatograph with a flame ionization detector was employed. A borosilicate glass coil (3 ft. × 0.25 in. o.d.) packed with 3% SE-30 on Chromosorb 750 (80/100 mesh) was silylated and conditioned before use, as previously described [26]. The nitrogen carrier gas flow was 40 ml min^{-1}. Column, injection port and detector temperatures were 200°, 210° and 210°C, respectively.

Petroleum oil samples

Light Arab and Bass-Strait crudes and fuel oils (identified as I, II and III) were generously supplied by Australian Oil Refining Pty. Ltd., Kurnell, Australia; heavy Arab crude and bunker fuel by the New South Wales Public Transport Commission's Redfern Laboratory, and Indonesian crudes by Pertamina Oil Company, Indonesia. Fuel oils SRM-1634 and GM-5 (National Bureau of Standards, Washington, DC) were also tested.

To minimize the effects of volatilization and sedimentation, sampling of the oils for analysis followed the recommended procedure [30] of the Institute of Petroleum.

Ashing procedures

Dry ashing. The ignition and dry ashing of oils [4] for colorimetric analysis was as follows: oil (20.00 g) and sulphur (2.0 g) were weighed into a clean 100-ml silica dish and ignited over a bunsen flame. After combustion, the residual coke was ashed at 500–520°C in a muffle furnace for 12 h.

For samples analysed by emission spectrography, lithium carbonate (30.0 mg) was added to the oil, as ash-collector, before ignition of the sample. The ash was weighed and then transferred quantitatively to a clean dry vial where it was finely powdered and homogenized with a dry rod.

For a.a.s. or g.c., convenient sample weights of oil (1—40 g) were taken, depending on the vanadium content of the oil.

Acid digestion. Rapid decomposition of oils for g.c. was accomplished as follows: oil (1.00 g) was weighed into a 100-ml Kjeldahl flask and heated with concentrated sulphuric acid (10 ml) on a ring-heater to fumes of sulphur trioxide. Perchloric acid (4 ml, 72%) was then added dropwise over about 15 min to maintain vigorous oxidation. On completion of oxidation, the clear solution was transferred to a 100-ml beaker and fumed to dryness on a hot plate. A "blank" was prepared at the same time.

Procedures for measurement of vanadium

Emission spectrography. To each sample ash (weighing approximately 30 mg) and standard (30.0 mg) containing 0.0010%, 0.0100%, 0.100% or 1.00% (w/w) vanadium in lithium carbonate, was added powdered graphite (40.0 mg, Ringsdorff RWIV) and the mixture was homogenized on a mechanical shaker for 2 min. Portions (20.0 mg) of the samples and standards were then loaded and compressed into graphite cup-electrodes machined from 0.25-in. RWIV graphite rods. Conical cathode counter-electrodes machined from similar graphite rods were used. After recording of the spectra and developing the plates [3], the densities of the vanadium lines at 318.34 or 265.62 nm were measured and the concentration of vanadium ($\mu g\ g^{-1}$) in each oil calculated from the calibration curve.

Colorimetry. The Institute of Petroleum Method IP285 [4] was used.

Atomic absorption spectrometry. Vanadium in fuel oil III was determined directly by the Institute of Petroleum Method IP288 [6]. Alternatively, the

oil (10 g) was ignited, dry-ashed, and the residue dissolved in 1 M hydrochloric acid (10.0 ml). Vanadium was determined from a calibration plot prepared with standard solutions of 0, 10, 20, 30, 40 and 50 μg V ml^{-1} in 1 M hydrochloric acid.

Gas chromatography. To each sample residue in a beaker (50 ml) was added 1.0 ml of concentrated sulphuric acid and the solution was evaporated to dryness on a hot plate. After cooling, sulphurous acid (5.0 ml; 6% (w/v) sulphur dioxide in water) was added to reduce vanadium to the oxovanadium(IV) state and the solution was carefully reheated to dryness. Buffer (5.0 ml) and ligand solution (3.0 ml; 4 g of I in 100 ml of methanol) were then added and the solution was warmed on the steam bath for 15 min to effect chelation. On cooling, the solution was diluted with distilled water (30 ml) and extracted with the internal standard n-octacosane solution (5.0 ml). With samples containing less than 5 μg V g^{-1}, the internal standard solution was diluted tenfold (to 0.00015% w/v n-octacosane) and the extract reduced to 0.5 ml. Vanadium was then determined from a calibration plot based on triangular peak area ratios (chelate/internal standard) obtained from standards containing 0, 10.0, 20.0, 30.0, 40.0 and 50.0 μg vanadium.

RESULTS AND DISCUSSION

Extraction and chromatographic behaviour of the vanadium derivative

Before the volatile complex was formed for g.c., it was necessary to decompose the petroleum oils by one of the ashing procedures described and then reduce the vanadium selectively to the oxovanadium(IV) state. The reduction was achieved conveniently with sulphur dioxide although other common reducing agents [31] such as H_2S, Fe(II), Sn(II), sodium hydrogen-sulphite or ascorbic acid may be suitable.

Derivatization was carried out in methanol because chelation was appreciably faster in this solvent than in an aqueous or aqueous–methanol medium. Consequently, it was necessary to evaporate the solution containing the reduced vanadium to dryness before derivatization. The buffer used in the derivatization was ammonium acetate–acetic acid although sodium acetate—acetic acid and triethanolamine acetate—acetic acid were equally satisfactory. However, sodium acetate or triethanolamine alone considerably reduced the yield of chelate. For efficient extraction with carbon disulphide, water was added to the methanol medium prior to extraction. The alternative procedure of extracting with a carbon disulphide solution of the ligand [23] was not attempted.

A chromatogram of the extracted chelate is shown in Fig. 1. Repeated injection of such small quantities of extracted chelate (1-μl volumes equivalent to 20 ng of vanadium) confirmed the absence of column loading and showed that the conditions were suitable for quantitative analysis. Another important detail is the stability of solutions of the chelate in carbon disulphide. Thus, extracts containing two different vanadium concentrations

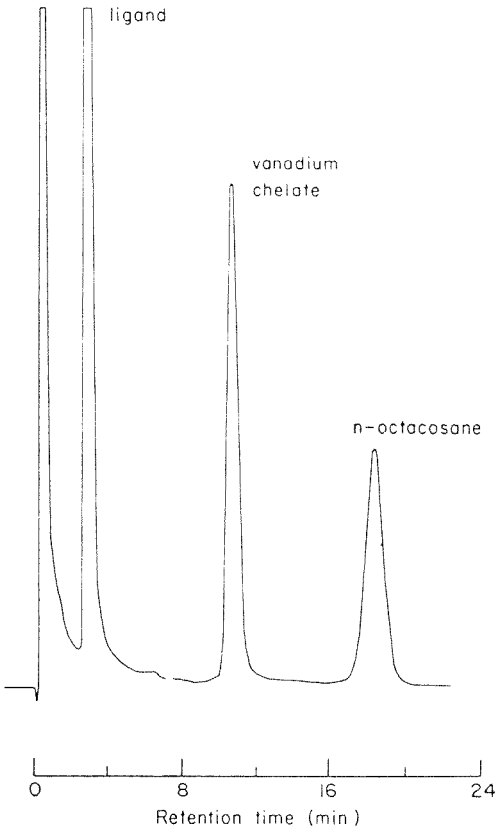

Fig. 1. Chromatogram showing peaks of solvent, unreacted ligand, oxovanadium(IV) derivative and internal standard. The peak for the derivative corresponds to about 40 ng of vanadium. Conditions as given in the Experimental section.

(Table 1) were stable for at least six days whereas solutions of II in benzene or chloroform gave highly insoluble solids and a diminished chromatographic response on standing for 1–2 days. Similarly, extracts of fuel oil III (containing about 60 μg V ml^{-1}) were stable over a six-day period. (The insoluble solids mentioned may be solvates or polymeric vanadium(V) compounds produced by aerial oxidation of the oxovanadium(IV) chelate [32, 33]; the corresponding nickel chelate is also unstable in solution [23].)

A typical calibration plot for the range 0—50 μg V ml^{-1} of extract was linear with peak height ratios in the range 0—40%.

When too great an excess of ligand was used, this interfered with measurements at lower concentrations because of tailing of the ligand peak. However, because of its low acidity, the unreacted ligand proved difficult to remove; for example, washing the extract with 1 M sodium hydroxide was ineffective. The problem was overcome by maintaining the concentration of ligand at approximately 100-fold excess over the metal ion concentration.

TABLE 1

Stability of carbon disulphide extracts of the vanadium chelate(II) over a six-day period

Time (h)	0	24	48	72	96	120	144
Ratio[a] (10 μg V ml^{-1})	0.39	0.39	0.42	0.45	0.45	0.41	0.40
Ratio[a] (50 μg V ml^{-1})	2.00	1.85	1.88	1.89	1.84	1.81	1.88

[a]Peak height relative to n-octacosane. Results are the means of three determinations.

Interference of common metal ions

The effect of thirteen metal ions on the procedure for determining vanadium was studied by measuring the change in the peak height of the extracted derivative II relative to that of the internal standard after the addition of each ion. The results (Table 2) show that Ti(IV) and Co(II) cause severe depression in the response. This type of interference may result from condensation of the oxovanadium(IV) chelate to form an involatile dimeric or polymeric species containing vanadium and titanium or cobalt. Since titanium alone is known [34] to react in this way, the related vanadium chelate may cease to be available in a monomeric, volatile form. Consequently, a suitable masking reaction to remove coordinative unsaturation in the cobalt and titanium chelates (e.g. pyridine and fluoride ion, respectively) may be required.

TABLE 2

Interference of various metal ions on the determination of vanadium by gas chromatography

Interfering ion	Colour of extract	Peak height ratio[a]	
		Ratio of interfering ion (w/w)	
		1:1	10:1
O	Pale green	1.52	1.52
Ti(IV)	Pale-yellowish green	0.39	0.32
Co(II)	Brown	0.78	0.72
Fe(II)	Yellow	1.49	1.57
Cr(III)	Pale green (pink aq. phase)	1.53	1.46
Mn(II)	Yellowish green	1.58	1.61
Cu(II)	Violet	1.51	1.52
Ni(II)	Pale green	1.53	1.59
Zn(II)	Pale green	1.61	1.65
Zr(IV)	Pale green	1.50	1.47
Sn(II)	Pale green	1.56	1.44
Mo(VI)	Pale green	1.49	1.57
W(VI)	Pale green	1.63	1.47
U(VI)	Pale green	1.55	1.55

[a]Relative to n-octacosane. Results are the means of three determinations using 100 μg of vanadium and 5.0 ml of internal standard solution with 100 μg or 1000 μg of interfering ions.

A second form of interference was observed with all the cations examined because of competition for the ligand. This also was resolved for all except the Ti(IV) and Co(II) ions by increasing the concentration of ligand to 100-fold excess over the total metal ion concentration. Among the thirteen metal ions studied, distinct chromatographic peaks were obtained for the derivatives of Cu(II) and Ni(II) but these did not interfere because of complete separation from the vanadium chelate (see Fig. 2). A peak for the cobalt(II) derivative was also observed when a ten-fold excess of Co(II) ion was employed. This was seen as a small peak with retention similar to that of the nickel chelate. Although the ligand obviously reacted with several other metal ions, the products evidently were not volatile. For example, chromium(III) reacted to form a pink species which was not extracted from the aqueous phase, whereas iron(II) gave a yellow species, extracted by carbon disulphide but not detected chromatographically. Thus, tetradentate Schiff bases are selective derivatizing agents for the determination of metals by g.c. and appear to be restricted, at present, to Cu(II), Ni(II), Co(II), Pd(II), Pt(II) and V(IV).

Analysis of petroleum oils for vanadium

Results for the analysis of fourteen petroleum oils, using g.c. and a direct calibration procedure are given in Table 3. The results for dry and wet ashing

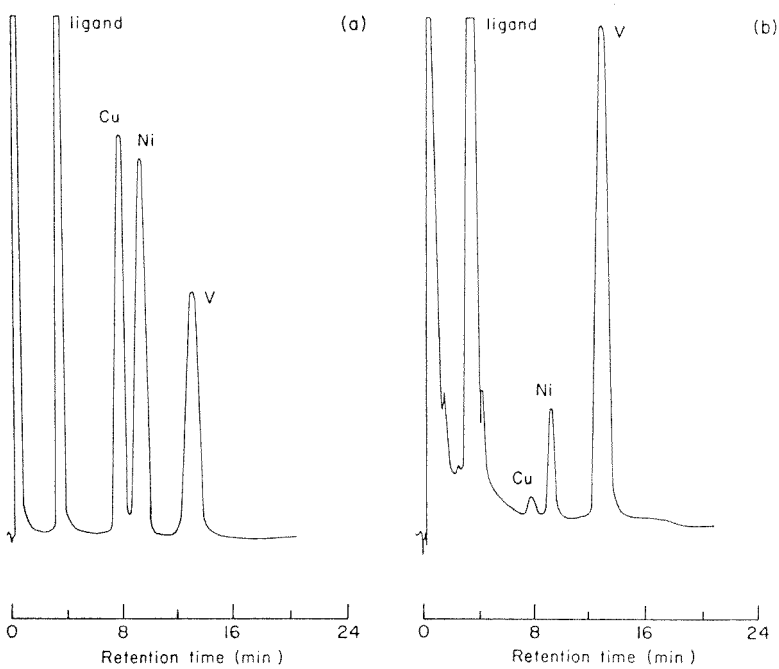

Fig. 2. Chromatograms of (a) a mixture of the ligand with the copper(II), nickel(II) and oxovanadium(IV) derivatives; (b) an extract originating from fuel oil III showing the presence of copper, nickel and vanadium in the oil.

TABLE 3

Determination of vanadium in various petroleum crudes and fuel oils by different methods

Sample	Vanadium content ($\mu g\ g^{-1}$)			
	Emission spectrography	Colorimetry (IP285)	Gas chromatography	
			Dry ashing	Wet ashing
Heavy Arab crude	20	20.5	17.8	20.2
Light Arab crude	16	15.1	15.3	14.7
Bass-Strait crude	0.6	—	0.3	—
Bunker fuel	8	7.2	9.8	9.6
Fuel oil I	19	24.2	23.6	23.1
Fuel oil II	27	24.2	22.3	22.9
Fuel oil III	28	31.2 ± 2.2[a]	30.0	29.5 ± 1.1[a]
Offshore Java crude	0.25	—	0.12	—
Java crude	0.08	—	—	—
Kalimantan crude	0.05	—	—	—
Irian Jaya crude	6.6	—	8.6 ± 0.5[a]	—
Sumatra crude	0.45	—	0.25	—
Residual fuel oil SRM-1634[b]	—	—	317 ± 6.0[a]	—
Residual fuel oil GM-5[c]	—	—	71.5 ± 2.3[a]	—

[a]Results (means ± std. deviations) based on four determinations. Other values represent single determinations. [b]NBS Certified value for SRM-1634 is 321 ± 15 $\mu g\ g^{-1}$. [c]The value given for GM-5 is 79 ± 1.2 $\mu g\ g^{-1}$.

are in close agreement, showing that negligible vanadium was lost during the combustion and ashing procedure. Furthermore, the values for the other oils (ranging from about 0.3 to 320 $\mu g\ V\ g^{-1}$) agree well with those obtained by the emission spectrographic and standard colorimetric methods, or with the certified value. However, of the two certified oils, the vanadium level found in GM-5 was significantly lower than the certified value despite the good precision of the four determinations. Nevertheless, the satisfactory results obtained by direct calibration for all other samples suggest that interference effects are negligible in most cases and that the determination of vanadium by g.c. is competitive with the present standard colorimetric methods IP285 and ASTM-D1548. It may be noted that the detection limit of about 0.1 $\mu g\ V\ g^{-1}$ is governed by the sensitivity of the flame-ionization detector. Extension of the method to ultra-trace determinations of vanadium will depend on the successful use of a fluorinated Schiff base and electron-capture detection.

Interlaboratory comparison

In an inter-laboratory study based on fuel oil III (see Table 4), the results for the colorimetric and g.c. methods again agree closely. In contrast, the standard a.a.s. method [6] yielded significantly higher values, varying widely about the mean of 42 $\mu g\ V\ g^{-1}$. These findings lend support to previous doubts expressed [11, 17, 18] concerning the accuracy of a.a.s.

TABLE 4

Vanadium concentrations ($\mu g\ g^{-1}$) obtained in an inter-laboratory study of fuel oil III

Laboratory[a]	A.a.s.		Colorimetry (ASTM-D1548)	G.c. (wet ashing)
	Organic (IP288)	Aqueous		
1	39	—	36	—
2	62	—	29	—
3	29	—	33	—
4	50	—	30	—
5	30	—	26	—
This work[b]	43 ± 5.5	31.2 ± 0.9	31.2 ± 2.2[c]	29.5 ± 1.1

[a]Industrial laboratories in Sydney (Australia). Values given for laboratories 1—5 represent single determinations. [b]Results (means ± std. deviations) based on four determinations. [c]IP285 method.

in the analysis of petroleum oils without prior decomposition of the sample. Indeed, Bowman and Willis [11], and more recently Fuller [35] and Lang et al. [36] have advocated prior digestion of the oils in acid, to avoid the difficulty involved in the use of organometallic standards. The need for this acid-digestion step was confirmed by further analysis of fuel oil III by a.a.s. (Table 4) using both a calibration and standard addition procedure; results obtained without acid digestion were 43 ± 5.5 μg V g^{-1} and 48 ± 6.5 μg V g^{-1}, respectively, and remained essentially unchanged after background correction. After acid digestion, the value obtained by a.a.s. was in good agreement with the results obtained by colorimetry and g.c.; the precision was better than that for the alternative a.a.s. procedure. In view of this evidence, and the relative instability of many vanadium chelates in organic solvents [2, 32, 37], it appears that the existing standard methods IP288 and D2787 [6, 7] require revision.

In conclusion, attention is drawn to an important feature of the gas chromatographic method. This is its capability for simultaneous determination of the three transition metals copper, nickel and vanadium, typically present in petroleum. Figure 2(a) shows a chromatogram obtained for a mixture of the Schiff base I and chelates of the three metals. Figure 2(b) shows a chromatogram from an extract originating from fuel oil III, with distinct peaks for copper and nickel, as well as vanadium. The efficacy of the chromatographic method for separating and determining these constituents of petroleum may be of value in characterizing crudes of different geographical environments, because the ratio copper : nickel : vanadium varies widely in such samples [22, 38]. For similar reasons, the determination of these elements by g.c. may be useful in the characterization of oil spills and oil-polluted waters, thereby complementing the chromatographic data concerning the organic constituents [39] of such samples. Another advantage of the chromatographic method lies in its potential for distinguishing V(IV) and V(V) species.

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SERINE-SELECTIVE MEMBRANE PROBE BASED ON IMMOBILIZED ANAEROBIC BACTERIA AND A POTENTIOMETRIC AMMONIA GAS SENSOR

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SUMMARY

A new class of bioselective membrane probes using anaerobic bacteria is introduced with the successful construction of a L-serine-selective probe consisting of *Clostridium acidiurici* cells coupled to a potentiometric ammonia gas sensor. The intact cells containing the enzyme serine dehydratase are physically immobilized at the electrode surface in conjunction with iron(II) stearate, which is shown to enhance response characteristics. The potential vs. log concentration plot is linear from 1.6×10^{-2} to 1.8×10^{-4} M serine with an average slope of 54 mV/decade and response times of 3—5 min. Optimal behavior of the probe is retained even in non-deaerated media for at least three days, and significant interference is posed only by L-glutamine. Quantitative conversion of serine is demonstrated over the linear concentration range, suggesting possible analytical or clinical applications for these probes utilizing anaerobic bacteria.

Bacterial cells have been coupled with potentiometric electrodes to produce sensors for the determination of glutamine, aspartate, arginine, cysteine, nitrate, histidine, NAD^+ and cholesterol [1—8]. The bacterial strains chosen as biocatalysts have all been cultured and employed aerobically. In this study, it is demonstrated for the first time that an anaerobically grown bacterium, *Clostridium acidiurici*, can be used in conjunction with a potentiometric gas sensor for the determination of the amino acid L-serine without vigorous deaeration of working or storage media. Serine is enzymatically converted to ammonia and other products by L-serine dehydratase (E.C. 4.2.1.13) according to

$$\text{L-serine} + H_2O \underset{\text{dehydratase}}{\overset{\text{serine}}{\rightleftharpoons}} \text{pyruvate} + NH_3 + H_2O \qquad (1)$$

The ammonia produced is then detected by an ammonia gas sensor, creating a serine probe with potentiometric response. While L-serine dehydratase occurs in both microbial and animal cells, the enzyme is found with high activity in the anaerobe *Clostridium acidiurici* [9], ATCC 7906.

A bacterial sensor can offer numerous advantages over other methods used to quantify amino acids. Classically, free amino acids have been separated on an ion-exchange column, reacted with ninhydrin and detected spectrophoto-

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metrically [10], a time-consuming and involved procedure. Other methods have included gas—liquid chromatographic separations of amino acid derivatives [11], high-performance liquid chromatographic separations [12], microbioassay [13], and mass spectrometry [14]. Some methods have failed to separate two amino acids, such as L-serine and L-threonine [15]. Potentiometric techniques offer several advantages over all of the above, including short assay time, adequate detection limit, low cost, and minimal sample preparation, as noted elsewhere [16]. Bacterial probes avoid the long and tedious enzyme separation and purification steps needed for immobilized enzyme probes; moreover, in the case of L-serine dehydratase, the enzyme is not commercially available.

EXPERIMENTAL

Apparatus and reagents

All potentiometric measurements were made with a Corning model 12 pH/mV meter in conjunction with a Heath-Schlumberger model SR-240 potentiometric recorder. Measurements were made in thermostatted cells controlled at 25 ± 0.1°C with a Haake model FS water bath. The ammonia gas sensor used for all work was the Orion model 95-10. An IEC model HT centrifuge was modified for refrigerated use. The bacteria were grown in a Torbal anaerobic jar model AJ-3; the jar was incubated in a National Appliance Co. (NAPCO) model 322 carbon dioxide incubator adjusted to 37°C.

All solutions were prepared with distilled, deionized water. Analytical-grade reagents were used except where otherwise noted. L-Serine and all other amino acids, dithiothreitol, glycylglycine, isonicotinic acid hydrazide, sodium thioglycollate, and uric acid were purchased from Sigma Chemical Co., St. Louis, MO. Yeast extract was obtained from Difco Laboratories, Detroit, MI.

The bacterium *Clostridium acidiurici*, ATCC 7906, was purchased from the American Type Culture Collection, Rockville, MD. A commercial anaerobic gas mixture consisting of 85% N_2, 10% H_2, and 5% CO_2 (Linde Division, Union Carbide Corp., NY) was used in the bacterial growth chamber at 6 psi.

Procedures

Clostridium acidiurici was grown in a medium consisting of 3.0 g of uric acid, 1.0 g of yeast extract, 4.0 g of K_2HPO_4, 0.1 g of $MgSO_4 \cdot 7H_2O$, and 0.005 g of $FeSO_4 \cdot 7H_2O$ per liter of water. The solution was heated with stirring to approximately 90°C for 20 min until all reagents dissolved. A 1.0-ml aliquot of aqueous 1% phenol red indicator was added and the solution was made alkaline dropwise with concentrated sodium hydroxide solution until a bright orange color appeared (pH 7.6—8.0). The solution was made 0.5% in sodium thioglycollate, an oxygen scavenger, divided among 125-ml culture flasks and autoclaved at 15 psi for 20 min. The flasks were cooled to 37°C before culturing aseptically with bacteria.

The inoculated flasks were placed in an anaerobic jar; the jar was evacuated and flushed with anaerobic gas mixture three times before being placed in a CO_2 incubator. Growth proceeded for approximately 24 h without disturbance; a prepared culture was indicated by a reduction of anaerobic jar pressure, the appearance of turbidity, and a shift in solution color toward red hues (pH $\approx$ 8.4).

The matured bacterial cultures were centrifuged and washed with a pH 7.0, 0.05 M potassium phosphate buffer. The washed cells were collected by centrifugation as a paste and approximately 10 μl was applied to the gas-permeable membrane of the ammonia electrode.

Iron(II) stearate was prepared by adding dropwise 2.5 ml of 0.01 M $FeSO_4 \cdot 7H_2O$ (dissolved in slightly acidified water) to 3.0 ml of 0.1% stearic acid dissolved in absolute ethanol. The resulting precipitate was centrifuged and approximately 5 μl was added to the bacterial paste on the ammonia gas-permeable membranes. The bacterial paste was fixed to the sensor membrane by means of a circular dialysis membrane, as described previously [17].

The probe was conditioned for 2 h in freshly prepared working solution prior to use. The working solution consisted of a pH 8.5 borate buffer containing 0.05 M boric acid, 0.05 M KCl, 0.01 M isonicotinic acid hydrazide (INH), 1.5×10^{-3} M dithiothreitol, and 4.5×10^{-4} M $FeSO_4 \cdot 7H_2O$. The iron(II) sulfate and dithiothreitol stock solutions were prepared daily and kept tightly stoppered between uses. The probe was stored in working solution between assays, and covered with parafilm if more than three hours elapsed between successive experiments.

RESULTS AND DISCUSSION

A typical calibration curve showing the bacterial probe response in a working solution of L-serine is shown in Fig. 1. The response was linear from 1.6×10^{-2} M to 1.8×10^{-4} M serine, with slope ranging from 52 to 56 mV/decade. The lower limit of detection of a new membrane averaged 4.0×10^{-5} M serine while the response time was between 3 and 5 min.

Both iron(II) ions and dithiothreitol were essential for the bacterial probe response. Iron(II) ions have been reported [9, 18] to activate the L-serine dehydratase enzyme believed to be responsible for the ammonia production [19]. Dithiothreitol was present in solution to protect the sulfhydryl groups on the enzyme from oxidation [20]. The order of addition of these two reagents was important; if iron(II) was added to working buffer containing dithiothreitol, an insoluble green iron(II) complex was formed which correlated with good membrane response and stability. The opposite order of addition resulted in no formation of green precipitate and poorer membrane characteristics.

The effect of various ions and buffer systems on enzyme activity was studied. Both potassium and ammonium ions have been shown [21] to

prevent or mitigate the reversible inhibition that has been reported with mammalian serine dehydratase [22]. These ions were said to lower the apparent Michaelis—Menten constant for the enzyme prosthetic group, pyridoxal phosphate, by a factor of 50 in alkaline media [21]. In the presence of potassium or ammonium ions, the half maximal rate of deamination was achieved at far lower pyridoxal phosphate concentrations. Potassium ions were chosen for the present study as they caused no interference at the ammonia gas sensor. Because serine deamination occurred freely without added pyridoxal phosphate under these working conditions, it is believed that the monovalent ion influence was important with this system as well.

The effect of various types of buffers on the response of the bacterial-based serine membrane probe was studied using the previously described initial rate method [23]. All buffers studied had a 0.1 M ionic strength and a pH of 8.5. The results of this study, which are summarized in Table 1, reveal that a glycylglycine buffer system yielded the greatest initial biocatalytic activity; however, a single exposure to L-serine was found to render the bacterial electrode inactive. This may have been due to complexa-

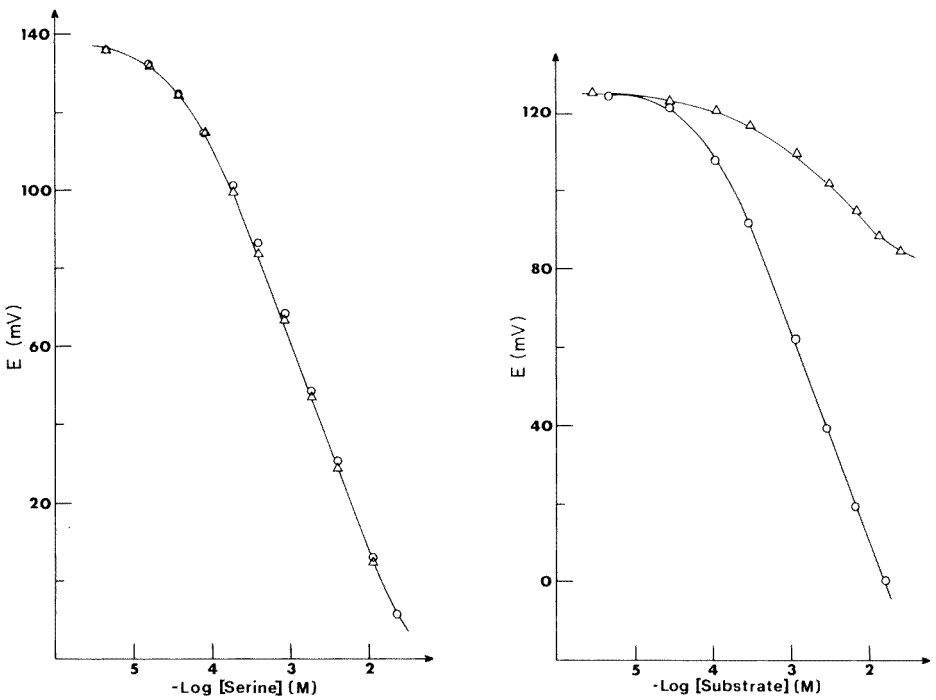

Fig. 1. Response of the bacterial probe for serine. Buffer system: 0.1 M borate—KCl, 0.01 M INH, 4.3 × 10^{-3} M dithiothreitol, 4.3 × 10^{-4} M Fe^{2+}, pH 8.5. Time after immobilization: (△) 25 h; (○) 72 h.

Fig. 2. Response of the bacterial probe (○) serine; (△) glutamine. (Conditions as in Fig. 1.) Negligible interference was shown by the L-isomers of asparagine, aspartate, ornithine, threonine, creatinine, urea, glycine, or tyrosine under the same assay conditions.

TABLE 1

Effect of buffers and potassium ions on the serine bacterial probe response

Buffer[a]	Initial rate of response (mV min^{-1})	
	Probe 1	Probe 2
0.1 M Borate	7.7	4.2
0.05 M Borate—0.05 M KCl	16.5	11.5
0.1 M Phosphate	6.0	6.0
0.1 M Glycylglycine	4.0	21.7
0.05 M Glycylglycine—0.05 M KCl	23.4	>50

[a]Each buffer contained 4.3×10^{-3} M dithiothreitol, plus 4.3×10^{-4} M Fe^{2+}.

tion of iron(II) ions by the glycylglycine buffer. Dialysis for 1 h in a fresh aliquot of glycylglycine buffer, dithiothreitol, and iron(II) ions failed to reactivate the biocatalytic activity. In contrast, a potassium chloride—borate buffer showed both good initial rates of deamination and reproducibility; therefore, the chloride—borate buffer was chosen for all subsequent work.

Isonicotinic acid hydrazide (INH) has been reported to inhibit bacterial ammonia assimilation [24] and has been used successfully in other bacterial electrode systems [6, 7]. The addition of 0.01 M INH to all working and storage solutions tended to diminish the change in the base-line potentials that was observed when no INH was present.

Different amino acids and biologically significant compounds were tested as possible interferences in the determination of L-serine, as shown in Fig. 2. The L-isomers of asparagine, aspartate, ornithine, threonine, creatinine, urea, glycine, and tyrosine showed negligible response, while glutamine caused interference at higher concentrations, as noted with other bacterial membrane probes [2, 3, 6].

The lifetime of the membranes depended critically on the addition of a minute amount of iron(II) stearate to the bacterial paste during the probe construction. Presumably the low concentration of iron(II) ions released to the bacterial enzyme by the stearate aided in stabilizing the dehydratase enzyme. Figure 3 shows successive serine calibration curves produced by a probe lacking the iron(II) stearate. The slope increased from 55 mV/decade to the super-Nernstian 62 mV/decade over the 45-h lifetime, while the linear range degenerated from almost 2 orders of magnitude to less than half an order of magnitude. The lower limit of detection rose more than one order of magnitude to 2.7×10^{-4} M serine. Figure 1 contrasts the stability of the probe when prepared with a 5-μl aliquot of the stearate salt. In this instance, the slope was maintained at 54 mV/decade, while the linear range and lower limit of detection actually improved after one day of use as noted with certain other biocatalytic systems [25]. The linear response of the iron(II) stearate-enhanced probe ranged over two decades change in concentration of substrate, while the lower limit of detection was 5×10^{-5} M serine.

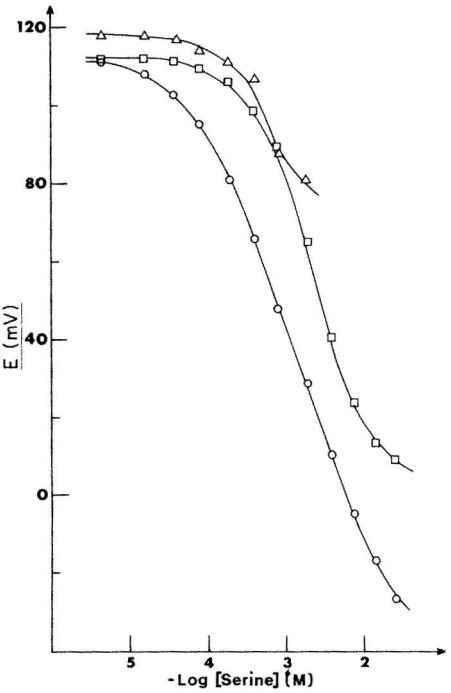

Fig. 3. Lifetime study showing the response of the bacterial serine probe prepared without iron(II) stearate: (○) 3 h; (□) 25 h; (△) 45 h after immobilization.

The analytical usefulness of this method hinges on the ability of the bacterial enzyme to convert serine quantitatively to ammonia. Percent recovery studies were performed on three serine samples at each of three values of 2.1, 3.4, and 5.8 μmol ml^{-1} added. In the linear portion of the logarithmic calibration curve, between 92% and 103% of added serine was recovered, with the imprecision of the data being ±2%.

The quantitative conversion of L-serine and the favorable lower limits of detection of the probe may make it possible to quantify free serine in biological fluids. Various workers have reported the concentration of serine in the serum of healthy adults as ranging from 9.4×10^{-5} M to 2.9×10^{-4} M [26], which is above the limits of detection of this membrane probe. Normal 24-h urinary serine output is in the 10^{-4} M range [27] with abnormal states such as Hartnup's disease increasing serine concentrations 5—20 fold [28]. Removal of the main interference, glutamine, could be effected through the addition of glutaminase followed by a short degassing period. The serine assay might also be coupled with a potentiometric glutamine assay, using any of the reported glutamine sensors [29].

The serine-selective membrane probe described here provides the first example of an anaerobic bacterial sensor. It is the only sensor with potentiometric response reported for the determination of serine, and is useful for

at least three days, with purging of oxygen being unnecessary during use and storage. The slope, lower limit of detection, and quantitative yield suggest that such a probe could be a viable analytical tool. The applicability of anaerobic bacteria as feasible biocatalysts encourages the future development of these sensors.

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CALCIUM-SELECTIVE ELECTRODES BASED ON CALCIUM TETRA(4-CHLOROPHENYL)BORATE COMPLEXES OF MACROCYCLIC POLYETHER DIAMIDES

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SUMMARY

The preparation of macrocyclic 7,19-dibenzyl-7,19-diaza-1,4,10,13,16-pentaoxacycloheneicosane-6,20-diones substituted in positions 2 and 3 with methyl groups, and their properties in PVC membranes as calcium sensors are described. Complexes of these polyether diamides (PEDA) with calcium and tetra(4-chlorophenyl)borate (TClPB) ions having the composition 2PEDA · Ca · 2TClPB were prepared. Calcium electrodes based on these complexes have selectivity coefficients for calcium over barium up to 10^3, and over alkali metals up to 3×10^4.

Some macrocyclic polyether diamides are known to exhibit a certain selectivity for calcium cations if applied in membrane sensors [1]. A study of the effect of structural changes on membrane selectivity revealed [2] that the best properties are exhibited by polyether diamides derived from dioxaoctanedicarboxylic acids. To establish the importance of substitution in the chain of dicarboxylic acid, the preparation and properties of ligands derived from 3,6-dioxaoctanedicarboxylic acid substituted with methyl groups in positions 4 and 5 were investigated in greater detail in this study. It was found, moreover, that these ligands yield crystalline complexes of defined composition with calcium cations and tetra(4-chlorophenyl)- or tetraphenylborate anions. The selectivity properties of these complexes with the selectivities of the free ligands are compared and discussed.

EXPERIMENTAL

Synthesis of ligands

Starting materials. Trimethyl- and tetramethyl-3,6-dioxaoctanedicarboxylic acids were prepared by the reaction of 2-methylbutanediol-2,3 or pinacol with ethyl diazoacetate as described earlier [2], the only difference being that the reaction was carried out in diethyl ether and more boron trifluoride etherate was added.

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4,4,5-Trimethyl-3,6-dioxaoctanedicarboxylic acid was purified by column chromatography on silica gel (benzene–methanol–acetic acid, 45:8:4). The viscous oil crystallized on prolonged standing (m.p. 38–40°C). [^{1}H-n.m.r. ($CDCl_3$): 1.1 (m, 9H, CH_3); 3.4 (q, 1H, CH); 4.1 (m, 4H, CH_2); 9.8 (s, 2H, OH). Elemental analysis: calc. for $C_9H_{16}O_6$ (220.2), 49.1% C, 7.3% H; found, 49.0% C, 7.5% H.]

4,4,5,5-Tetramethyl-3,6-dioxaoctanedicarboxylic acid was recrystallized from an ethyl acetate-hexane mixture (m.p. 148–149.5°C). [^{1}H-n.m.r. ($CDCl_3$): 1.2 (s, 12H, CH_3); 4.1 (s, 4H, CH_2); 8.4 (bs, 2H, OH). Elemental analysis: calc. for $C_{10}H_{18}O_6$ (234.2), 51.3% C, 7.7% H; found, 51.2% C, 7.8% H.]

Other diacids as well as diamines were prepared as described earlier [2].

Macrocyclic polyether diamides. Diamides (PEDA, I–IV) were prepared by reacting the acid dichlorides with N,N′-dibenzyl-1,11-diamino-3,6,9-trioxaundecane at high dilution [2]. The products were purified by chromatography on silica gel using benzene–methanol (5 + 1) as the eluent. Diamides I and II have been described [2].

7,19-Dibenzyl-2,2,3-trimethyl-7,19-diaza-1,4,10,13,16-pentaoxacyclohene-icosane-6,20-dione (III) was obtained in 48% yield as an oil. [^{1}H-n.m.r. ($CDCl_3$): 1.15 (m, 9H, CH_3); 3.5 (m, 17H, NCH_2CH_2O, OCH_2CH_2O, $CHCH_3$); 4.1–4.9 (m, 8H, OCH_2CO, CH_2Ar); 7.2 (s, 10H, Ar). M.s.: M^+ 556, 512, 487, 469, 429, 307. Elemental analysis: calc. for $C_{31}H_{44}N_2O_7$ (556.7), 66.9% C, 8.0% H, 5.0% N; found, 67.2% C, 8.2% H, 4.9% N.]

7,19-Dibenzyl-2,2,3,3-tetramethyl-7,19-diaza-1,4,10,13,16-pentaoxacyclo-heneicosane-6,20-dione (IV) was obtained in 61% yield as an oil. [^{1}H-n.m.r. ($CDCl_3$): 1.2 (d, 12H, $(CH_3)_2C$); 3.5 (m, 16H, NCH_2CH_2O, OCH_2CH_2O); 4.1–4.8 (m, 8H, OCH_2CO, CH_2Ar); 7.2 (s, 10H, Ar). M.s.: M^+ 570, 512, 487, 469, 429, 379. Elemental analysis: calc. for $C_{32}H_{46}N_2O_7$ (570.7), 67.3% C, 8.1% H, 4.9% N; found, 67.3% C, 8.0% H, 4.25% N.]

Preparation of complexes of PEDA with calcium tetra(4-chlorophenyl)borate

Potassium tetra(4-chlorophenyl)borate (0.2 mmol) dissolved in 1 ml of methanol was added to a solution of polyether diamide (0.2 mmol) and calcium thiocyanate (0.1 mmol) in 1 ml of methanol. The precipitated complex was isolated by filtration, washed with a small volume of cold methanol and dried at 80°C. Yield was 70–80%. The melting points and elemental analyses of the complexes were as follows.

2PEDA-I · Ca · 2TClPB: m.p. 95–97°C; calc. for $C_{104}H_{108}N_4O_{14}Cl_8B_2Ca$ (1983.4), 63.0% C, 5.5% H, 2.8% N; found, 62.95% C, 5.5% H, 3.0% N.

2PEDA-II · Ca · 2TClPB: m.p. 104–106°C; calc. for $C_{108}H_{116}N_4O_{14}Cl_8B_2Ca$ (2039.4), 63.6% C, 5.7% H, 2.7% N, 13.9% Cl; found 63.3% C, 5.9% H, 2.8% N, 13.7% Cl.

2PEDA-III · Ca · 2TClPB: m.p. 238–241°C; calc. for $C_{110}H_{120}N_4O_{14}Cl_8B_2Ca$ (2067.6), 63.9% C, 5.85% H, 2.7% N; found 63.7% C, 6.1% H, 2.7% N.

2PEDA-IV · Ca · 2TClPB: m.p. 227–229°C; calc. for $C_{112}H_{124}N_4O_{14}Cl_8B_2Ca$ (2095.7), 64.2% C, 6.0% H, 2.7% N, 13.5% Cl; found, 64.2% C, 6.2% H, 2.6% N, 13.6% Cl.

Preparation of electrodes

The free ligand (2 mg) or the complexed ligand (10 mg) was dissolved in 0.2 ml of *o*-nitrophenyl-n-octyl ether and mixed with 2 ml of a 5% (w/v) poly(vinyl chloride) in cyclohexanone. The membranes were cast in a glass ring (4.0 cm i.d.) resting on a glass plate and allowed to dry for at least 24 h at room temperature. Discs (7 mm) were then cut with a cork borer and mounted in a Philips electrode body (type IS561) using 0.01 M $CaCl_2$ as the internal filling solution.

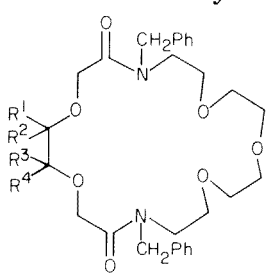
Evaluation of the electrode behaviour

All e.m.f. measurements were made at 25°C by means of a digital pH meter (Radiometer, Model pHM4) on cells of the type Hg, Hg_2Cl_2, KCl (satd.)/0.1 M NH_4NO_3/sample solution//membrane//0.01 M $CaCl_2$, AgCl, Ag. A saturated calomel electrode OP815 (Radelkis) in double-junction configuration was used as the external reference electrode. Selectivity coefficients were obtained by the separate solutions method in 0.1 M metal chloride solutions as described in detail earlier [2].

RESULTS AND DISCUSSION

Free ligands

The favourable steric effect of some substituents of dicarboxylic acids used in the preparation of macrocyclic polyether diamides on the selectivity for calcium has already been reported [2]. To investigate this effect in greater detail, a series of polyether diamides I–IV was prepared, with an increasing number of methyl substituents in the ring.



(I) $R^1 = R^2 = R^3 = R^4 = H$
(II) $R^1 = R^3 = H$, $R^2 = R^4 = CH_3$
(III) $R^1 = H$, $R^2 = R^3 = R^4 = CH_3$
(IV) $R^1 = R^2 = R^3 = R^4 = CH_3$

For the investigation of ion-selective properties, the compounds thus obtained were incorporated in PVC membranes using *o*-nitrophenyloctyl ether as solvent and plasticizer. Electrodes prepared with these membranes showed a linear Nernstian response in the concentration range 10^{-1}–10^{-5} M $CaCl_2$. The selectivity coefficients with respect to calcium obtained by the separate solutions method are given in Table 1. Atomic models of diamides

TABLE 1

Membrane selectivity coefficients of polyether diamides (PEDA); values expressed as $-\log k_{Ca,M}$

Ion	PEDA			
	I	II	III	IV
Mg^{2+}	2.86	2.41	3.71	2.19
Sr^{2+}	0.31	0.90	0.92	0.83
Ba^{2+}	−0.05	1.01	1.53	1.11
Li^{+}	1.17	1.30	0.72	−0.53
Na^{+}	1.64	2.10	2.19	0.94
K^{+}	1.25	2.10	2.61	1.09
Rb^{+}	1.40	2.50	2.86	1.35
Cs^{+}	1.35	2.50	2.95	1.29

TABLE 2

Membrane selectivity coefficients of complexes of polyether diamides with calcium and tetra(4-chlorophenyl)borate; values expressed as $-\log k_{Ca,M}$

Ion	Complex of PEDA			
	I	II	III	IV
Mg^{2+}	3.27	3.11	3.63	3.69
Sr^{2+}	1.82	0.69	0.71	0.91
Ba^{2+}	2.82	1.01	1.28	2.00
Li^{+}	2.48	2.82	2.21	2.24
Na^{+}	2.76	3.36	2.94	3.53
K^{+}	2.21	3.07	2.84	3.56
Rb^{+}	2.25	3.07	2.84	3.88
Cs^{+}	1.76	2.77	2.68	3.86

I—IV show that an increase in the number of methyl substituents of the ethylene oxide bridge in the vicinity of the carbonyl groups leads to steric stress which makes oxygen atoms approach each other and causes rigidity of the ring. This fact should be reflected in an increased preference for small ions. The measured selectivity coefficients for the calcium ion with respect to the small lithium ion agree with such a view; the preference for calcium is decreased in the case of diamide III with three methyl groups, and the attachment of a fourth methyl group in IV leads to a ligand which is more sensitive to lithium than to calcium. In the case of diamide II, containing two methyl groups, the Ca/Ba selectivity is increased compared to the unsubstituted ligand I and reaches its maximum for the trimethyl derivative III. The selectivity of diamide IV for calcium with respect to barium decreases again. A similar trend is exhibited by the selectivities for calcium compared to sodium and further alkali metals.

Complexes of ligands

Tetra(4-chlorophenyl)borate complexes of ligands I—IV and calcium ions were obtained by precipitation of a methanolic solution of the ligand and calcium thiocyanate with a solution of potassium tetra(4-chlorophenyl)borate in methanol. The complexes thus formed are insoluble in water and very poorly soluble in alcohol and benzene. They can, however, be dissolved in acetone, cyclohexanone, ethers and nitrated hydrocarbons. According to the results of elemental analyses, they contain two ligands and two borate anions for each calcium ion. The isolated complexes were incorporated into PVC membranes similarly to the free ligands. Electrodes prepared with these membranes showed linear Nernstian response in the 10^{-1}—10^{-5} M $CaCl_2$ range.

The selectivity coefficients obtained are summarized in Table 2. Compared with the selectivities of free ligands, some remarkable selectivity changes occur in the complexes (Fig. 1). The selectivity for calcium with respect to alkali metals is generally increased, sometimes by as much as 2.5 orders of magnitude. The largest increase takes place in the complex of diamide IV, while for the complex of diamide III the changes are the smallest. The dependence of the selectivities for calcium with respect to lithium on the number of methyl groups in the ring observed with free ligands (cf. Fig. 1) does not appear when the complexes are used. The selectivity for calcium over barium shown by ligands II and III is scarcely affected by the complex formation, but with the complex of ligand IV, the calcium selectivity

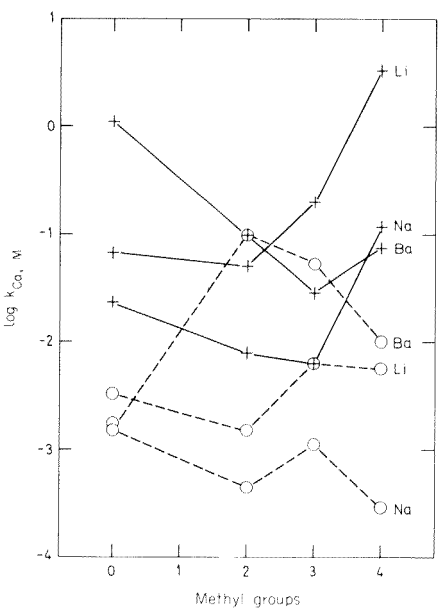


Fig. 1. Dependence of the selectivity coefficients on the number of methyl substituents in polyether diamides I—IV. (——) Free diamides; (- - -) complexes with calcium and tetra(4-chlorophenyl)borate.

is increased by one order of magnitude, and with ligand I complex formation leads to a dramatic enhancement of the calcium selectivity by three orders of magnitude. The changes in selectivities just described are most probably due to differences in the stabilities of the ligand—tetra(4-chlorophenyl)borate complexes compared with the complex-forming properties of the free ligands. The pronounced changes in the Ca/Ba selectivity in the case of the complex of ligand I cannot be explained by a change in the stoichiometry of the complex, because the barium complex was isolated and shown to have the same stoichiometric composition as the complex with calcium. It can be assumed that formation of the same complexes occurs in equilibrated membranes into which the individual components have been incorporated separately. Membranes prepared with the same molar ratio of the ligand to the tetra(4-chlorophenyl)borate exhibited changes in selectivity similar to those observed if the previously isolated complex was used. From this viewpoint, the favourable effect of the tetraphenylborate anion on the selectivity of calcium ligands with respect to the monovalent ions (and in some cases also with respect to barium) observed by Simon et al. [3, 4] can be explained by the formation of ligand—cation—tetraphenylborate complexes with different stabilities. For instance, under the conditions of the preparation of the complexes described in this study, ligand 4 described by Simon et al. [4] yields an insoluble oily product.

Consequently, in the preparation of calcium sensors, it is more advantageous to use crystalline complexes than a separate addition of tetraphenylborate into the membranes. Utilization of the complex guarantees an optimal stoichiometry of the ligand—cation—tetraphenylborate system, and at the same time removes the danger of overdosage of the anion, which leads to a loss of calcium selectivity because of the ion-exchange effect.

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DIRECT CURRENT AND DIFFERENTIAL PULSE POLAROGRAPHIC BEHAVIOUR OF BENZYLPENICILLOIC ACID

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SUMMARY

The differential pulse and direct current polarographic behaviour of benzylpenicilloic acid (BPA) is discussed. In pH 9.2 borate buffer, the single anodic wave ($E_{1/2}$ = —0.25 V) obtained is diffusion-controlled at concentrations less than 2×10^{-5} M but adsorption-controlled in 10^{-4} M solution. Cyclic voltammetry at a hanging mercury drop electrode shows that the electrode reaction is reversible at pH 9.2. The differential pulse peak current is linearly related to concentration in the range 10^{-6}—2×10^{-5} M. Penicillamine yields an anodic peak well separated from the BPA peak. Both substances may be determined in each other's presence. Intact penicillin decreases the BPA peak but the linearity between i_p and BPA concentration is maintained.

Benzylpenicilloic acid (BPA) is the major degradation product of benzylpenicillin and is therefore of interest in studies concerning penicillin degradation as well as penicillin allergy [1]. The stability of benzylpenicillin in aqueous medium is pH-dependent and is greatest at pH 6—7. In alkaline solutions, the main degradation products are BPA and penicillamine, whereas in acidic solutions not only BPA but also benzylpenicillenic, benzylpenillic and benzylpenilloic acid may be formed [2]. Intact benzylpenicillin is not polarographically active, but a method involving its polarographic reduction after nitrosation has been reported [3]. Among the degradation products, penicillamine has been investigated polarographically [4]. This compound gives anodic waves by formation of a mercury complex. Benzylpenicillenic acid also yields anodic waves [5].

Benzylpenicilloic acid reacts with mercury(II) ions in solution. This reaction was utilized in a titrimetric method for the determination of penicillins after hydrolysis to the corresponding penicilloic acids [6]. Given the strong interaction between benzylpenicilloic acid and mercury(II) ions in solution, this acid should produce anodic waves at a dropping mercury electrode. In the present paper, the polarographic behaviour of benzylpenicilloic acid in alkaline and acidic medium is reported, and the effects of intact penicillin and penicillamine on the polarographic determination of BPA are discussed.

EXPERIMENTAL

Equipment

A PAR Model 174 polarographic analyzer was used with a PAR 174/70 drop timer and a Coulter Electronics x-y recorder. In the PAR 174 the current signal is amplified 10 times in the differential pulse (d.p.) mode; all d.p. currents reported here are these amplified values. The current sampling interval was modified from 16.7 ms to 20 ms in order to optimize discrimination against 50-Hz noise. The dropping mercury electrode had a natural drop-time of 5.0 s and a mass flow of 1.73 mg s^{-1} at a mercury height (h) of 50 cm; this height was used unless otherwise specified. The counter electrode was a platinum wire and the saturated calomel reference electrode was connected through a salt bridge containing the buffer solution. A PAR Model 170 analyzer was used for cyclic voltammetry and a.c. polarography.

Samples and reagents

Benzylpenicillin (Astra Pharmaceuticals) was shown by acidimetric titration [7] to be more than 99% pure. Benzylpenicilloic acid (BPA) was prepared by alkaline hydrolysis of benzylpenicillin [8]. About 40 mg of benzylpenicillin was weighed accurately into a 100-ml volumetric flask, 10 ml of 1 M NaOH was added, and after 5 min, the mixture was neutralized with 10 ml of 1 M HNO_3 and diluted to the mark with buffer solution. Titration of the hydrolysate with mercury(II) solution [6] indicated 100% yield. Penicillamine (Fluka) stock solutions (10^{-3} M) were prepared in deaerated distilled water. New stock solutions of all the derivatives were prepared daily.

Borate buffer pH 9.2 was 0.01 M in disodium tetraborate and 0.1 M in potassium nitrate. Citrate buffer pH 6.5 was prepared from 0.1 M trisodium citrate dihydrate by adjustment of pH to 6.5 with 0.1 M citric acid. Acetate buffer pH 4.6 was 0.05 M acetic acid—0.05 M sodium acetate. Nitrogen for deaeration was of SR quality. Mercury (Kebo) was of double-distilled quality. Unless otherwise stated, all chemicals were of analytical grade.

Procedure

Appropriate amounts of BPA stock solution were added to 10 ml of deaerated buffer solution to yield the desired concentration. Potentials were scanned towards positive potentials. A scan rate of 2 mV s^{-1} was used for the d.c. mode. All d.p. polarograms were recorded at 5 mV s^{-1} with a drop time of 1 s and a pulse amplitude of 25 mV. All experiments were done at 22 ± 1°C.

RESULTS AND DISCUSSION

Influence of pH

The influence of pH on the polarographic behaviour of BPA was studied in pH 4.6 acetate, pH 6.5 citrate and pH 9.2 borate buffers. Typical d.c.

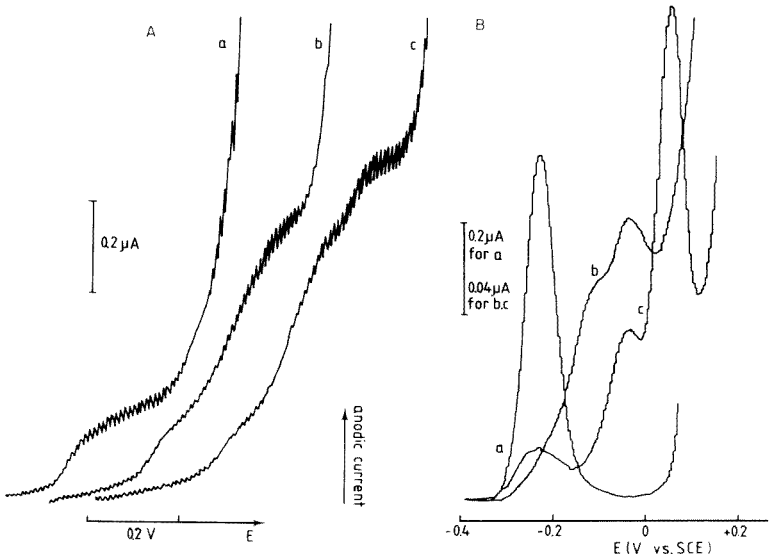

Fig. 1. (A) Direct current polarograms of 10^{-4} M BPA (scans start at −0.4 V). (B) Differential pulse polarograms of 10^{-5} M BPA. (a) pH 9.2; (b) pH 6.5; (c) pH 4.6.

and d.p. polarograms are shown in Fig. 1. Anodic waves are obtained in all three buffers. At pH 4.6, there are three d.c. waves or three d.p. peaks. The d.c. waves are poorly defined and cannot be measured accurately; only the d.p. peak at +0.05 V is easily measured. At pH 6.5, the two waves observed are again poorly defined. At pH 9.2, the polarograms show a single d.c. wave at −0.25 V and a single d.p. peak at −0.24 V. The polarograms obtained at pH 4.6 and pH 6.5 are complex and their analytical applicability is therefore limited. The well-defined wave or peak found with pH 9.2 buffer is far more suitable for evaluation purposes, and this buffer was used in all subsequent studies.

Current as a function of BPA concentration

When the solutions were buffered at pH 9.2, the calibration graph based on d.p. peak current (i_p) was linear over the concentration range 10^{-6}—2×10^{-5} M BPA; within this interval, the sensitivity was 36.8 ± 0.4 μA mM^{-1}. At higher concentrations, however, the sensitivity decreased gradually, and above 10^{-4} M the peak height was almost independent of concentration. The sensitivity of d.c. polarography is too low for measurable waves to be achieved at concentrations below 10^{-5} M, but the levelling-off effect found for d.p. polarography at higher concentrations was also observed for the d.c. polarograms.

The effect of the mercury pressure on the d.c. wave was investigated both for a 2×10^{-5} M BPA solution, i.e. within the linear range, and for a 10^{-4} M solution. For the former solution, the linear relationship obtained between

i_d and $h^{1/2}$ indicated a diffusion-controlled current. With the 10^{-4} M solution, however, i_d was directly proportional to h, indicating an adsorption-controlled process. The drop time vs. potential curves for the pure pH 9.2 buffer solution and for 10^{-4} M BPA showed a significant decrease in drop time at potentials more positive than −0.25 V when BPA was present; this again indicated adsorption effects at such potentials. Thus, the decreased i_d/C ratio found for BPA concentrations above 2×10^{-5} M is probably due to adsorption of the reaction product.

Cyclic voltammetry

To elucidate further the electrode reaction of BPA, cyclic voltammograms were recorded at a hanging mercury drop electrode in pH 9.2 and in pH 4.6 buffers. The sweeps were all started at −0.8 V and the scan was reversed at a potential of +0.00 V (pH 9.2) or +0.20 V (pH 4.6). The voltammogram obtained at pH 9.2 (Fig. 2, a) shows an oxidation peak at −0.22 V in the forward scan and a reduction peak at −0.27 V in the reverse scan; the peak separation was slightly affected by scan rate but the electrode reaction of BPA at this pH seems to be quite reversible. At pH 4.6, the cyclic voltammogram is more complex: in the first forward scan three peaks are observed at positive potentials, and in the reverse scan, there are peaks in the positive region as well as a peak at −0.42 V. A second forward scan on the same drop yielded an anodic peak at −0.41 V whereas the more positive peaks disappeared. This behaviour indicates that the anodic reaction of BPA at pH 4.6 comprises an irreversible breakdown of the penicilloic molecule with formation of a reversible redox system.

The reaction between penicilloic acid and mercury(II) ions in solution initially yields a mercury complex of penamaldic acid. This complex then decomposes to a mercury complex of penicillamine and penilloaldehyde [1, 2]. The stability of the penamaldic acid complex is pH-dependent and is greatest at alkaline pH [9]. It has been proposed that some penamaldic acid always co-exists in equilibrium with penicilloic acid [1]. The reversibility of the electrode reaction at pH 9.2 can thus be explained by assuming that the major reaction product at the electrode is the mercury penamaldate complex. The low stability of this complex at pH 4.6 is probably why the polarographic behaviour is so complicated at this pH.

Presence of penicillamine

The influence of penicillamine on the determination of BPA was investigated at pH 9.2. Penicillamine produces a single peak at −0.55 V over the concentration range 2×10^{-6}–10^{-4} M. The calibration curve is linear within the entire interval. A mixture of 10^{-5} M penicillamine and 10^{-5} M BPA gives two well-separated peaks (Fig. 3), but the height of the BPA peak decreases as the penicillamine concentration increases, although the penicillamine peak is unaffected by the BPA concentration. The decreased sensitivity of the BPA peak is probably due to strong adsorption of a penicillamine–mercury

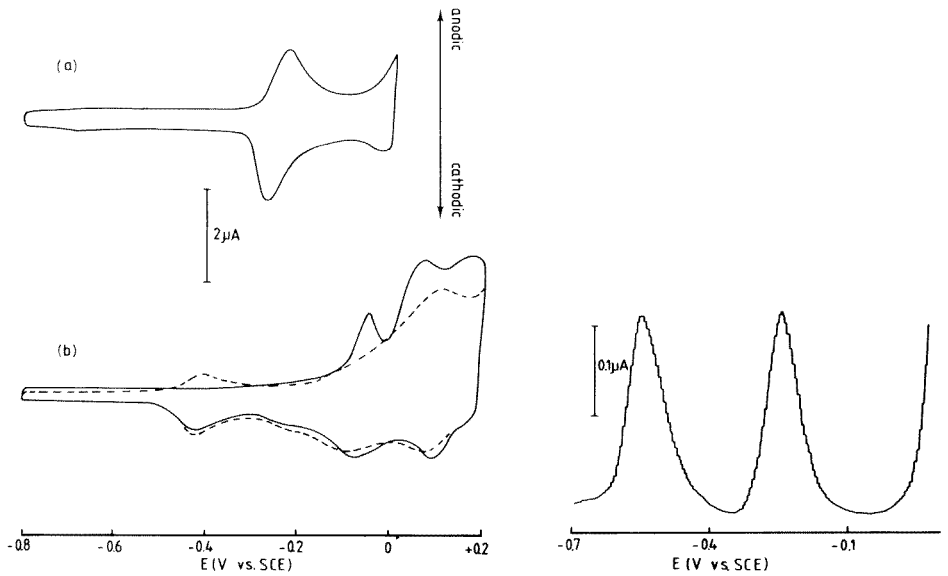

Fig. 2. Cyclic voltammograms of 10^{-4} M BPA at (a) pH 9.2, and (b) pH 4.6. (——) First cycle; (---) second cycle. Scan rate 200 mV s^{-1}.

Fig. 3. Differential pulse polarogram of 10^{-5} M BPA and 10^{-5} M penicillamine at pH 9.2.

complex [4]. Despite this decreased sensitivity of BPA caused by penicillamine, the i_p vs. BPA concentration relationship remains linear (Fig. 4A). Thus both BPA and penicillamine in mixtures may be determined simply by first evaluating the BPA peak by standard addition, and then evaluating the penicillamine peak.

Presence of intact penicillin

Intact benzylpenicillin does not give rise to any waves at a mercury electrode [3]. However, two small d.p. peaks at —0.2 V and about —1.0 V, respectively, were observed here for solutions at pH 9.2. The peak at —0.2 V was too high to be explained exclusively as being due to an impurity of BPA. It is suggested that the two peaks are of a capacitive nature as a result of adsorption/desorption processes of benzylpenicillin. This suggestion is supported by the results obtained by a.c. polarography. When the a.c. current was recorded 90° out of phase compared to the imposed alternating voltage, both peaks were observed, whereas with in-phase measurements, only slight humps appeared on the background.

The sensitivity of the d.p.p. measurements of BPA decreased when benzylpenicillin was present. Calibration graphs for 10^{-6}—2×10^{-5} M BPA in presence of 10^{-5}—10^{-3} M benzylpenicillin are shown in Fig. 4B. The peak heights have been corrected for the initial peak of benzylpenicillin. As can be seen, the linearity of the calibration graphs is not affected, and with benzylpenicillin concentrations above about 10^{-4} M, the decrease in sensitivity for

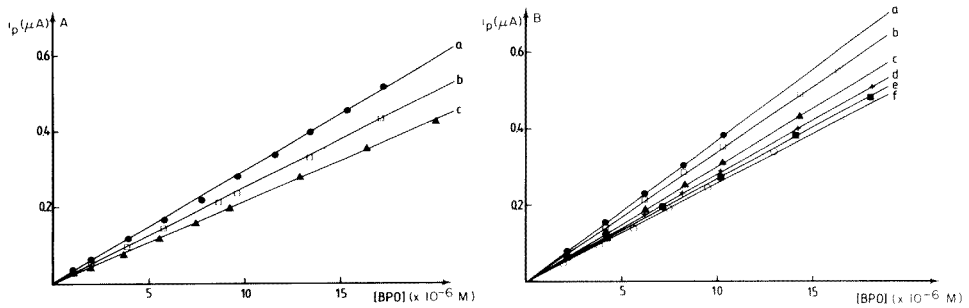

Fig. 4. Differential pulse peak current for BPA as a function of BPA concentration: (A) in presence of (a) 5×10^{-6} M, (b) 10^{-5} M, (c) 10^{-4} M penicillamine; (B) in presence of (a) 10^{-5} M, (b) 2×10^{-5} M, (c) 5×10^{-5} M, (d) 10^{-4} M, (e) 2×10^{-4} M, (f) 10^{-3} M benzylpenicillin.

BPA is small. The influence of benzylpenicillin on the BPA peak height is probably due to surface activity of the intact penicillin, which seems to reach a plateau at a bulk concentration of 10^{-4} M. This is indicated by the fact that the capacitive peak at —0.2 V ceases to grow with concentration at this level, as well as by the stabilization of the d.p.p. sensitivity for BPA.

The degradation of the penicillin to BPA can be followed easily as a function of time by recording polarograms continuously in a single sample volume. This approach was demonstrated for a 10^{-4} M benzylpenicillin solution at pH 9.2. The BPA peak at —0.25 V increased with time, corresponding to a 0.7% degradation per hour. This experiment also showed that small amounts of BPA in the presence of benzylpenicillin should be measured shortly after addition of the penicillin to the borate buffer in order to avoid errors from degradation during the analysis.

Other penicilloates

Preliminary investigations made on penicilloates obtained by alkaline hydrolysis of penicillin V and aminobenzylpenicillin showed that these penicilloates yield an anodic peak at the same potential as BPA. The peak currents obtained were found to be proportional to concentration in the same manner as BPA. No other penicillins were tested but it is probable that all penicilloates that react with mercury(II) ions will yield anodic waves at a mercury electrode.

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DETERMINATION OF HYDROGEN PEROXIDE IN PICKLING BATHS FOR COPPER ALLOYS BY LINEAR SWEEP VOLTAMMETRY

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SUMMARY

Hydrogen peroxide in pickling baths for copper and copper alloys can be determined by linear sweep voltammetry with a glassy carbon electrode. The oxidation mechanism changes around 0.15 M H_2O_2. Catalytic decomposition was found to be much smaller at glassy carbon electrodes than at platinum electrodes. An almost linear calibration curve was obtained up to 60 mM H_2O_2. Interferences from Cu^{2+}, Zn^{2+}, Ni^{2+}, Al^{3+}, Fe^{3+} and Pb^{2+} as well as from the stabilizers were small. All measurements were made in sulphuric acid solutions.

The use of hydrogen peroxide in pickling baths has increased during the past few years, as it causes fewer environmental problems than some other oxidants, e.g. chromates. The hydrogen peroxide in the baths can usually be determined precisely by visual titration using permanganate. Nevertheless, there is a need for an instrumental method which can be used either manually or automatically at the site of the baths.

Pickling baths for copper and copper alloys, as well as baths for some other metals, contain 0.5—2 M sulphuric acid and 0.1—0.6 M stabilized hydrogen peroxide. The metal concentration will increase with use up to the limit set by the regeneration routine, usually 0.5 M for copper. The metal interferences considered in this work were from Cu^{2+}, Zn^{2+}, Ni^{2+}, Fe^{3+}, Al^{3+} and Pb^{2+}. Only a few of the known methods for the determination of hydrogen peroxide can be used in samples with the mentioned compositions. Direct electrochemical oxidation of hydrogen peroxide seemed to be the most promising one.

Harrar [1] developed a coulometric determination of hydrogen peroxide in which the oxidation was made at a platinum anode at +0.93 V vs. SCE. Guilbault and Lubrano [2] used an amperometric oxidative detection method for enzymatically produced hydrogen peroxide. A patent has also been granted for an electrochemical method for hydrogen peroxide determinations [3]. Very many papers on the electrochemical properties of hydrogen peroxide have been published. Hickling and Wilson [4] studied the anodic decomposition at Pt, Au, Ni and graphite electrodes. Lingane and Lingane [5] used chronopotentiometry in a mechanistic study of the oxida-

tion at a platinum anode in sulphuric or perchloric acid. The mechanisms of catalytic decomposition and oxidation at carbon electrodes have been studied recently by two research groups [6—10].

The overall reaction consumes 4 electrons and the final products are oxygen and water. There is also a non-electrolytic decomposition to oxygen and water and the electrode material will often catalyze this reaction. In the case of carbon electrodes, the carbon can be oxidized to carbon dioxide. The half-wave potential as well as the anodic current depend on the surface condition of the electrode.

EXPERIMENTAL

Voltammetric measurements

Voltammetric measurements were made with a polarograph (Princeton Applied Research, model 174A) and an x-y recorder (Houston 2000). The voltage was scanned in the positive direction from 950 mV to 1700 mV vs. Ag/AgCl at a rate of 200 mV s^{-1}. A more powerful potentiostat (80 mA output) was used in some experiments; the start potentials and scan rates were also varied as indicated in each case under Results.

Working electrodes were made from glassy carbon rods (3-mm diameter; Le Carbone Lorraine, Paris). Smaller electrodes made from glassy carbon of unspecified origin were also used. The carbon was either glued into a glass tube using an epoxy resin or enclosed with heat-shrinkable tubing (Alpha, USA, Fit-221). A commercial glassy carbon electrode (Princeton Applied Research) was also used. The electrodes made by the different methods had almost the same properties. The glassy carbon surface was polished with 1- and 0.25-μm alumina (Struers, Copenhagen), cleaned in an ultrasonic bath, and oxidized at +1.7 V during 15 min in a solution containing 0.1 M H_2O_2 and 1 M H_2SO_4, which was stirred vigorously. The reference electrode was an Orion double-junction type (model 90-02). The outer chamber was filled with 1 M H_2SO_4. The auxiliary electrode was a platinum foil separated from the solution by a clay filter. The cell solution volume was 30 ml.

Three types of hydrogen peroxide solutions were used. Perhydrol (Merck, Cat. No. 7209) of p.a. quality and two moderately stabilized types for technical purposes, viz., HyBrite (FMC Corp., USA) and MS400 (Nord Nero AB, Sweden). A solution of an auxiliary stabilizer, MS402, was added in some cases. It contained no hydrogen peroxide, as it is intended for rejuvenation of baths or for producing special surface effects.

Hydrogen peroxide stock solutions were standardized by visual titration with permanganate standardized against oxalate. The procedures followed essentially those described by Kolthoff et al. [11].

RESULTS AND DISCUSSION

Exploratory studies

Ideally a method for use in the factory should be as direct as possible and the reading should be a direct measure of the required quantity, i.e. the hydrogen peroxide concentration. Voltammetric measurements were therefore made directly in the undiluted baths.

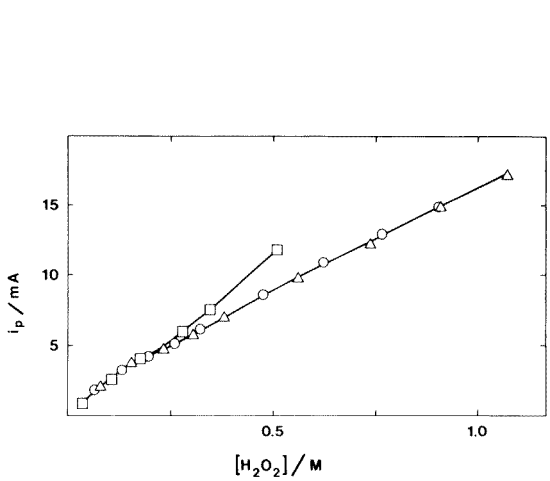
The catalytic decomposition of hydrogen peroxide interfered strongly at platinum electrodes. Bubbles of oxygen formed so rapidly that the peak trace became very noisy even at high sweep rates. In more dilute solutions, the condition of the electrode surface was important as noted by earlier investigators. Addition of stabilizer or metal ions also changed the peak current and the peak potential. The catalytic hydrogen peroxide decomposition was small on gold electrodes as has been observed before [12]. However, the overvoltage was larger and the wave was distorted. Moreover, the addition of stabilizer or metal ions again affected the response. Therefore gold seems to be unsuitable for the intended application.

The oxidation wave was well-behaved and much more reproducible on glassy carbon electrodes. Figure 1 shows calibration curves obtained with the three types of hydrogen peroxide. It can be seen that the slope is changed at around 0.15 M H_2O_2. The calibration as well as the peak potential are fairly independent of the stabilizer below this concentration. Above 0.15 M H_2O_2 the kind and amount of stabilizer are important for the response. Above 0.15 M H_2O_2, the peak potential increased with the concentration of hydrogen peroxide until the peak almost merged with the oxygen evolution wave. A high scan rate, 2 V s^{-1}, had to be used in order to avoid excessive interference from oxygen bubbles on the electrode.

The reaction is of the first order [10] with respect to the hydrogen peroxide concentration at carbon electrodes (in contrast to the half order [13] at platinum electrodes). The reaction proceeds in steps and the reacting species are weakly adsorbed at low concentrations but firmly adsorbed at high concentrations. In the solutions used in this work, there seem to exist at least two different mechanisms for the oxidation, and the second type dominates above 0.15 M H_2O_2.

After a period of use in strong hydrogen peroxide solution, the glassy carbon surface becomes covered with a loose layer of carbon dust caused by pitting during the oxidation of carbon to carbon dioxide [9].

The results suggest that the oxidation current at glassy carbon electrodes should be fairly independent of the stabilizer and the metal content at a hydrogen peroxide concentration of 0.1 M or less. The peak potential, however, will still be dependent on the composition of the bath. A method for hydrogen peroxide determination should therefore involve a dilution to 0.1 M or less as a first step, and the peak current should be used in the evaluation.



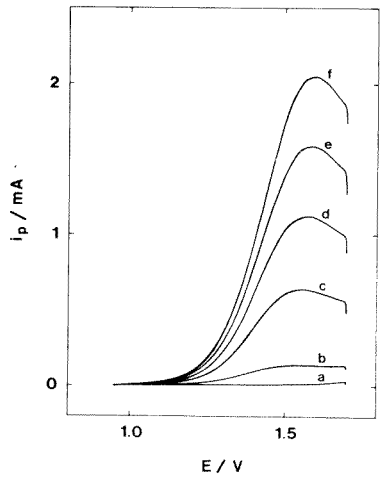


Fig. 1. Calibration curves for hydrogen peroxide at a glassy carbon electrode (diam. 1 mm). Sweep rate 2 V s^{-1}. (□) Perhydrol; (△) HyBrite; (○) MS 400.

Fig. 2. Linear sweep voltammograms of hydrogen peroxide in 1 M H_2SO_4 at a glassy carbon electrode (Le Carbone). Sweep rate 200 mV s^{-1}. (a) Background; (b) 3.57 mM; (c) 17.7 mM; (d) 31.7 mM; (e) 45.5 mM; (f) 59.1 mM H_2O_2. The potential is measured vs. Ag/AgCl.

Method for determination of hydrogen peroxide

Figure 2 shows a series of voltammograms recorded in solutions of varying hydrogen peroxide concentration up to 0.06 M. The sweep rate affects both the shape and the position of the wave. It is easier to read the peak current at low sweep rates, especially if the hydrogen peroxide concentration is low. The pretreatment described in the Experimental section is essential for a reproducible wave; at an untreated glassy carbon electrode, the wave may not appear at all. Even after pretreatment there is a small decrease in peak current from the first to the following sweeps. A series of measurements were therefore started by three scans of the background in a solution without hydrogen peroxide; the value of the background current was taken from the third scan. Two scans were always made in sample solutions containing hydrogen peroxide and the peak current was measured from the second one. The solution was stirred vigorously between scans to remove oxygen bubbles from the electrode surface. The electrode surface should not be touched between measurements; polishing is not necessary even after extended use.

A typical calibration graph for HyBrite-stabilized hydrogen peroxide was linear over the range 4×10^{-3}—6×10^{-2} M with peak currents ranging up to 2 mA, and passed through the origin. The other two types of hydrogen peroxide gave identical calibration curves. The peak can be observed down to about 2 mM H_2O_2. The detection limit is estimated to be 0.1 mM H_2O_2 if the peak potential is known from a calibration curve. The variations in background current and electrode condition during 8 h have been taken into

account in this estimation. The noise of the baseline itself is very low so that even lower detection limits could be obtained with a slightly modified procedure. The slope of the calibration curve will increase slightly (5%) with time for a new electrode until a steady state is obtained (±2%). However, there may sometimes be sudden larger changes (10%), especially when the electrode is used on real pickling baths in the factory. The electrode was therefore calibrated once a day.

The temperature coefficient of the peak current was +1% K^{-1}. All measurements reported in this paper were made at room temperature, 23 (±1) °C.

Interferences

Table 1 shows that the variation in peak current is small when the concentration of sulphuric acid is varied up to 2 M. If the baths are diluted with 1 M H_2SO_4, variations in acid concentrations of the baths will be further levelled out. For actual pickling baths, the overall error from this source was estimated to be less than 1%. The error is always negative if calibration curves are made in 1 M H_2SO_4.

Variations in the acid concentration have a rather large effect on the peak potential but this will not affect the results if the evaluation is made from the difference between the peak current and the background current at the same potential.

The interferences from metal solutions were studied in peroxide solutions containing 1 M H_2SO_4 to which the metal salts had been added in the form of sulphates. The peak current decreased 2% when Cu^{2+}, Zn^{2+}, Ni^{2+}, Al^{3+} or Fe^{3+} was added at the 50 mM level. The decrease in current was thus about the same for all the metals and was a linear function of the metal concentration. Considering the dilution which precedes the measurement, the interference from any of the metals mentioned as well as from a saturated solution of lead ions, should be negligible.

The slope of the calibration curve was the same for all the three peroxide solutions tested. If additional stabilizer is added, however, this may no longer be true. When the sample solutions contained 1% (v/v) MS402 as auxiliary stabilizer, the peak current for 60 mM H_2O_2 decreased 13%. The

TABLE 1

Variation of i_p (in mA) as a function of the sulphuric acid concentration for the P.A.R. glassy carbon electrode at a sweep rate of 0.5 V s^{-1}

$[H_2O_2]$(mM)	$[H_2SO_4]$(M)			
	0.3	1.0	2.0	3.0
17.6	4.28	4.42	4.12	3.75
52.5	12.2	12.9	12.1	11.5

decrease was linearly dependent on the concentration of the stabilizer. Thus if baths containing large amounts of stabilizer are to be analyzed, a separate calibration curve should be made in solutions to which the stabilizing agent has been added.

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SUBTRACTIVE ANODIC STRIPPING VOLTAMMETRY WITH TWIN IDENTICAL MERCURY-FILM ELECTRODES DIFFERING IN THEIR CONVECTION TRANSPORT DURING DEPOSITION

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SUMMARY

Subtractive anodic stripping voltammetry based on the use of different convection rates at the surface of two identical working electrodes during the deposition step is described. Twin mercury-film glassy-carbon disc electrodes, one rotated and one stationary, are used to demonstrate this technique. All background currents arising from nonconvective-dependent sources are effectively compensated. Deposition times of 3—5 min suffice for measuring heavy metal ions at sub-μg ml^{-1} concentration levels.

Anodic stripping voltammetry (a.s.v.), because of its inherent sensitivity, has become commonly used for the determination of heavy metal traces. In recent years, various approaches have been suggested for increasing the sensitivity (or shortening deposition times) of a.s.v.: these include improved mass-transport [1] and increase of electrode surface area [2] to enhance the amount of metal deposited; the use of different potential—time waveforms, such as differential pulse [3], staircase [4] or phase-selective a.c. [5] to replace the conventional linear scan during the stripping step; the rotating ring-disc electrode [6] and subtractive anodic stripping voltammetry [7]. The last technique seems to be very promising: in addition to compensating for the charging current (as do the various potential-pulse approaches) it also compensates for other non-capacitive background currents, such as the oxidation of mercury or the reduction of hydrogen ions, or reactions of other interfering electro-active compounds.

Subtractive a.s.v. involves the use of different deposition times on twin matched electrodes immersed in the sample solution. To increase the sensitivity of the subtractive mode, various electrodes have been examined: two hanging mercury drop electrodes (HMDE) [7, 8], two rotating mercury-film glassy-carbon disc electrodes [9], a rotating (glassy-carbon or gold) split-disc electrode [10, 11], and two stationary mercury film electrodes in a flow-through cell [12].

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In this paper, another approach to subtractive a.s.v. is described. The method is based on transporting the metal ions to the surfaces of twin matched electrodes at different convection rates during the deposition time. For currents limited by convection, the amounts of material deposited during the plating period are proportional to the rate of convection, with cubic or square-root dependence, depending on the electrode geometry; this is a consequence of hydrodynamic theory [13]. Since the background in a.s.v. is free of any contribution related to transport-controlled processes during the deposition [14], flat base lines are obtained in the potential region of interest. This subtractive a.s.v. approach is applicable to the various electrode configurations employed in conventional a.s.v.: different rotation speeds in the case of twin rotating disc electrodes; different flow rates for two stationary electrodes in a flow cell, or different stirring rates for twin HMDEs. The characteristics of the approach based on different convection rates are explored in this paper for twin rotated disc electrodes. Preliminary results have already been reported [9].

THEORY

The total current recorded during the stripping step in conventional a.s.v. is given by

$$i_t = i_p + i_c + i_{elec} + i_{f,r} \quad (1)$$

where i_p is the analytical stripping current resulting from the re-oxidation of the metal from the electrode, and i_c, i_{elec}, and $i_{f,r}$ are background components: i_c is the double-layer charging current; i_{elec} is due to background reactions which limit the accessible working potential (i.e., electrolyte decomposition or mercury oxidation); the component $i_{f,r}$ is due to potential-dependent redox reactions in which the reactant and product remain in solution, e.g., reduction of dissolved oxygen or iron(III).

For the mercury film electrode (MFE), the analytical response of interest, i_p, is given [15] by $i_p = 1.1157 \times 10^6\, n^2 A C_e l v$, where C_e is the concentration of the metal in the mercury, A and l are the surface area and the thickness of the mercury film, respectively, and v is the potential scan rate. For the HMDE, i_p is given [15] by $i_p = 2.72 \times 10^5\, n^{3/2} A D^{1/2} C_e v$. According to Faraday's law, $C_e = i_L t_{dep}/nFV$, i_L being the limiting current for the deposition of the metal in question, t_{dep} the deposition time, and V the volume of the mercury electrode.

Under the forced-convection conditions of the deposition step i_L is given [16] by $i_L = nFAC_bM$, where C_b is the bulk concentration of the ion in question and M is the mass-transport coefficient, which is proportional to the flux of the ion at the electrode surface. M has the following general forms for the rotating electrode, for the flow-through electrode and for the HMDE, respectively: $M = K\omega^{\alpha}$, $M = K'U^{\alpha'}$, and $M = K''m^{\alpha''}$. Here ω is the rotation speed, U the solution flow rate, m the stirring rate during the

deposition step, and K, K', K'', α, α' and α'' are constants, characterizing the hydrodynamic pattern and electrode geometry.

Combination of eqn. (1) with the above-mentioned relationships enables the total currents recorded during the stripping step, at the rotating MFE, flow-through stationary MFE and HMDE, respectively, to be expressed by

$$i_{t(\mathrm{rot,MFE})} = 1.1157 \times 10^6 n^2 A v C_b t_{dep} K \omega^{\alpha} + i_c + i_{elec} + i_{f,r} \quad (2)$$

$$i_{t(\mathrm{flow,MFE})} = 1.1157 \times 10^6 n^2 A v C_b t_{dep} K' U^{\alpha'} + i_c + i_{elec} + i_{f,r} \quad (3)$$

$$i_{t(\mathrm{HMDE})} = 2.72 \times 10^5 n^{3/2} A^2 D^{1/2} v^{-1} v C_b t_{dep} K'' m^{\alpha''} + i_c + i_{elec} + i_{f,r} \quad (4)$$

A practical and effective way of isolating the analytical stripping current from the various background components is to impose different rates of convective transport toward the surfaces of two matched working electrodes, during the deposition step.

This approach can be described by

$$\Delta i_t = [(i_{P(M_H)} + i_{bkgd}) - (i_{P(M_L)} + i_{bkgd})] = i_{P(M_H)} - i_{P(M_L)} \quad (5)$$

where the subscripts M_H and M_L represent the high and the low rates of mass transport during the deposition step, respectively. The various background sources ($i_c + i_{elec} + i_{f,r}$) which are independent of mass transport during the deposition step, are compensated.

Subtractive a.s.v. with different rates of mass transport furnishes the following responses when employed with the rotating MFE, the flow-through stationary MFE and the HMDE, respectively:

$$\Delta i_{t(\mathrm{rot,MFE})} = 1.1157 \times 10^6 n^2 A v C_b t_{dep} K (\omega_H^{\alpha} - \omega_L^{\alpha}) \quad (6)$$

$$\Delta i_{t(\mathrm{flow,MFE})} = 1.1157 + 10^6 n^2 A v C_b t_{dep} K' (U_H^{\alpha'} - U_L^{\alpha'}) \quad (7)$$

$$\Delta i_{t(\mathrm{HMDE})} = 2.72 \times 10^5 n^{3/2} A^2 D^{1/2} V^{-1} v C_b t_{dep} K'' (m_H^{\alpha''} - m_L^{\alpha''}) \quad (8)$$

EXPERIMENTAL

Apparatus and reagents

Measurements were made in a 300-ml pyrex glass cell, containing 100 ml of solution. Twin glassy carbon disc electrodes (GC 30S, Tokai Electrode Mfg. Co., Tokyo; 3 mm diameter, sealed inside appropriate glass tubes) were inserted through the teflon cover of the cell, at a distance of 3 cm from each other. The newly cut faces of the GC were polished until a mirror-like surface was obtained. The salt-bridge of the reference electrode (Ag/AgCl, sat. KCl) was immersed in the sample solution, symmetrically flanking the working electrodes. A glass partition was placed between the working electrodes to minimize the mutual effect of electrode rotation on the diffusion layer at each electrode. The synchronous motor used was capable of independently rotating each electrode at 0, 1410 and 2765 rpm. The electro-

chemical apparatus used for the a.s.v. and subtractive a.s.v. measurements was similar to that described previously [12].

All solutions were prepared from triply-distilled water. All chemicals were Merck Suprapur. Stock solutions (10^{-3} M) of metal ions were prepared by dissolving the pure metal in nitric acid and making up to volume with water. A 5.7×10^{-4} M Hg^{2+} solution, used for the in situ mercury film deposition, was similarly prepared. Supporting electrolytes were prepared from 0.4 M acetic acid—ammonium acetate (pH 4.8) or 0.5 M ammonia—ammonium chloride (pH 8) stock buffer solutions.

Procedure

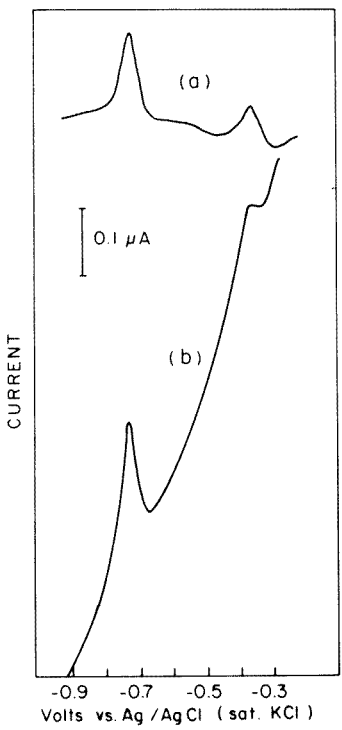
A 4-ml aliquot of the buffer stock solution was pipetted into the cell, followed by 5 ml of the mercury(II) solution. The mixture was then diluted to 100 ml and deaerated by purging with nitrogen. During deaeration, the working electrodes were kept at +0.4 V. The nitrogen delivery tube was then raised above the solution. This was followed by checking the identity of the twin electrodes by imposing equal deposition conditions on both electrodes. The subsequent stripping usually resulted in a straight horizontal line, indicating complete electrode balance. In case of incomplete balance, correction was effected by using the electrode-balance dial of the PAR 174/51 accessory. Once balance was achieved, the electrodes were ready for use for the analytical run. The plating potential, chosen appropriately for the ions to be determined, was imposed on both electrodes for a period depending on the concentrations of the former. During this period, one of the working electrodes was rotated at the highest speed available (2765 rpm) while the other, the "background" electrode, was kept at rest (0 rpm). At the end of the deposition period electrode rotation was stopped; after a 15-s rest period, an anodic potential scan to −0.05 V was initiated, together with simultaneous recording of the subtractive stripping voltammogram. Keeping the electrodes at −0.05 V between successive measurements cleaned co-deposited metals from the mercury films. Standard additions were introduced through an opening in the cell cover.

RESULTS AND DISCUSSION

Typical voltammograms obtained by the proposed method and by a.s.v. for 2.3×10^{-9} M (0.25 ppb) Cd(II), obtained under identical experimental conditions, are compared in Fig. 1. The charging current, which is the main source of background over this potential range, is effectively compensated by the subtractive mode. This allows the use of fast scan rates to increase the peak current (eqn. 6) and to decrease the deposition times (3—5 min for the sub-ppb concentration level and 30 s—2 min for the ppb level). The noise level (around 5 nA) corresponds to a concentration of the order of 1.1×10^{-10} M. To obtain higher peak currents by the proposed subtractive method, the difference between the two convection rates (rotation speeds in this case)

must be increased (eqn. 6). This is best achieved with the "background" electrode at rest (ω = 0 rpm) during the deposition. Such an operation is also most convenient from the experimental point of view because it allows the use of an unmodified motor.

Figure 2 illustrates the capability of the proposed subtractive approach to discriminate against the mercury oxidation background current, permitting measurement of copper ions at the ppb concentration level, after deposition for only 1 min (Fig. 2, curve d); deposition intervals of 3 min are inadequate with conventional a.s.v. (Fig. 2, curve c). The peak currents for copper in the subtractive mode are proportional to deposition time (curves d—f). The application of conventional differential pulse a.s.v. (d.p.a.s.v.) at a single working electrode does not provide sufficient sensitivity for measurements in the vicinity of the limits of the useful potential range; copper is obscured



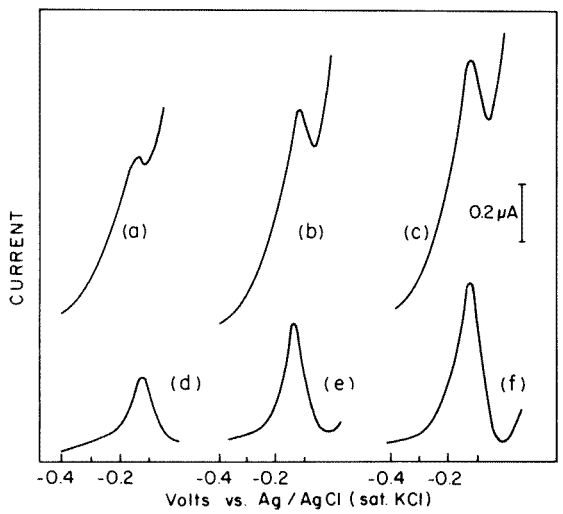


Fig. 1. Comparison of voltammograms obtained by (a) subtractive a.s.v.; (b) a.s.v., 2.3 × 10^{-9} M Cd(II) in 0.02 M NH_3/0.02 M NH_4Cl solution. Depositions for 5 min at —1.0 V. Rotation speed (a) 2765—0 rpm; (b) 2765 rpm. Scan rate, 12 V min^{-1}. (The smaller peak at ~0.35 V, is due to Cu(II) present in the blank solution.)

Fig. 2. Comparison of a.s.v. (a, b, c) and the proposed subtractive a.s.v. (d, e, f) in overcoming mercury oxidation current interference. Stripping currents of 5 × 10^{-8} M Cu(II) in 0.02 M acetic acid/0.02 M ammonium acetate solution. Deposition at —0.6 V for: (a, d) 1 min; (b, e) 2 min; (c, f) 3 min. Scan rate, 6 V min^{-1}. Rotation speed, 2765 rpm (a.s.v.) 2765—0 rpm (subtractive a.s.v.).

by mercury oxidation and zinc by hydrogen evolution. The potential—time waveform employed by d.p.a.s.v. is, of course, designed mainly for compensating charging current. Measuring the current difference between points before and during the potential pulse (with amplitudes of 10—100 mV) results in large d.p.a.s.v. background currents, because of the steep currents of the mercury oxidation and/or the hydrogen evolution. Since these currents are independent of mass transport during the deposition step, they are compensated by the proposed subtractive a.s.v. approach. In addition to its enhanced sensitivity, stripping times in the suggested mode are very short (5—10 s) as a result of the fast scan rates employed. In contrast, d.p.a.s.v. employs slow scan rates, i.e. prolonged stripping steps (2—3 min), resulting in relatively long overall analytical cycles.

The peak current and peak charge in subtractive a.s.v. with different rates of mass transport are linearly correlated to the metal ion concentration over a fairly wide concentration range (Fig. 3). The slopes of these calibration curves are 41.5 nA nM^{-1} and 21 nQ nM^{-1}, respectively. The well-defined peaks, rising above a horizontal baseline, permit the convenient use of peak height (current) for the quantification of results.

Comparison between the present results and previously reported data for subtractive a.s.v. with different deposition times shows that the detection limits of these two approaches are comparable. Both share the important advantage of efficient discrimination against the major components of the background current. However, the main advantage of the approach suggested here is the fact that both electrodes are kept at the same potential throughout

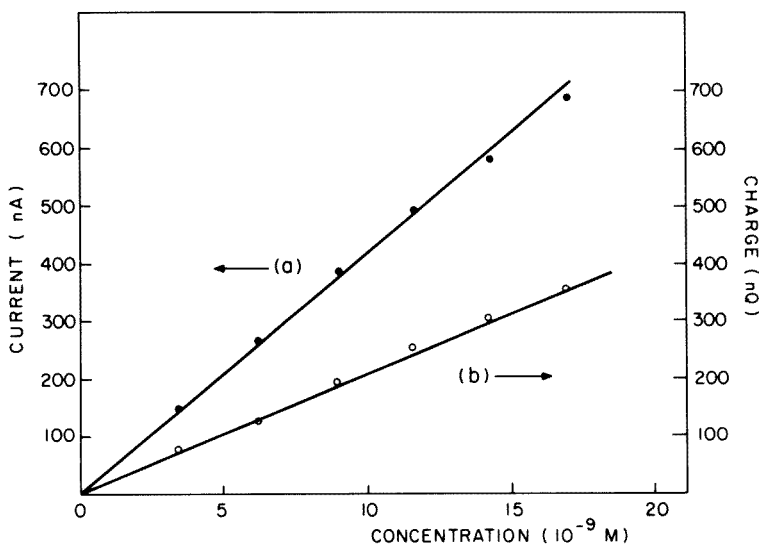


Fig. 3. Dependence of (a) peak current and (b) charge on lead(II) concentration. Depositions for 3 min at —1.1 V; scan rate, 6 V min^{-1}; rotation speed, 2765—0 rpm; 0.02 M acetic acid—0.02 M ammonium acetate buffer solution.

the analytical cycle. Such an operation is essential for achieving flat base lines in subtractive a.s.v., for this constitutes the primary problem of these subtractive measurements. Application of large potential differences on the electrodes, which is the case in subtractive a.s.v. with different deposition times, affects their electrochemical identity very rapidly and makes elimination of background current impossible [11]. The use of cumbersome potential—polarization programs to minimize this effect significantly increases the complexity of operation. In addition, subtractive a.s.v. with different deposition times usually requires an additional potential source for controlling the potential of the "background" electrode.

The approach outlined above is presently being incorporated in a flow-through cell, with twin disc electrodes, one rotated and the other stationary, in the course of the deposition step.

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SIMULTANEOUS DETERMINATION OF CADMIUM AND LEAD IN URINE BY MEANS OF COMPUTERIZED POTENTIOMETRIC STRIPPING ANALYSIS

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SUMMARY

In computerized potentiometric stripping analysis for cadmium and lead in urine the samples are acidified with hydrochloric acid to a total concentration equal to 0.5 M. The sample is pre-electrolyzed at −1.25 V vs. SCE for 2 min without prior sample heating or deoxygenation, the working electrode being a mercury pre-coated glassy-carbon electrode. The lead and cadmium concentrations are evaluated by means of standard addition. Detection limits are 1 nM for both elements. Results obtained by potentiometric stripping analysis and by solvent extraction/atomic absorption are compared for samples from unexposed persons and from one patient under penicillamine treatment. The relative merits of the potentiometric stripping, anodic stripping and atomic absorption techniques are discussed.

Determination of lead and cadmium in urine is frequently employed to detect metal poisoning, and to check the effects of clinical treatment used in connection with such poisoning. The determinations are performed either by atomic absorption spectrometry or electroanalytically. The different techniques used have been summarized in recent articles by Lund and Eriksen [1] and Jagner et al. [2].

The most successful attempt hitherto to determine cadmium and lead in urine electroanalytically would appear to be that of Lund and Eriksen [1], who determined cadmium and lead simultaneously without prior digestion with mineral acids. In order to achieve this, it was, however, necessary to heat and deoxygenate the sample and to use a relatively long time for pre-electrolysis.

It has been demonstrated previously [2] that potentiometric stripping analysis [3, 4] can be used for the determination of lead in urine. However, a urine sample normally contains high concentrations of oxidizing agents, and so it was necessary to deoxygenate the urine sample and to use pre-electrolysis times of 4 min or more in order to determine lead at the concentrations normal in urine. For the determination of cadmium it was concluded that "the sensitivity must be improved if potentiometric stripping analysis is

to become a suitable method for routine determination of cadmium in urine". The incorporation of a microcomputer in the potentiometric stripping system has increased the sensitivity by more than two orders of magnitude [5, 6]. The present paper demonstrates how computerized potentiometric stripping analysis can be used for a rapid simultaneous determination of cadmium and lead in urine after very simple pretreatment of the sample.

EXPERIMENTAL

Chemicals and apparatus

Stock solutions (1000 mg l^{-1}) of mercury(II), lead(II) and cadmium(II) in 0.2 M nitric acid were prepared from Titrisol ampoules (Merck). Working standards of suitable concentration were prepared by dilution with doubly-distilled water. All mineral acids used were of analytical grade.

A potentiometric stripping analyzer (Radiometer ISS820) was used in connection with a laboratory-constructed microcomputer system [5]. The microcomputer registered the potentiometric stripping curve, subtracted the capacitance background, and displayed the resulting curve at a decreased rate on the strip-chart of the ISS820 [5]. The glassy-carbon working electrode was made by pressure-fitting a 12-mm glassy-carbon rod, with a diameter of 2.1 mm, through a hole bored in a 150-mm teflon rod with a diameter of 12.5 mm; the center of the glassy-carbon rod was placed excentrically 4 mm from the center of the teflon rod. The glassy-carbon electrode was rotated at a constant rate of 2200 rpm during pre-electrolysis and stripping. The Radiometer TTA80 titration unit, which forms a part of the ISS820 system, was used for rotation of the electrode. A platinum wire was used as counter electrode with a saturated calomel reference electrode (Radiometer K4040 and P101, respectively).

A Perkin-Elmer 403 spectrometer equipped with a heated graphite atomizer HGA 2100 was used for the atomic absorption measurements.

Samples

Approximately 300-ml samples were taken from laboratory personnel and acidified with hydrochloric acid to a total concentration of 0.5 M immediately after sampling. Samples were also taken from a lead-poisoned patient undergoing penicillamine treatment. Acidified samples could be stored for several weeks without any observable changes in lead and cadmium concentrations.

Mercury pre-coating procedure

For the analysis of urine, the glassy-carbon working electrode must be pre-coated with a mercury film. This was achieved by pre-electrolysis for 2 min at −0.6 V vs. SCE in a solution containing mercury(II) (200 mg l^{-1}) in 0.1 M hydrochloric acid. Prior to pre-coating, the glassy-carbon electrode was polished for a couple of seconds on 3-μm diamond paste and cleaned with

ethanol. Once pre-coated, the electrode could be used for several urine samples, provided that it was rinsed with ethanol after each sample and stored in doubly-distilled water when not in use. The mercury pre-plated electrode should not be left in the pre-plating solution or in the acidified urine samples for a long period of time without a reducing potential applied to it, because these samples slowly oxidize the mercury film.

Procedures

Potentiometric stripping. The acidified urine sample (25 ml) was pre-electrolyzed −1.25 V vs. SCE for 2 min prior to the recording of the stripping curve. Standard aliquots of cadmium(II) and lead(II), corresponding to additions of approximately 10 nM cadmium(II) and 50 nM lead(II), were added, and the pre-electrolysis/stripping cycle was repeated. The lengths of the stripping plateaux were evaluated as described elsewhere [3, 4] and the concentrations of lead(II) and cadmium(II) were evaluated by the normal equations for standard addition.

Atomic absorption. The acidified urine sample (10 ml) was neutralized with ammonia and buffered to pH 4.6 with acetic acid. To this solution 0.2 ml of an aqueous solution containing 1% (w/v) of ammonium pyrrolidine-dithiocarbamate and 1% (w/v) of diethyldithiocarbamate was added and the trace metal analytes were extracted into 5 ml of methyl isobutyl ketone by vigorous shaking for 30 s. After phase separation by centrifugation, the organic phase was analyzed by atomic absorption spectrometry within 1 h after extraction; 20-μl injections were used. A deuterium background corrector was used to minimize the effect of non-specific absorption. The experimental parameters for the atomization cycle were as follows: drying at 90°C (15 s), ashing at 200°C (15 s), and atomizing at 2200°C for Cd or 2400°C for Pb (12 s). The wavelengths were 217.3 nm for Pb and 228.8 nm for Cd. The lead(II) and cadmium(II) concentrations were determined by standard addition; known aliquots of the analytes were added to another subsample of the same urine sample and the extraction procedure was repeated.

RESULTS AND DISCUSSION

Potentiometric stripping curves in acidified urine samples

Figure 1 shows the potentiometric stripping curve recorded after 2 min of pre-electrolysis at −1.25 V vs. SCE in an acidified urine sample containing 5.4 nM cadmium(II) and 46 nM lead(II). Also shown in Fig. 1 is the potentiometric stripping curve recorded under the same experimental conditions after the standard addition of 9.0 nM cadmium(II) and 50 nM lead(II). As can be seen, well shaped stripping plateaux are obtained for both lead and cadmium in the acidified samples.

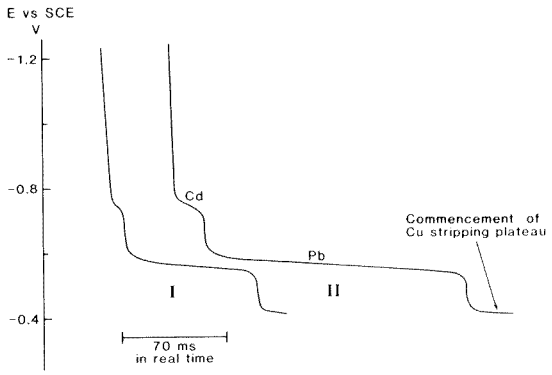

Fig. 1. Potentiometric stripping curves recorded after 2 min of pre-electrolysis at −1.25 V vs. SCE in (I) an acidified urine sample containing 5.4 nM cadmium(II) and 46 nM lead(II); (II) after the standard addition of 9.0 nM cadmium and 50 nM lead(II).

Accuracy, precision and detection limit

The accuracy of the potentiometric stripping technique was tested by comparison with the results obtained by solvent extraction/atomic absorption. The results for urines from seven different persons, summarized in Table 1, show satisfactory agreement between the cadmium values obtained by the two techniques. The agreement between the lead values is not as good as for the cadmium values, probably because of the difficulties in background correction in the atomic absorption measurement.

Lead poisoning is usually treated with EDTA or penicillamine. It has previously been shown that EDTA does not affect the potentiometric stripping signal in acidified urine samples [2]. In order to investigate the possible effects of penicillamine on the potentiometric stripping signal, urine samples from a patient under treatment were analyzed by the potentiometric stripping method and by atomic absorption spectrometry. The results are summarized

TABLE 1

Comparison between the results obtained by potentiometric stripping analysis (p.s.a.) with pre-electrolysis for 4 min, and solvent extraction/atomic absorption spectrometry (a.a.s.) for seven different samples of urine

Person no.	Pb (nM)		Cd (nM)		Person no.	Pb (nM)		Cd (nM)	
	P.s.a.	A.a.s.	P.s.a.	A.a.s.		P.s.a.	A.a.s.	P.s.a.	A.a.s.
1	24	20	1.8	1.7	5[a]	26	22	3.6	2.1
2	12	9	1.3	1.1	6	41	43	7.6	7.1
3	23	17	1.8	1.3	7[a]	38	41	1.8	2.2
4[a]	33	22	6.7	6.3	Reagent blank	2	3	0.4	0.4

[a]Smoker.

TABLE 2

Comparison between the results obtained by potentiometric stripping analysis and flame atomic absorption spectrometry on samples obtained from a patient under penicillamine treatment

Days after penicillamine treatment started	Pb (μM)	
	P.s.a.	Flame a.a.s.
Before treatment	1.9	2.2
5	7.2	7.5
9	5.8	6.0
19	3.9	4.6
25	2.5	3.0

in Table 2. As can be seen, very high urine lead levels were obtained during the treatment. The penicillamine treatment did not affect the shape of the potentiometric stripping curves and the results obtained by the two techniques agree satisfactorily.

The precision of potentiometric stripping analysis was estimated by repetitive pre-electrolysis/stripping in the same acidified urine sample and by repetitive analysis of subsamples originating from the same urine sample. Ten repetitive pre-electrolysis/stripping cycles in an urine sample containing 14.4 nM cadmium(II) and 96 nM lead(II) (cf. curve II, Fig. 1) gave a relative standard deviation of 0.05 for cadmium and 0.02 for lead. Measurements of ten subsamples gave an average value for cadmium(II) of 6.7 nM and for lead(II) of 26 nM, the relative standard deviations being 0.13 and 0.04, respectively.

The detection limit in potentiometric stripping analysis is inversely proportional to the time of pre-electrolysis. The detection limits for cadmium(II) and lead(II) after 2 min of pre-electrolysis can be estimated from Fig. 1 as 1 nM for both elements. This detection limit is sufficient for the determination of both lead and cadmium at normal concentrations. If only elevated cadmium and lead values are to be determined, a pre-electrolysis time of 1 min is more than sufficient. Although there are great individual variations in urine samples, the sensitivity of the potentiometric stripping determination is not affected to any great extent. It is, however, necessary to use a standard addition procedure and not a calibration plot procedure.

Sample throughput

The time needed for sample pretreatment, i.e., addition of hydrochloric acid, is almost negligible in computerized potentiometric stripping analysis. Given a pre-electrolysis time of 2 min and one standard addition, the time needed for one determination is about 5 min, excluding the time required for mercury pre-plating. Since most of the potentiometric stripping experiment is performed automatically, it is possible to evaluate the results from

the previous determination and to prepare for the next determination while one determination is running. Consequently, a throughput of 10—12 determinations per hour is feasible. This rate can be increased slightly if the pre-electrolysis time can be decreased to 1 min for samples with high concentrations of analyte.

Effect of pre-electrolysis potential

Since the composition of urine samples can differ considerably, the pre-electrolysis potential should be kept as negative as possible during each determination in order to achieve optimum sensitivity in all samples. Experiments showed that it was not possible to operate at a potential below −1.40 V vs. SCE because the hydrogen gas formed affected the shape of the stripping curve.

By investigation of several different samples, it was found that the sensitivity of the potentiometric stripping procedure did not increase when the working electrode potential was decreased below −1.25 V vs. SCE. In some samples, the maximum sensitivity was, however, obtained at −1.10 V vs. SCE, i.e. the same length of the stripping plateaux was obtained at −1.10 V vs. SCE as at −1.25 V vs. SCE. The use of a pre-electrolysis potential of −1.25 V vs SCE in all samples is therefore recommended.

Precipitation. Maintenance of the working electrode

When urine samples were analyzed immediately after sampling, a white precipitate was sometimes formed during pre-electrolysis, especially when pre-electrolysis times longer than 4 min were attempted. No precipitate was formed in another portion of the same sample, which had been acidified at the same time, hence the initiation of precipitation was attributed to the pre-electrolysis process. The formation of the precipitate did not affect the length of the stripping plateaux. If, however, several pre-electrolysis/stripping cycles were applied in a sample where precipitation had started, the potentiometric stripping curves became distorted. Taking the working electrode out of the sample and washing it with ethanol restored the electrode performance. It is therefore recommended that the electrodes be rinsed with ethanol between each sample.

The mercury film on the glassy-carbon electrode is slowly oxidized by urine constituents. If the same mercury film is to be used for a large number of samples, the working electrode should not be left in the acidified urine samples without a reducing potential applied to it. If the mercury-coated glassy-carbon electrode is stored in doubly-distilled water, the mercury film will not be oxidized and the same mercury film can be used for several days.

Comparison between the potentiometric stripping, anodic stripping and atomic absorption techniques

Computerized potentiometric stripping analysis has a number of advantages compared to anodic stripping voltammetry and differential-pulse

anodic stripping voltammetry. A main advantage is that it is not necessary to deoxygenate or heat the sample prior to measurement. Furthermore, the time needed for pre-electrolysis is shorter in the potentiometric stripping than in the anodic stripping technique. Recording of a differential-pulse anodic stripping voltammogram under optimum conditions takes several minutes while the recording of a potentiometric stripping curve is made in less than 1 s. Moreover, the potentiometric stripping technique is likely to give more accurate results because interferences from electroactive species are less probable; no current is drawn through the working electrode during stripping.

Determination of cadmium and lead in normal urines by means of direct injection into the heated graphite furnace atomizer does not give reliable results, mainly because of the salt matrix. Thus it is necessary to use a combination of solvent extraction and atomic absorption spectrometry. Compared with this procedure, the computerized potentiometric stripping method is very advantageous in that the sample pretreatment is considerably simpler and cadmium and lead can be determined simultaneously. Moreover, the potentiometric stripping analyzer is simpler to handle in that very few experimental parameters have to be adjusted.

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FLOW INJECTION METHOD FOR SULFIDE DETERMINATION BY THE METHYLENE BLUE METHOD

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SUMMARY

Flow injection determination of sodium sulfide and hydrogen sulfide in solution through the methylene blue spectrophotometric procedure is described. The carrier streams are *N,N*-dimethylaniline sulfate (5.4 mM, HCl solution) and iron(III) ammonium sulfate (14.2 mM, HCl solution) and are merged before injection of sulfide (in 0.01 M NaOH). The sampling rate is 210 per hour. The effect of reagent concentrations and interferents on the determination has been investigated.

Flow injection analysis (f.i.a.) is a name given to a method that depends on rapid injection of a solute sample into a reagent stream without air segmentation. It is simple, with potential sampling rates of the order of 150—200 per hour. The underlying theory and applications of f.i.a. have been described and reviewed by Růžička and Hansen [1]. The present paper describes how a conventional colorimetric method used for the determination of sulfide ions has been adapted for flow injection analysis.

The methylene blue method of determining the sulfide content in solutions has been thoroughly reviewed [2, 3]. The method involves treating sulfide fixed as a suspension of ZnS in solution with iron(III) and *p*-aminodimethylaniline (as its sulfate or chloride), the latter acting as the chromogenic reagent. Color development can require 1 min to 3 h, depending on the concentrations of sulfide and the reagents. Various reagent compositions have been proposed by previous workers [4—17] for preparing the amine and iron(III) solutions under a wide range of conditions, and there is quite a large variation in the concentrations of reagents employed for producing methylene blue. An attempt has been made in this work to rationalize the optimum conditions for the formation of the dye in the range of sulfide concentrations employed in the present investigations, and details are described later in this paper.

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EXPERIMENTAL

Reagents

Rapid color development required suitable selection of the concentrations of amine and iron(III) solutions. After careful manipulation, the following reagent compositions were found to be appropriate for sulfide solutions in the range 1—50 ppm of sulfur.

Amine solution (5.4 mM). *N,N-p*-dimethylaniline sulfate (Eastman Reagent, 1 g) was dissolved in 80 ml of concentrated hydrochloric acid and the solution was diluted to 500 ml with water.

Iron(III) solution (14.2 mM). Iron(III) ammonium sulfate (Fisher Reagent, 3.5 g) was dissolved in 40 ml of concentrated hydrochloric acid and the solution was diluted to 500 ml with water.

Sulfide standards. (I) Sodium sulfide solutions were prepared fresh, whenever required, by dissolving about 0.5 g of Analar $Na_2S \cdot 9H_2O$ (MC/B) in 250 ml of degassed water. The surface of crystals was initially rinsed with water and washings were discarded. The sulfide content was determined iodimetrically using published procedures [18].

(II) Hydrogen sulfide, generated by the action of 6 M HCl on sodium sulfide, was bubbled through 500 ml of degassed water for 1—2 min in a Dreschel bottle. The bottle was stoppered and the sulfide content was determined iodimetrically [18]. All measurements were made in triplicate.

Instrumentation

In the experimental set-up shown in Fig. 1, the carrier stream was pumped at a rate of 3.5 ml min^{-1} by a Buchler Polystaltic Pump. The manifold was made from polyethylene tubing (Norton Plastic and Synthetics Division, OH) with short pieces of the pumping tubes (i.d. 2.0 mm) serving as connectors between the mixing coils. The mixing coil (i.d. 0.8 mm) used had a length of 2 m. The sample polyethylene loop (i.d. 1.0 mm) could deliver 130 μl. A load time of 15 s was maintained while 65 μl of sample was injected for each run. The amine and iron(III) solutions had to be taken in two streams (ca. 50 cm). Attempts to run a mixture of the two reagents in a single stream had a deleterious effect on the peak heights. The peak height continuously decreased, indicating a deterioration of reagent strength. However, if the two solutions were run separately and mixed before the point of injection (through a glass Y-joint, i.d. 2.0 mm) the peaks were found to be highly reproducible for each sulfide concentration.

A Bausch-Lomb (Spectronic 20) spectrophotometer, coupled with a Servo recorder (Kipp & Zonen, The Netherlands), was used for absorbance measurements at 662 nm. The full scale response of the recorder was less than 0.5 s. A flow-through cell (Fig. 2) was constructed from a solid aluminum rod. The design permits the polyethylene tube to be inserted into the rod through a small hole drilled in the bakelite cover of the sample compartment while the rod is in position in the cell compartment. A suitable exit hole is situated

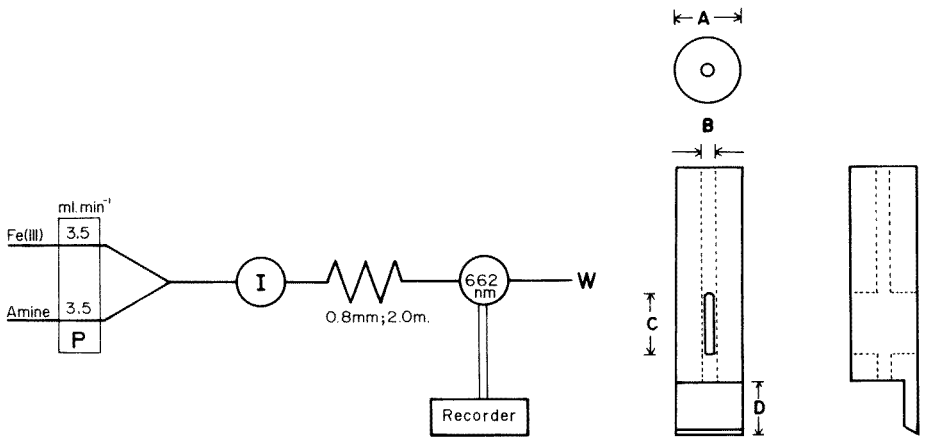

Fig. 1. Flow manifold for the determination of sulfide ions. The sample, 65 μl, is injected (I) into a carrier stream containing 5.4 mM *N,N-p*-dimethylaniline sulfate, 14.2 mM Fe(III) and 1.0 M HCl. The detection wavelength is 662 nm.

Fig. 2. Flow cell for determination of sulfide ions. Machined from a solid aluminum rod with A = 13 mm, B = 3 mm, C = 12.5 mm, D = 20 mm, and overall length = 93 mm.

below the lamp assembly. The detector response was found to be excellent despite the small path length (0.8 mm) provided by the tube.

Samples were injected using a FIA-100 apparatus (Lachat Instruments, WI) through a type 50 teflon rotary valve. This is a pneumatic actuator consisting of a double-action air cylinder coupled to a crank arm to produce the required rotary supply pressure of 30—125 psi. An Intermatic Autoflator 150 PSI Compressor (W. R. Brown Co., Chicago) was employed for this purpose, along with a pressure control valve (ca. 100 psi). Since water vapor was found to condense in the air line connected to the flow injection apparatus, a drying tube containing anhydrous calcium sulfate was placed at the air inlet to the Autoflator, and changed frequently.

Preparation of calibration samples

Aliquots of the solution (0.2—5.0 ml for Na_2S solutions; 0.5—5.0 ml for H_2S solutions) were pipetted into 5 ml of 0.05 M NaOH, and each solution was diluted to 25.0 ml with distilled water.

RESULTS AND DISCUSSION

All absorbance measurements were made at 662 nm [3]. This wavelength was further verified with solutions of methylene blue on a Cary Model 14 recording spectrophotometer and the Bausch-Lomb Spectronic 20 used.

Since the carrier stream not only provided the mobile phase but also served as a chromogenic reagent, the pumping rate was kept constant and a

continuous recording of transmittance vs. time was made. The calibration graph obtained by plotting absorbance vs. sulfur content of solutions (0—45.1 ppm sulfide) was linear. A linear regression treatment of the data, assuming both variables are subject to error [19], yielded the relationship between absorbance, A, and sodium sulphide concentration, C_{Na_2S},

$$A = (0.00498 \pm 0.00046)C_{Na_2S} + (0.01941 \pm 0.02)$$

with standard error of 0.0005 and correlation coefficient = 0.9973. Similar observations were made with H_2S solutions (0—41.4 μmol) giving the relationship

$$A = (0.00687 \pm 0.00045)C_{H_2S} + (0.00758 \pm 0.01)$$

with standard error of 0.0005 and correlation coefficient = 0.9986.

The maximum sampling rates that can be achieved correspond to about 210 samples per hour. The results obtained confirmed that the output of the instrument follows the Beer—Lambert law and there was no significant carry-over between samples even at this high sampling rate. It was easy to follow the formation of sample zone as the formation of the dye was clearly visible. A distinct flow injection signal could be obtained with solutions containing 1 ppm sulfide but below 0.5 ppm, noise interfered with the signal measurement.

Interferences

The methylene blue method has been employed for the determination of sulfate ion, after reducing it to sulfide. Since interference by nitrogenous materials in the determination has been noted by several workers [9—11], the effect of urea, potassium nitrate, and ammonium chloride on the flow injection determination of sulfide was investigated. The results (Table 1) indicate that while nitrate and urea have only marginal influence, ammonium chloride interferes at higher concentrations. Budd and Bewick [12] determined sulfide in carbonaceous ores. The determination of sulfide solutions was carried out in presence of added sodium carbonate. Although the sulfide ion can be recovered with reasonable accuracy in presence of 200 mg of carbonate, bubbles of CO_2 interfere with the determination when the amount exceeds 280 mg.

Influence of sulfhydryl and disulfide groups on the determination of sulfide was checked. While penicillamine disulfide (ca. 11 mg) and diethyldithiocarbamate (ca. 100 mg) had no effect, traces of CS_2 increase the peak height. However, addition of thiourea (>0.3 mg) decreases the peak height almost linearly.

Mixtures of sulfite, thiosulfate, and sulfide ions in solution are commonly encountered. It was of interest to study the determination of sulfide ion in the presence of added sulfite and thiosulfate. Table 1 shows the recoveries of sulfide obtained. It can be seen that the ions do not interfere seriously with the determination of sulfide, up to 200 and 80 ppm, respectively.

TABLE 1

Determination of sodium sulfide in presence of potential interferences

Species	Amounts (mg)	Number of determinations	Sulfur (ppm)	
			Average	S.d.
KNO_3	0.0—110	4	16.7	0.4
	151.5		15.5	
Urea	0.0—62	5	16.0	0.5
NH_4Cl	0.0—98.0	5	17.2	0.8
	147.0		14.2	
Na_2CO_3	0.0—216	5	16.4	0.9
	280		CO_2 bubbles	
Na_2SO_3	0.0—2.48	4	15.3	0.4
	4.13		13.5	
Na_2SO_3[a]	0.0—3.18	5	22.1	1.2
$Na_2S_2O_3$	0.0—0.91			

[a]Mixture of Na_2SO_3 and $Na_2S_2O_3$.

Trace amounts of copper ion in the solutions were found to be deleterious for the determination of sulfide by the methylene blue method by Johnson and Arkley [20] and Gustafsson [9]. However, in the present determinations, solutions of 0.1 mM Cu^{2+} ion had no significant effect, but the peak height decreased considerably in 0.3 mM Cu^{2+} solutions.

Optimum conditions

A wide range of experimental conditions has been employed for the formation of methylene blue during sulfide determination [4—17]. The range of iron(III), amine and hydrochloric acid concentrations within which the absorbance of the resulting methylene blue was greatest for samples containing 23.0 ppm of sulfide were investigated. With sulfide and hydrochloric acid concentrations constant, 36 determinations were done for all combinations of iron(III) (4, 8, 12, 16, 20, 24 mM) and amine concentrations (2, 4, 6, 8, 10, 12 mM). Each combination of amine and iron(III) was run ten times to average the peak heights. These peak heights were converted to absorbances, additional values interpolated using a conventional cubic spline algorithm, and the resulting 21 × 21 data matrix is plotted in Fig. 3 as a pseudo three-dimensional figure viewed from a 20° elevation. It can be seen from the absorbance surface that within the region of 2—8 mM amine and 4—16 mM iron(III), the absorbance is not particularly sensitive to the actual amine or iron(III) concentrations. Similar experiments were done to ascertain the sensitivity of color development to hydrochloric acid concentration. It was found that provided the acid concentration was above 0.6 M, the absorbance was at a maximum and unchanged. These results indicate that the concentration ratios of amine : iron(III) : hydrochloric acid used in this study were optimum and furthermore that the method does not require strict control of these concentrations for optimum conditions to be attained.

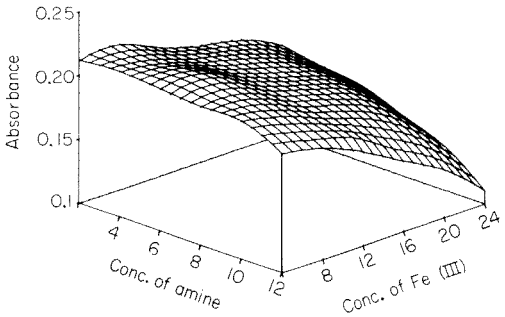

Fig. 3. Response surface for the determination of sulfide ions using the manifold shown in Fig. 1. All ratios of amine: Fe(III) were run at constant hydrochloric acid (1.0 M) and sulfide (23.0 ppm) concentrations.

In conclusion, the flow injection determination of sulfide through production of methylene blue offers a convenient and rapid procedure. It is likely that the technique could be useful for assay of H_2S in industrial waste and when present as an air pollutant.

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DIRECT SPECTROPHOTOMETRIC DETERMINATION OF THIOCYANATE IN SERUM AND URINE WITH A CONTINUOUS-FLOW ANALYZER

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SUMMARY

A spectrophotometric method for the rapid measurement of thiocyanate in serum and urine without separation from interfering substances is described. Thiocyanate reacts immediately with chloramine-T in presence of iron(III) chloride catalyst to give cyanogen chloride, which reacts with a mixture of γ-picoline (4-methylpyridine) and barbituric acid to form a soluble violet—blue product, which is measured at 605 nm. Other components of physiological fluids react more slowly and do not interfere if the reaction time of the chlorinating step is kept very short. The proposed procedure is compared with a highly selective method, based on the oxidation of thiocyanate to cyanide, and good agreement was obtained for both serum and urine. The method is readily adapted to a continuous-flow procedure with a Technicon AutoAnalyzer.

Thiocyanate is a detoxication product of cyanide, and determinations of thiocyanate in serum or urine have consequently been used for monitoring exposure to hydrogen cyanide from tobacco smoke [1—3], fire atmospheres [4], and certain vegetables that contain cyanogenic glucosides [5]. Moreover, thiocyanate is known to accentuate the anomalies of iodine deficiency, and the antithyroid properties of thiocyanate have been well-documented [6, 7]. Thus numerous methods for the determination of thiocyanate in body fluids have been developed. Of these, colorimetric methods based on the formation of a red complex with iron(III) ions [1, 8] are inselective and only applicable to serum. Several methods [2, 9—16] have been based on the König synthesis of pyridine dyes [17] in which cyanogen bromide or chloride produced by the reaction of thiocyanate (or cyanide formed from thiocyanate) with bromine or chlorine, reacts with pyridine and an aromatic amine to produce a coloured reaction product. Boxer and Rickards [10] developed a highly selective method based on the oxidation of thiocyanate to hydrogen cyanide under mild conditions; hydrogen cyanide was then separated from interfering compounds by aeration into sodium hydroxide and determined by a König reaction as modified by Epstein [13], with chloramine-T as the chlorinating agent and 1-phenyl-3-methyl-5-pyrazolone as the coupling agent.

The method of Boxer and Rickards was later improved [11, 14] with barbituric acid as the coupling compound. Although the method of Boxer and Rickards is sensitive and very selective, it is fairly laborious to perform and is applicable only to small series of samples.

During investigations on the role of thiocyanate in the aetiology of endemic goitre, the need arose for an automatic method for the determination of thiocyanate, applicable to physiological fluids, that was reliable and insensitive to interference, and that did not require carcinogenic reagents. Among the methods reported, those suitable for automation give reliable results on serum samples, but their selectivity has not been documented when applied to urine. A potentiometric method [18] and a fast kinetic method [19] suffer from similar drawbacks, while a recently reported gas-chromatographic method [20] is fairly laborious to perform. A simple and specific method for thiocyanate in serum and urine has been described by Lundquist et al. [21], based on the separation of thiocyanate from interfering substances by adsorption on a weakly basic anion-exchange resin, but in its existing form the method is not suitable for automation.

In the method described below, thiocyanate in serum and urine is determined by a recently reported modification of the König reaction [16] in which the stability of the dyestuff was improved by replacing pyridine with γ-picoline (4-methylpyridine). The proposed method requires that only two reagents be added to the sample: a catalyzed chlorinating reagent (chloramine-T/iron(III) chloride) and a chromogenic reagent (barbituric acid). In the conditions employed, thiocyanate reacts immediately to form cyanogen chloride whereas other components of physiological fluids react slowly and do not interfere.

EXPERIMENTAL

Apparatus

Spectrophotometric measurements were done with a Varian Cary 219 double-beam spectrophotometer in 10-mm cells. Test tubes (15 × 130 mm) fitted with ground-glass stoppers, were used as reaction tubes. For the continuous-flow procedure, the manifold consists of units of the Technicon AutoAnalyzer system as shown in Fig. 1. Glass and tygon tubing are used throughout, with a Technicon AutoAnalyzer proportioning pump. The transmittance is read in the colorimeter (flow cell path length 15 mm) through an interference filter (peak at 600 nm) and recorded linearly. The waste lines from the analyzer are fed into a bottle fitted with an acid-trapped vent; no smell of γ-picoline is thus discernible in the room in which the analyzer is operating.

Reagents

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

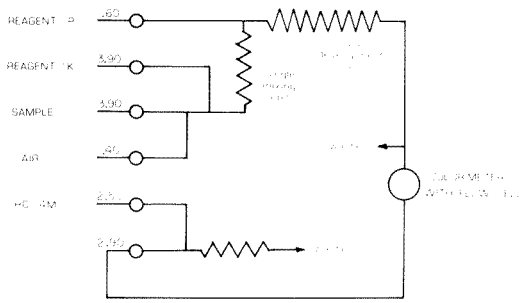

Fig. 1. The analytical manifold. The numbers on the pump tubes correspond to the flow rates in ml min^{-1}. At the flow rates indicated, the mixing coils for the chlorinating step (0.5 m) and for the colour development (5.5 m) corresponds to reaction times of 20 s and 3 min, respectively.

Standard thiocyanate solutions. Dissolve 1.67 g of potassium thiocyanate in water and dilute to exactly 1 l (1000 mg SCN^- l^{-1}). Prepare working solutions by suitable dilution with water.

Buffer solution (pH 4.1). Dissolve 10.8 g of sodium acetate trihydrate and 24.0 ml of anhydrous acetic acid in water and dilute to 1 l.

Catalyzed chlorinating reagent (reagent K). The procedure of Van Peborgh [12] was adopted. Mix equal volumes of a 1% (w/v) solution of chloramine-T and a 0.1% (w/v) solution of iron(III) chloride. Allow the mixture to stand overnight and filter off the precipitate. The clear supernatant solution is the reagent K. This is equivalent to the chloramine-T and iron(III) chloride reagents of Epstein [13], pre-mixed to avoid precipitation in the manifold. For the analysis of serum samples, dilute this reagent (1 + 2) to obtain the "diluted reagent K". The reagent is active for at least a week when kept at room temperature.

γ-Picoline—barbituric acid reagent (reagent P). Dissolve 6.0 g of barbituric acid in a mixture of 30 ml of γ-picoline (4-methylpyridine) and 64 ml of water, and add 6.0 ml of concentrated hydrochloric acid (d = 1.18). The reagent obtained is light yellow and is stable for a week in the refrigerator, despite its colour change from light yellow to orange-yellow.

Sample preparation

The samples should be free from suspended matter, which causes a noisy transmittance trace, and proteins, which are liable to precipitate in the manifold. Urine samples are simply diluted and buffered as follows: to 0.5 ml of sample add 2.0 ml of the acetate buffer and 7.5 ml of water to achieve a final dilution of 1 + 19.

For serum samples, the following deproteinization procedure is used: to 500 μl of serum add 250 μl of 0.8 M $NaClO_4$ and 250 μl of 20% trichloroacetic acid, and centrifuge the sample for 20 min at 2000 rpm; then, to 1.0 ml of the clear supernatant liquid add 100 μl of 1 M NaOH and 8.9 ml

of water to achieve a final dilution of 1 + 19. The sodium hydroxide added was just enough to neutralize the bulk of acid while still maintaining the slight acidity required for the thiocyanate—chloramine-T reaction. As previously reported [1, 19], the addition of sodium perchlorate is necessary to ensure that all of the thiocyanate is determined by this method.

Procedure

Urine. In a dried reaction tube, place 5.0 ml of diluted urine sample, add 5.0 ml of the catalyzed chlorinating reagent K, stopper the tube and shake gently. After 20—30 s, add 2.0 ml of the γ-picoline—barbituric acid reagent, stopper the tube again and mix. After 2—5 min, measure the absorbance at 605 nm against a reagent blank.

Serum. In a dried reaction tube, place 5.0 ml of the deproteinized and diluted serum sample, add 5.0 ml of the diluted reagent K, stopper the tube and shake gently. Then proceed as for urine samples.

RESULTS AND DISCUSSION

The reaction between chloramine-T and thiocyanate ions is fairly slow unless catalyzed by iron(III) ions [13, 14]. However, the reaction should be done under acidic conditions, because the thiocyanate chlorination is not complete at pH > 5 (Fig. 2); the urine samples were therefore acidified with acetate buffer. When iron(III) chloride is used as catalyst with pure thiocyanate solutions, the formation of cyanogen chloride from thiocyanate is practically instantaneous (Fig. 3) and the concentration of chlorinating reagent is not critical (Fig. 4).

When the reaction is applied to a sample containing a large amount of reducing substances, the chlorinating agent is not completely available for the formation of CNCl from thiocyanate, and an underestimate of the thiocyanate concentration is obtained with a short reaction time unless a large

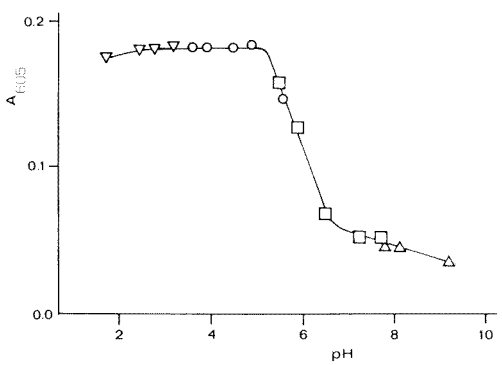

Fig. 2. The effect of the pH of the solution on chlorination. (▽) Chloroacetate buffer; (○) acetate buffer; (□) phosphate buffer; (△) Tris buffer. All buffers were 0.05 M; SCN^- concentration was 0.2 mg l^{-1}. Chlorinating conditions as described in the procedure for serum samples.

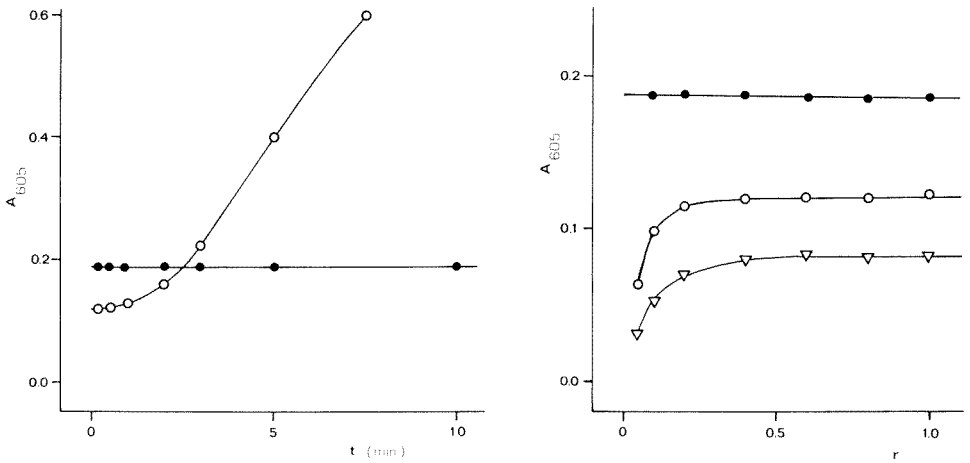

Fig. 3. The effect of time of chlorination on the colour development for: (•) standard thiocyanate solution, 0.2 mg l^{-1}; (○) a urine pool (dil. 1 + 19) of thiocyanate concentration 2.59 mg l^{-1}. Except for time, the chlorinating conditions were as in the procedure.

Fig. 4. The effect of increasing the concentration of reagent K on the colour development for: (•) a standard thiocyanate solution, 0.2 mg l^{-1}; (○) a urine pool (dil. 1 + 19), 2.59 mg l^{-1}; (▽) a serum pool (dil. 1 + 19), 1.78 mg l^{-1}. Chlorination time 20 s. Factor r is the volume ratio of reagent K to sample; r = 1 corresponds to the conditions described in the procedure. For the serum pool, diluted reagent K is used.

excess of chloramine-T is employed. Figure 4 shows the results obtained with serum and urine pools. The first rapid reaction of chloramine-T with reducing substances in the sample successively leads to an increasing absorbance of the solution with longer chlorinating time (Fig. 3). This increase of absorbance can be ascribed to the slower formation of CNCl from reducing substances, because the absorption spectra of the dyes obtained by allowing a longer reaction time in the chlorinating step are exactly the same as those obtained from thiocyanate only. This increase in absorbance with increasing time of chlorination is more marked in urine samples than in serum samples. Thus, given the instantaneous reaction of thiocyanate with chloramine-T to form cyanogen chloride and the need for an excess of chlorinating reagent to overcome the effects of reducing agents in physiological fluids, a reliable estimate of thiocyanate in such samples requires a large excess of chloramine-T and a very short time for the chlorinating step. Accordingly, a 1:1 ratio of K reagent to diluted sample was employed (undiluted and diluted, respectively, for urine and serum) and a working dilution of (1 + 19) was used for both serum and urine, to avoid underestimates in samples with high concentrations of reducing substances. When the 1:1 ratio of K reagent to sample was used, maximum absorbance was achieved within 2—3 min after the addition of the γ-picoline—barbituric acid reagent, and the absorbance remained constant for about 5 min, though it decreased by about 3% after 30 min.

Precision and recovery

The precision of the method was evaluated by analyzing 8 aliquots of a urine pool. The result obtained, 2.13 ± 0.034 mg l^{-1} (mean ± SD), corresponds to a coefficient of variation of 1.6%. The recovery of thiocyanate (1.50 mg l^{-1}) added to a urine sample containing 1.25 mg SCN^- l^{-1} was 101.3 ± 2.3%, in five separate experiments. In further tests, 0.50, 1.00, 1.50, 2.00 and 3.00 mg SCN^- l^{-1} were added to a urine sample containing 1.83 mg SCN^- l^{-1}; the regression line between the observed (y) and expected (x) values was: $y = (1.013 \pm 0.021)x + (0.012 \pm 0.017)$. The recovery of thiocyanate (1.00 mg l^{-1}) added in 6 separate experiments to a serum sample containing 1.64 mg SCN^- l^{-1} was (99.7 ± 2.2)%.

Selectivity

Cyanide reacts immediately with chloramine-T to form CNCl and thus behaves like thiocyanate in the present method. However, in the routine procedure the interference caused by cyanide can be neglected because the concentration of cyanide in serum and urine is exceedingly low [22]. To validate the proposed method further, serum and urine samples were analyzed by the present method and by the highly selective oxidation method of Boxer and Rickards [10]. The regression lines between the values obtained by the present method (y) and those obtained by the oxidation method (x) are: $y = (1.010 \pm 0.009)x + (0.102 \pm 0.115)$ for serum samples ($n = 14$) and $y = (0.973 \pm 0.012)x + (0.193 \pm 0.106)$ for urine samples ($n = 11$).

Continuous-flow procedure

The performance parameters of any AutoAnalyzer system vary from day to day, necessitating the inclusion of a set of standards and, if relevant, drift correction samples and interaction test patterns [23] in daily runs. The standard sampling rate normally adopted is 25 samples/h, with a sample/wash ratio of 1:1, a sample cup alternating with a cup containing distilled water. The degree of interaction that occurs when a low sample follows a high sample in the physiological range of thiocyanate concentrations under various sampling conditions was obtained by running an interaction test pattern consisting of a low (1.0 mg l^{-1}) standard, followed by a high (5.0 mg l^{-1}) standard which was in turn followed by another low (1.0 mg l^{-1}) standard. If R_B is the reading produced by the low standard before the high standard (reading R_H), and R_A is the reading produced by the low standard after (and affected by) the high standard, then the percentage of interaction is defined as $100(R_A - R_B)/R_H$. As long as the alternating sample/water configuration is used, sampling rates up to 50 samples/h (25 actual samples/h) are readily usable (percentage interaction less than 0.8).

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OPTIMIZATION OF A KINETIC-CATALYTIC METHOD BY USE OF A NUMERICAL MODEL AND THE SIMPLEX METHOD

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SUMMARY

Optimization of a kinetic-catalytic method based on a numerical model is demonstrated for the copper(II)-catalyzed decomposition of hydrogen peroxide in the presence of pyridine. The responses are calculated as initial rates of the catalytic reaction after calculating the equilibrium distributions of all species in the reaction mixture. Simulation of response surfaces as well as simplexes showed optimal concentrations for hydrogen peroxide, pyridine and pH. Experimental verifications of the simulated reaction conditions showed that the catalytic determination of copper down to 1.5×10^{-7} M is possible.

The optimization of kinetic-catalytic methods [1] is usually carried out by systematic variation of a single factor while all other variables are held constant. This technique, however, is less suitable for catalytic methods because the latter are based on chemical reactions which are often too complex to be treated precisely by one-factor experiments. On the other hand, the complexity of kinetic-catalytic procedures also limits the possibilities for their description by numerical models. To date, optimized kinetic-catalytic procedures have been elaborated only with the use of black-box approaches, such as the modified sequential simplex method [2, 3].

Increasing knowledge of the mechanisms and kinetics of catalytic methods of analysis, however, is gradually enabling these relatively complicated catalyzed reactions to be described by precise analytical mathematical expressions.

Kinetic studies of the copper-catalyzed decomposition of hydrogen peroxide in the presence of pyridine as an activator [4] have rendered possible the numerical modelling of a kinetic-catalytic method based on the rate equation found experimentally. The analytical response is the measured reaction rate, which should be a maximum if trace metal concentrations are to be determined. The reaction rate, which is monitored in the present study with a Clark-type oxygen electrode, is affected by such factors as pH, reagent and buffer concentrations and temperature. In order to characterize the influence of these factors in more detail, the numerical model was used to construct graphical representations of response surfaces, and the catalytic

determination was optimized theoretically by the simplex method. The simulated optimization steps markedly shortened the necessary labour required for the experimental runs used to find the optimum conditions. The experimental verification, however, shows the limitations of such a mathematical model at the present stage of knowledge as applied to homogeneously-catalyzed reactions.

Based on the optimized conditions, an analytical procedure is proposed for kinetic-catalytic determination of traces of copper.

EXPERIMENTAL

Computation

The program for calculation of the initial reaction rates and for the optimization with the simplex method was written in FORTRAN IV. For evaluation of equilibrium concentrations the computer program COMICS [5] was used. All computations were done with an ES 1040 computer (VEB Robotron, G.D.R.).

Kinetic determination

The preparation of the reagent solutions and details of the experimental technique have been described elsewhere [4]. Every kinetic run was carried out by pipetting the following solutions into a thermostatted 50-ml glass vessel: 25 ml of phosphate buffer (final concentration 0.0476 M), 1 ml of 0.875 M pyridine and 5—50 μl of a 10^{-3} M copper(II) solution. Different amounts of water were added to the reaction mixture, to give a volume of 35 ml when the hydrogen peroxide had been added. The solution was thermostatted for 10 min and oxygen was removed simultaneously by bubbling pure argon through the solution. The reaction was started by addition of the appropriate amount of hydrogen peroxide (30%) to the stirred reaction mixture. The formation of oxygen with time was followed with a Clark-type oxygen electrode amperometrically over 10 min. From the recorded kinetic curves, the initial slope (tangent method [6]) or the oxygen concentration at constant time (fixed time method [6]) was used as a measure of the reaction rate. The rate of the catalyzed reaction was obtained by subtracting the rate of the blank reaction from the overall reaction rate.

RESULTS

The numerical model

The decomposition of hydrogen peroxide is catalyzed by many metals [1, 6]. In weakly alkaline solutions and in the presence of pyridine as an activator, the decomposition of hydrogen peroxide should enable traces of copper to be determined at the nanogram level, based on the reaction

$$2H_2O_2 \xrightarrow[\text{pyridine}]{\text{Cu(II)}} O_2 + 2H_2O$$

Kinetic measurements with calorimetric or amperometric monitoring of the course of the reaction [4] gave the rate expression $v_0 = k[Cu(py)^{2+}]_0 [HOO^-]_0$, with $k = (3.52 \pm 0.32) \times 10^5\ M^{-1}\ s^{-1}$. In this equation, v_0 stands for the initial reaction rate, and the concentrations of the 1:1 copper—pyridine complex and the hydrogen peroxide anions correspond to initial equilibrium concentrations. Because the stability of the catalytically-active copper—pyridine complex is relatively low, and copper forms many binary and ternary complexes in the presence of peroxide, pyridine and phosphate buffer, only a fraction of the catalyst is present in its catalytically-active form. For that reason, under any reaction conditions, the equilibrium concentrations of all species have first to be computed and then the initial reaction rates calculated according to the above equation.

For the present reaction conditions, the total concentrations of pyridine and hydrogen peroxide as well as the pH were put into the computer program COMICS [5], which takes into account 23 equilibria in the system [4]. The output of that program gave the sought-for equilibrium concentrations which could be used for evaluation of the initial reaction rates.

Response surfaces

Initially the variation of only two interdependent factors was simulated, giving response surfaces of the catalytic reaction. Such surfaces are presented in Fig. 1 where the initial reaction rate v_0 is plotted against hydrogen peroxide and pyridine concentrations and pH.

The flared shape of all surfaces with variation of pyridine concentrations (Figs. 1a, c) is caused by the dependence of the concentration of catalytically-active copper—pyridine complex on the total pyridine concentration. From these graphs it may be concluded that the pyridine concentration optimally should be about 2.5×10^{-2} M.

It is also evident that inside the boundaries of the kinetic model (0.02—0.2 M hydrogen peroxide, pH 6—9, 10^{-3}—0.1 M pyridine) the response increases at higher peroxide concentrations (Figs. 1b, c) as well as at higher pH values (Figs. 1a, b).

Simplex method

For simultaneous variation of the three factors (pH and concentrations of hydrogen peroxide and pyridine) simulations with a fixed-size simplex and variable-size simplex (modified simplex) [7] were undertaken. In these simulations the boundaries given by the kinetic investigations [4] were not followed strictly because the simplex moved toward higher hydrogen peroxide concentrations than were previously studied, which could provide further information relevant to the analytical application. The simulated responses obtained at each vertex for the 3-dimensional optimization are shown in Fig. 2 for the fixed-size and variable-size simplexes.

As can be seen from Fig. 2, the variable-size simplex (curve a) converged better to an optimal value than the fixed-size simplex (curve b). Furthermore

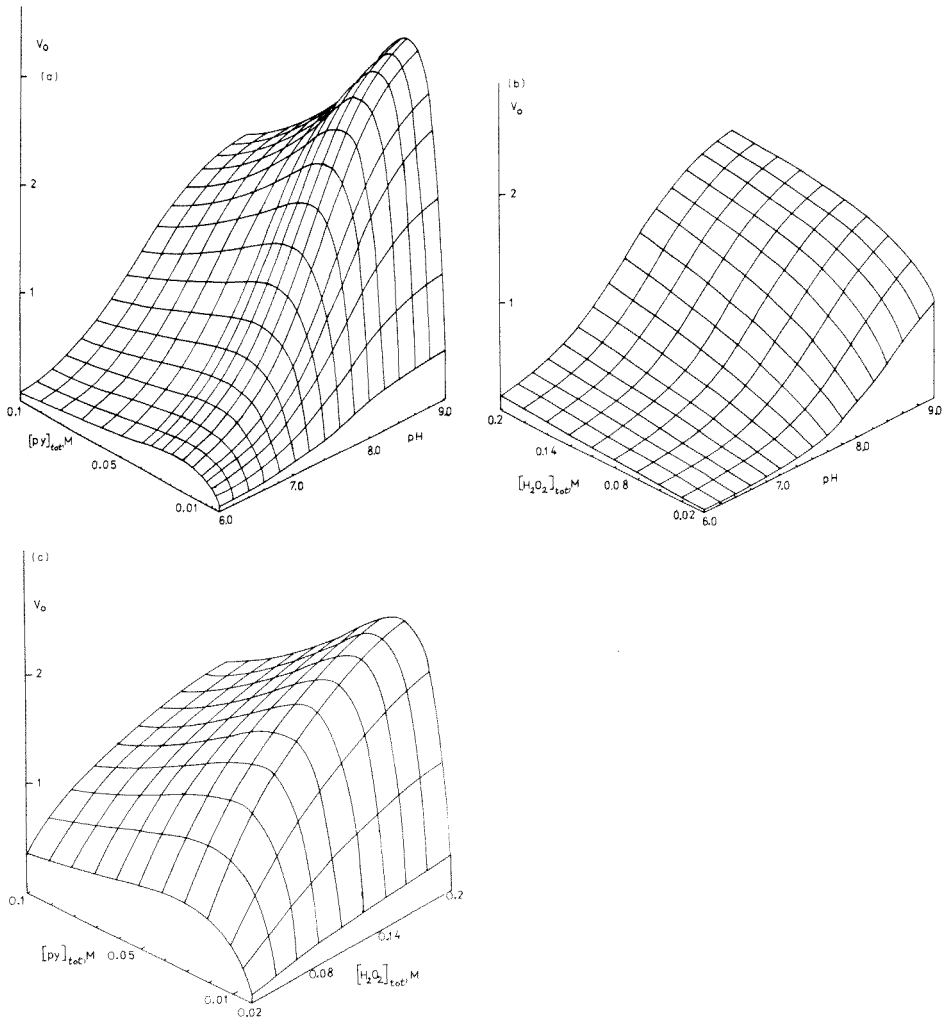

Fig. 1. Response surfaces for the initial reaction rate (v_0, in units of 10^{-5} M min^{-1}) on variation of hydrogen peroxide and pyridine concentration and pH; 2.4×10^{-6} M Cu(II), 0.0476 M phosphate; (a) 0.191 M H_2O_2; (b) 2.5×10^{-2} M pyridine; (c) pH 8.0 (phosphate).

the fixed-size simplex shows a strong oscillation in response near the optimum. This oscillation is caused by the high sensitivity of the response (the initial rate) to the pyridine concentration. Following the total pyridine concentration during optimization (Fig. 3), it is evident that this factor is much better controlled by modifying the step width with the variable-size simplex (Fig. 3, curve a) than with the fixed-size simplex (Fig. 3, curve b). The pyridine concentration in the variable-size simplex moved towards a constant value of $(2.5 \pm 0.1) \times 10^{-2}$ M. From the simulated response surfaces and

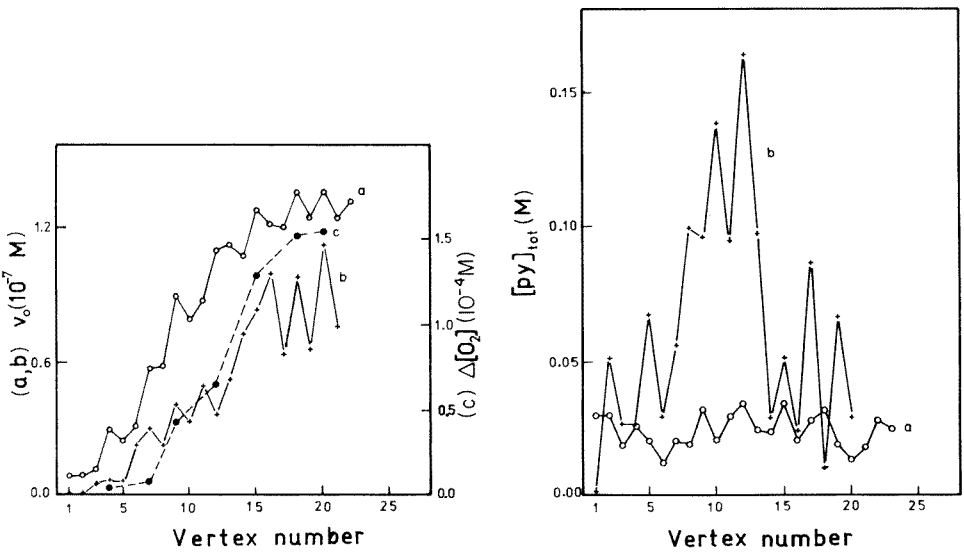

Fig. 2. Responses for the 3-dimensional optimization of the copper-catalyzed decomposition of hydrogen peroxide. (a) Simulated variable-size simplex, responses expressed as initial rates v_0; (b) simulated fixed-size simplex, responses expressed as initial rates v_0; (c) experimentally verified points of variable-size simplex, response measured as evolution of oxygen $\Delta[O_2]$ after 2 min.

Fig. 3. Change of total pyridine concentration during simplex optimization with (a) the variable-size simplex, (b) the fixed-size simplex.

from the simplexes, it can be concluded that the experimental verification of the data can be restricted to the influence of peroxide concentration and the pH value, the pyridine concentration being fixed at 2.5×10^{-2} M.

The experimental verification of the optimization steps of the variable-size simplex showed some unexpected features: at concentrations of hydrogen peroxide higher than were studied in the kinetic investigation [4] (>0.2 M), the initial rates were up to three times greater than predicted by this model. Thus, outside the boundaries of the kinetic model a new reaction mechanism seems to be involved, perhaps because of additional catalytic activity of species other than the 1:1 copper—pyridine complex. Because of these high reaction rates, it was not possible to measure the initial rates very precisely and the fixed time method had to be applied. The experimental conditions and the measured responses are given in Table 1. A comparison of these responses with the simulated curves (Fig. 2) reveals a qualitative coincidence between theory and experiment but, of course, the agreement is not good quantitatively.

TABLE 1

Experimentally obtained responses for the simplex optimization[a]

Vertex	pH	$[H_2O_2]$ (M)	$\Delta[O_2]$ ($\times 10^{-5}$ M)
4	7.19	0.115	0.41
7	7.47	0.179	1.06
9	7.88	0.236	4.3
12	8.13	0.269	6.4
15	8.46	0.340	12.2
18	8.76	0.408	15.1
20	8.81	0.390	15.4

[a] 2.5×10^{-2} M pyridine, 0.0476 M phosphate, 1.37×10^{-6} M copper(II), 25°C, 2 min reaction.

Calibration, detection limit and interferences

The parameters of vertex 20 (Table 1) were used as optimal conditions. For analytical purposes, the phosphate buffer used in the optimization experiments was replaced by a borate buffer because of its higher buffer capacity in this pH region. By plotting the evolution of oxygen after 10 min ($\Delta[O_2]_{10}$) against copper concentrations ranging from 1.4×10^{-7} to 1.4×10^{-6} M, a straight line was obtained with the following regression equation: $\Delta[O_2]_{10} = 409.1[Cu]_{tot}$ (the concentrations of oxygen and copper being expressed in M). The detection limit of the method calculated according to the 3σ-criterion was 9.5 ng Cu ml^{-1}, which is comparable to other spectrophotometrically-monitored kinetic-catalytic determinations of copper which enable 3—5 ng Cu ml^{-1} to be determined [1, 6].

A comparison of the sensitivity after optimization, with that found in the kinetic study [4], shows an increase in sensitivity of one order of magnitude.

The selectivity of the method was tested with regard to those species which interfered significantly in the determination of copper with a quite similar indicator reaction, the spectrophotometrically-monitored copper-catalyzed oxidation of sulphanilic acid with hydrogen peroxide in the presence of pyridine [8]. Table 2 gives the results for six metals and three non-metallic species. Although the present method is somewhat less selective with respect to manganese and iron, the influence of nickel and zinc is significantly less than in the sulphanilic acid reaction [8]. Anions do not show any influence up to 10^{-2} M but strong complexing agents such as EDTA mask copper at stoichiometric concentrations.

Conclusions

The study has demonstrated that determinations based on homogeneously-catalyzed reactions can be described with a numerical model. For such a model the mechanism, the equilibria and the kinetics of the reaction under study must be known in order to simulate the course of the catalytic

TABLE 2

Maximum tolerable molar ratios of foreign species in the determination of copper[a]

Species	[Cu(II)] : [ion][b]		Species	[Cu(II)] : [ion][b]	
	Amperometric	Spectrophotometric [8]		Amperometric	Spectrophotometric [8]
Co(II)	1:0.7	1:0.34	Zn(II)	1:200	1:16
Mn(II)	1:20	1:37	Cl^-	1:14 000	—
Mo(VI)	1:20	1:4	SO_4^{2-}	1:14 000	—
Fe(III)[c]	1:2	1:10	EDTA	1:0.13	—
Ni(II)	1:400	1:34			

[a] 7.14×10^{-7} M Cu(II), 0.390 M H_2O_2, 2.5×10^{-2} M pyridine, pH 8.82 (borate).
[b] Criterion for interference was a difference of two standard deviations of the method.
[c] Unlike the other species, iron catalyzes the reaction.

reaction. The relatively simple example of the copper(II)-catalyzed decomposition of hydrogen peroxide described gives encouragement that more complicated systems can also be simulated by the technique described.

The use of response surfaces as well as of multidimensional optimization methods is helpful in selecting the optimum conditions from simulated data or, as was demonstrated by Frazer et al. [9], from data obtained with on-line computerization of experiments.

Comparison of the simulated with the experimental data results showed only a qualitative coincidence outside the boundaries of the previous kinetic investigation. Nevertheless, the simulation allowed evaluation of highly sensitive conditions for the catalytic determination of copper down to 1.5×10^{-7} M. The reproducibility of the method is about 6.6% and the selectivity is comparable to that of other catalyzed reactions.

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LIQUID—LIQUID DISTRIBUTION BEHAVIOR OF ION-PAIRS OF TRIPHENYLMETHANE DYE CATIONS AND THEIR ANALYTICAL APPLICATIONS

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SUMMARY

The extraction of ion-pairs of monovalent anions (chloride, bromide, iodide, perchlorate, benzenesulfonate, naphthalenesulfonate and octanesulfonate ions) with pararosaniline, crystal violet, ethyl violet and methylene blue cations is described. The extraction constants (K_{ex}) for these ion-pairs between an aqueous phase and several organic phases (1,2-dichloroethane, chloroform, *o*-dichlorobenzene, chlorobenzene, benzene and toluene) were determined. The values of log K_{ex} for ion-pairs with ethyl violet were on average about 2.7 times larger than those of crystal violet, which were in turn about 6 times larger than those for pararosaniline. The order of the extractability of ion-pairs was 1,2-dichloroethane > chloroform > *o*-dichlorobenzene > chlorobenzene > benzene > toluene, and the differences of the log K_{ex} between two successive solvents averaged 0.4, 1.9, 2.1, 2.6 and 0.8, respectively. Ethyl violet and crystal violet are useful extraction-spectrophotometric reagents for iodide, perchlorate and alkyl or arylsulfonate ions.

Ion-pairs have become widely used in analytical chemistry, especially for the separation of ions by extraction, precipitation, flotation, partition chromatography and ion-selective electrodes. Liquid—liquid distribution is one of the most important separation techniques for trace amounts of ions, because the extract can be used subsequently for spectrophotometric, fluorimetric and atomic-absorption determinations. Recently, Sandell and Onishi [1] have discussed the analytical importance of ion-associates (ion-pairs). Of the many suitable cationic or anionic reagents which can react with counter ions to form ion-pairs, cationic dyes have been most frequently used in extraction—spectrophotometric determinations of metal chelates, metal complexes and non-metallic ions [2] and many organic anions, because of their high sensitivity (high molar absorptivity), ready availability and versatility.

Recently, the extraction behavior of ion-pairs of azo-dye cations and their analytical applications were reported [3]. In the work described here, cationic triphenylmethane dyes were studied in detail, because the extraction behavior of such dyes has not been examined systematically, despite their frequent utility in analytical chemistry.

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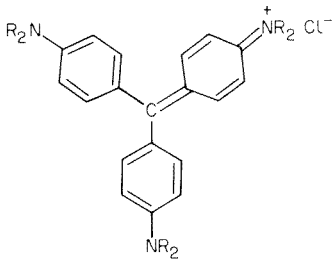
EXPERIMENTAL

Apparatus and reagents

Absorbances were measured on a Hitachi EPS-3T recording spectrophotometer and a Shimadzu UV-100 spectrophotometer in glass cells of 10-mm pathlength. A Hitachi—Horiba, Model F-5SS, pH meter equipped with a combined electrode (6026-05T) was used for pH measurements. An Iwaki (Model V-S Type KM) shaker was used for horizontal shaking of the 25-ml stoppered test tubes or the 150-ml separatory funnels used for extraction.

Cationic dyes. Triphenylmethane dyes (I_a, I_b, I_c) and methylene blue were obtained commercially. I_a, I_b and I_c were recrystallized from water; recrystallized I_b and I_c were needle-like crystals and I_a was amorphous. Methylene blue (analytical-reagent grade) was used without further purification. These dyes were dissolved in distilled water.



I_a, R = H (Pararosaniline, PR)
I_b, R = CH_3 (Crystal violet, CV)
I_c, R = C_2H_5 (Ethyl violet, EV)

Anion solutions. Sodium salts of monovalent anions were dissolved in distilled water.

Extracting solvents. Commerically available 1,2-dichloroethane (DCE), chloroform, *o*-dichlobenzene, chlorobenzene, benzene, toluene and methyl isobutyl ketone (MIBK) were used without further purification. These solvents were saturated with water by shaking with water before use.

All reagents used were of analytical-reagent grade.

Determination of the extraction constants, K_{ex}

An extraction system in which a monovalent cation (C^+) reacts with a monovalent anion (A^-) to form only one kind of ion-pair (C^+A^-) in the aqueous phase, and in which the extracted ion-pair does not dissociate or aggregate, involves the following equilibria.

The ion-association constant in aqueous solution, K_A, refers to the reaction

$$C^+ + A^- \rightleftharpoons C^+A^-; \quad K_A = [C^+A^-]/[C^+][A^-] \tag{1}$$

The distribution coefficient of the ion-pair (C^+A^-) between the aqueous and organic phases, K_D, refers to the equilibrium

$$C^+A^- \rightleftharpoons (C^+A^-)_{org}; K_D = [C^+A^-]_{org}/[C^+A^-] \tag{2}$$

where the subscript org refers to the organic phase and the aqueous concentrations are unmarked.

The distribution ratio of C^+ between the aqueous and organic phases, D, and the extraction constant, K_{ex}, are described by

$$D = [C^+A^-]_{org}/([C^+] + [C^+A^-]) \quad (3)$$

$$K_{ex} = [C^+A^-]_{org}/[C^+][A^-] = K_D \times K_{ex} \quad (4)$$

From eqns. (1—4), the following equation can be derived

$$D^{-1} = K_D^{-1} + (K_{ex}[A^-])^{-1} \quad (5)$$

The plots of D^{-1} against $[A^-]^{-1}$ must be linear; from the intercept of the y-axis and the slope, K_D and K_{ex} can be calculated.

Procedure A. An appropriate amount of the aqueous dye solution (2 ml of 10^{-3} M ethyl violet, crystal violet or methylene blue solution, or 5 ml of 10^{-3} M pararosaniline solution) was transferred to a separatory funnel and the sodium salt solution of the anion (A^-) was added in a 10—100-fold molar excess over the dye. Then 5 ml of acetic acid buffer solution (pH 5—6; 10^{-2} M) was added and the solution was diluted to 25 ml with distilled water. This solution was shaken with 50 ml of organic solvent. The separated organic phase was then shaken with a fresh solution of anion A^- containing a 10—100-fold molar excess of the dye. Portions (5-ml) of the organic phase and of an aqueous solution containing various amounts of the anion (A^-) and buffer solution (pH = 6; 10^{-3} M) were shaken in a 25-ml stoppered test tube for about 30 min to complete the extraction equilibrium. After phase separation, the absorbance of the organic or aqueous phase was measured at the maximum wavelength of the dye, and the distribution ratio (D) was calculated from the original absorbance of the organic phase and the absorbance of the organic or aqueous phase after shaking with the anion solution. The concentration of the anion (A^-) in the aqueous phase was obtained by adding the concentration of the dye in the aqueous phase to the original concentration of anion in the aqueous phase. In almost all cases, the concentration of anion was much higher than that of the dye in the aqueous phase, and the concentration of the ion-pair (C^+A^-) in the aqueous phase could be neglected compared with the concentration of the anion (A^-). The values of D^{-1} were plotted against $[A^-]^{-1}$ and the intercept and slope were calculated by the least-squares method.

When the distribution coefficient of the ion-pair, K_D, was very large, the intercept of the y-axis was near to zero, and the value of K_D became uncertain. Even in such cases, the extraction constants could be calculated from the slope; the accuracy was relatively good.

Procedure B. When the extraction constant of the ion-pair, K_{ex}, was small, procedure A was not satisfactory because only a little of the C^+A^- ion-pair could be extracted and so the distribution ratio of the dye could not be measured properly. In such cases, extraction constants were determined approximately by calculation from the absorbances of the dye in the aqueous and organic phases and the concentration of anion in the aqueous

phase after 5 ml of aqueous solution containing the dye (chloride), anion (sodium salt) and buffer solution (pH 6; 10^{-3} M) had been shaken with 5 ml of the organic solvent.

RESULTS AND DISCUSSION

Effect of pH

Cationic dyes may be present in aqueous solutions in various forms; they are protonated in acidic solution, and change gradually to the neutral molecule in alkaline solution. In the pH range 4—7, the dyes tested are in the monovalent cationic form and the absorbances of the ion-pairs in the organic phase were constant. In this work, the extractions were carried out at pH 5—6. In Fig. 1, the effect of pH on the extraction of the methylene blue chloride ion-pair is shown as an example.

Determination of the extraction constants for monovalent anions

Three triphenylmethane dyes and methylene blue were examined, and the extraction constants were calculated. The plots of D^{-1} against $[A^-]^{-1}$ for crystal violet are shown in Fig. 2 as examples. As expected, the plots were all linear. The results obtained for the four dyes are listed in Table 1.

It is generally considered that the bulkier and more organophilic the ions are, the better will be the extraction of the ion-pair. Thus the extraction of the ion-pair increases as the radius of the inorganic ion increases or as the

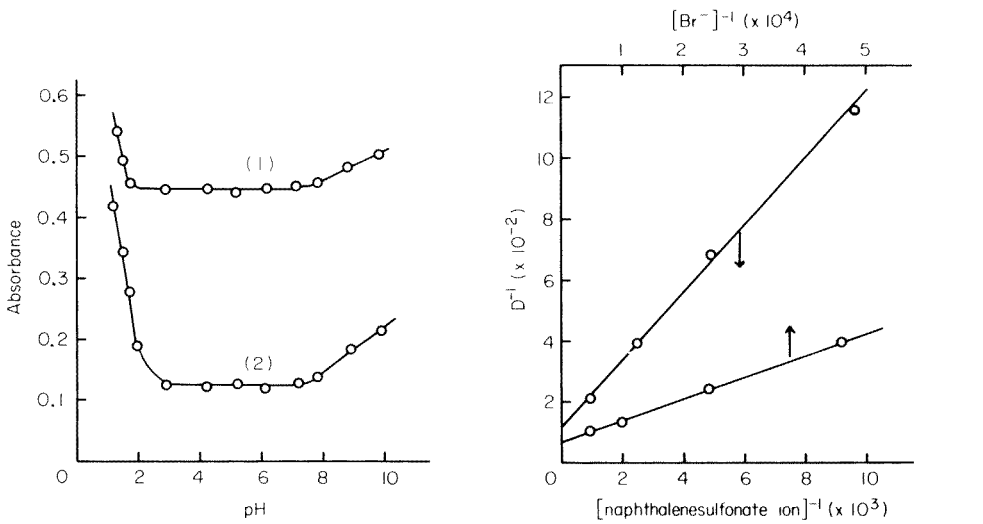


Fig. 1. Effect of pH on extraction of methylene blue ion-pair into 1,2-dichloroethane (1) $[Cl^-] = 2 \times 10^{-3}$ M; (2) $[Cl^-] = 1 \times 10^{-4}$ M; methylene blue; measured at 657 nm against solvent.

Fig. 2. The plots of D^{-1} against $[A^-]^{-1}$ for crystal violet.

dye cation becomes increasingly organophilic. These trends are clarified in Fig. 3 for halide ions. A reliable measurement of bulk is difficult to find for organic anions, but in all extraction systems, the extractability of the organic sulfonates was in the order benzenesulfonate < naphthalenesulfonate < octanesulfonate, and the extractability of the dye cations was in the order pararosaniline < methylene blue < crystal violet < ethyl violet.

The differences in log K_{ex} between cationic dyes Δ log K_{ex}, are listed in Table 2. The difference between ethyl violet and crystal violet, log $K_{ex(EV-CV)}$, was almost constant despite the variety of solvents and anions. For crystal violet and pararosaniline, the values of Δ log $K_{ex(CV-PR)}$ in chloroform were larger than those in 1,2-dichloroethane. The reason for this is not apparent. Schill [4], Gustavii [5] and Modin and Schill [6] reported that the contribution of the methylene group to the extraction constant (log K_{ex}) of the ion-pair with alkylammonium cations (i.e., Δ log K_{ex}) was 0.44 to 0.63.

TABLE 1

Extraction constants (log K_{ex}) obtained between aqueous and organic phases for ethyl violet, crystal violet, pararosaniline and methylene blue
(Values in parentheses were obtained by Procedure B)

Solvent	Anion						
	Cl^-	Br^-	I^-	ClO_4^-	Benzene-sulfonate	Naphthalene-sulfonate	Octane-sulfonate
Ethyl violet							
$C_2H_4Cl_2$	8.02	QE[a]	QE	QE	QE	QE	QE
$CHCl_3$	7.61	QE	QE	QE	QE	QE	QE
$C_6H_4Cl_2$	5.74	7.08	QE	QE	QE	QE	QE
C_6H_5Cl	3.69	4.99	7.19	QE	6.03	7.70	8.02
C_6H_6	(0.6)	(2.08)	4.98	6.39	(3.35)	5.35	5.70
C_7H_8	(−0.4)	(1.4)	4.13	5.77	(2.60)	4.61	5.01
Crystal violet							
$C_2H_4Cl_2$	5.30	6.52	QE	QE	7.29	QE	QE
$CHCl_3$	4.80	6.15	QE	QE	6.59	QE	QE
$C_6H_4Cl_2$	2.89	4.47	6.73	QE	5.43	6.97	7.19
C_6H_5Cl	(0.8)	(2.27)	4.73	6.41	3.37	4.93	5.13
C_6H_6	(−1.9)	(−0.5)	(1.71)	(3.72)	(0.9)	(2.43)	(2.60)
C_7H_8		(−0.9)	(1.3)	(2.99)	(0.2)	(1.80)	(1.99)
Pararosaniline							
$C_2H_4Cl_2$	(−0.4)	(1.3)	(3.2)	(4.6)	(2.2)	(3.4)	(4.0)
$CHCl_3$	(−1.9)	(−0.7)	(0.9)	(1.0)	(−0.1)	(1.5)	(2.1)
Methylene blue							
$C_2H_4Cl_2$	(1.4)			QE	(3.4)	4.6	
$CHCl_3$	(0.0)			(3.4)	(1.8)	(3.2)	
C_6H_5Cl	(−2.4)			(2.2)	(−0.5)	(0.9)	
MIBK	(0.0)			(4.4)	(1.9)	(3.3)	

[a]Quantitative extraction.

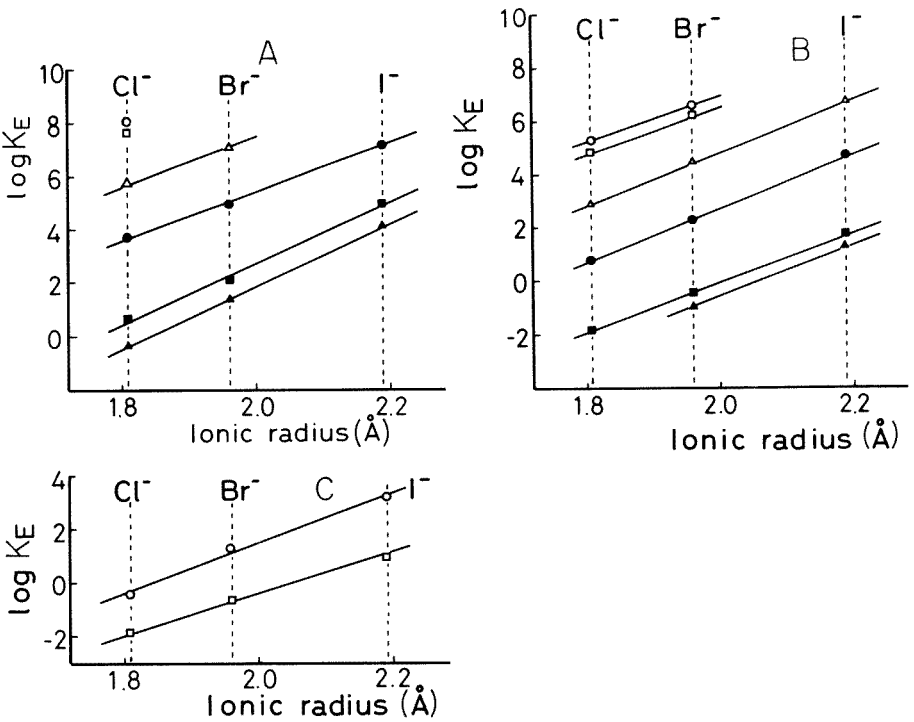


Fig. 3. The plots of log K_{ex} against the ionic radius of the halide ion for (A) ethyl violet; (B) crystal violet; (C) pararosaniline. (○) 1,2-Dichloroethane; (□) chloroform; (△) *o*-dichlorobenzene; (●) chlorobenzene; (■) benzene; (▲) toluene.

Davis et al. [7] also found that the contribution of methylene group to the partition coefficient, log F_{CH_2}, was 0.33 to 0.64. In previous work [3, 8], it was found that the addition of a methyl or methylene group to either anion or cation resulted in an increase in log K_{ex} of about 0.6. Earlier work [8] also showed that the difference in extraction constants between diethylamino and dimethylamino groups, $\Delta \log K_{ex(-N(C_2H_5)_2 - -N(CH_3)_2)}$, was about 1.0, and the difference between dimethylamino and amino groups, $\Delta \log K_{ex(-N(CH_3)_2 - -NH_2)}$ was about 1.8; thus the value of $\Delta \log K_{ex(-N(C_2H_5)_2 - -N(CH_3)_2)}$ is in agreement with the expected value, 0.6 × 2 = 1.2, while the value of $\Delta \log K_{ex(-N(CH_3)_2 - -NH_2)}$ is larger than expected. This is probably because of the abnormal hydrophilic character of the amino group. In the present work, the differences in extraction constants between crystal violet and pararosalinine, and between ethyl violet and crystal violet were 5.3 (in 1,2-dichloroethane) or 6.8 (in chloroform), and about 2.7, respectively. The differences, 2.7 and 5.3, are in good agreement with expected values, 3 (= 1.0 × 3) and 5.4 (= 1.8 × 3). The difference in chloroform, however, is much larger than expected.

The differences in log K_{ex} between extracting solvents are shown in Table 3. With all the ion-pairs examined, the order of extractability of the

TABLE 2

The differences in log K_{ex} between ethyl violet (EV) and crystal violet (CV) and between crystal violet and pararosaniline (PR)

Anion							
Average	Cl^-	Br^-	I^-	ClO_4^-	Benzene-sulfonate	Naphthalene-sulfonate	Octane-sulfonate
Δ log $K_{ex(EV-CV)}$	2.76 ± 0.26	2.55 ± 0.25	2.85 ± 0.42	2.73 ± 0.06	2.50 ± 0.16	2.83 ± 0.09	3.00 ± 0.11
Solvent							
Average	$C_2H_4Cl_2$	$CHCl_3$	$C_6H_4Cl_2$	C_6H_5Cl	C_6H_6	C_7H_8	
Δ log $K_{ex(EV-CV)}$	2.72	2.81	2.73 ± 0.12	2.73 ± 0.27	2.81 ± 0.46	2.69 ± 0.39	
Overall average Δ log $K_{ex(EV-CV)}$ = 2.74 ± 0.53							
Anion							
Average		Cl^-	Br^-	Benzene-sulfonate			
Δ log $K_{ex(CV-PR)}$	in $C_2H_4Cl_2$	5.70	5.22	5.09			
	in $CHCl_3$	6.70	6.85	6.69			
Average Δ log $K_{ex(CV-PR)}$ = 5.34 ± 0.36 ($C_2H_4Cl_2$) and 6.75 ± 0.10 ($CHCl_3$)							

solvents was 1,2-dichloroethane > chloroform > *o*-dichlorobenzene > chlorobenzene > benzene > toluene. It was also found that with ethyl violet and crystal violet the values of Δ log $K_{ex(sol.\ 1-sol.\ 2)}$ were almost equal. With pararosaniline, though few data were obtained, the values of Δ log K_{ex} for the differences between 1,2-dichloroethane and chloroform are abnormally large.

TABLE 3

The differences in log K_{ex} between extracting solvents

Solvent	E_T[a]	ϵ[b]	μ[c]	δ[d]	Δ log $K_{ex(sol.\ 1-sol.\ 2)}$		
					EV	CV	PR
$C_2H_4Cl_2$	41.9	10.36	1 86	9.8			
$CHCl_3$	39.1	4.86	1.15	9 3	0.41	0.52 ± 0.18	2.21 ± 1.4
$C_6H_4Cl_2$	—	9.93	2 27	10 0	1.87	1.58 ± 0.42	
C_6H_5Cl	37.5	5.62	1.54	9.5	2.07 ± 0.02	2.08 ± 0.12	
C_6H_6	34.5	2.28	0	9.2	2.59 ± 0.50	2.67 ± 0.35	
C_7H_8	33.9	2.38	0.31	8 9	0.76 ± 0.39	0.56 ± 0.16	

[a]Transition energy, in kcal mol^{-1} [9]. [b]Dielectric constant [10]. [c]Dipole moment [10]. [d]Solubility parameter [11].

The relations between the several parameters for the extracting solvent and the extractability of the ion-pair with ethyl violet and crystal violet are shown in Fig. 4 (A—D); the solvent parameters are listed in Table 3. The plot of the average values of $\Delta \log K_{ex(sol.-benzene)}$ against E_T parameters (Fig. 4A) shows that only chloroform behaves abnormally. The plot against dielectric constants (Fig. 4B) again illustrates the deviating behavior of chloroform. The plot against dipole moments (Fig. 4C) shows an approximately linear relationship between the benzene derivatives, but 1,2-dichloroethane and chloroform deviate far from the line. Similar deviations are obvious from the plot against the solubility parameters (Fig. 4D).

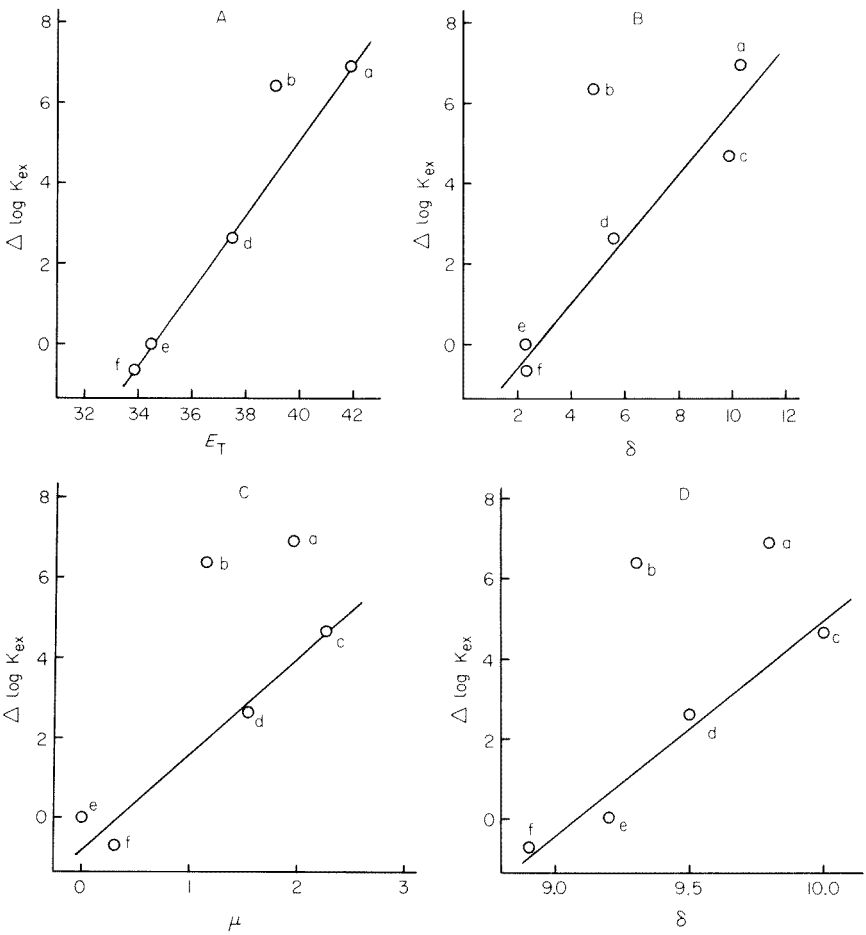


Fig. 4. Plots of $\Delta \log K_{ex(solvent-benzene)}$ against different solvent parameters: (A) transition energy E_T; (B) dielectric constant ϵ; (C) dipole moment μ; (D) solubility parameter δ. (a) 1,2-Dichloroethane; (b) chloroform; (c) *o*-dichlorobenzene; (d) chlorobenzene; (e) benzene; (f) toluene.

TABLE 4

Analytical parameters for several anions

Anion	Dye[a]	Solvent	Log K_{ex}	λ_{max} (nm)	Blank absorbance	ϵ[b] ($\times 10^4$ l mol^{-1} cm^{-1})
I^-	EV	C_6H_6	4.98	618.0	0.012	2.55
		C_6H_5Cl	7.19	602.0	0.281	8.81
ClO_4^-	EV	C_6H_6	6.39	615.5	0.012	11.40
		C_7H_8	5.77	614.2	0.011	10.50
	CV	C_6H_5Cl	6.41	596.4	0.058	11.00
	MB	$C_2H_4Cl_2$		660.1	0.069	10.90
Naphthalene-sulfonate	EV	C_6H_6	5.35	616.0	0.009	7.39
		C_6H_5Cl	7.70	600.0	0.255	10.40
	CV	C_6H_5Cl	4.39	600.9	0.048	7.60
	MB	$C_2H_4Cl_2$	4.6	660.0	0.070	7.02
Octane-sulfonate	EV	C_7H_8	5.01	615.0	0.010	3.76
		C_6H_6	5.70	616.0	0.010	7.14
		C_6H_5Cl	8.02	601.0	0.245	9.70
	CV	C_6H_5Cl	5.13	599.0	0.045	7.52
ReO_4^-	EV	C_6H_6		616.0	0.010	10.80
SCN^-	EV	C_6H_5Cl		602.0	0.281	10.40

[a]EV ethyl violet; CV crystal violet; MB methylene blue. [b] Molar absorptivity calculated from slope of calibration graph. [c]The concentration of anion was varied from 0 to 1×10^{-5} M, and the calibration graphs were all linear. Experimental conditions: 5 ml of sample solution + 0.5 ml of buffer solution (pH 5) containing 1 M Na_2SO_4 + 0.5 ml of 10^{-3} M dye solution, extracted with 5 ml of solvent.

Extraction—spectrophotometric determination of monovalent anion with cationic dyes

The cationic dyes examined here have large molar absorptivities above 10^5 l mol^{-1} cm^{-1}, and have been widely used in the spectrophotometric determination of many inorganic and organic anions.

When the aqueous and organic phases are of equal volume, and when the concentration of cationic dye in the system is about 10^{-4} M while the anion is in the range $0—10^{-5}$ M, the following conditions must be satisfied to develop a sensitive and reproducible analytical procedure. First, log K_{ex} of the ion-pair of the dye cation with the counter ion (chloride in this case) must be below 2, i.e., the absorbance of the reagent blank will be $\leqslant 0.1$. Secondly, log K_{ex} of the ion-pair containing the anion to be determined must be above 6, i.e., more than 99% of this anion will be extracted. From Table 1, the extraction systems satisfying the above two conditions are selected in Table 4. The molar absorptivities shown were obtained by calculating the slope of the linear calibration curve; also given are the absorbance of the reagent blank and the maximum absorption wavelength. Some extraction systems which do not satisfy completely the two above conditions are also shown in Table 4 for information. From Table 4, it is clear that determinations of iodide, per-

chlorate, naphthalenesulfonate and octanesulfonate ions with triphenylmethane dyes provide excellent sensitivities.

Conclusions

The results obtained for the extraction of ion-pairs formed between the dye cations and monovalent anions provide useful information for the selection of dyes and solvents for particular purposes, especially in the design of extraction-spectrophotometric methods for inorganic and organic anions. In this work, only a few organic sulfonates were examined. However, by combining earlier results on the contribution of various organic groups to extractability [3—8] with the results obtained here, further useful spectrophotometric methods would be possible. For example, the log K_{ex} value of an alkylsulfonate ion with more than nine methylene and methyl groups should exceed 6, and such alkylsulfonate ions will be extracted quantitatively into benzene with ethyl violet; analogously, the log K_{ex} value of an alkylbenzenesulfonate with more than 5 methylene and methyl groups should exceed 6, and such ions will be extracted quantitatively into benzene. In the same sense, perrhenate ion, the ionic radius of which is larger than that of perchlorate, can be extracted into benzene with ethyl violet and determined spectrophotometrically, whereas thiocyanate, with an ionic radius larger than iodide, can be extracted into chlorobenzene with ethyl violet (see Table 4).

The extraction behavior of ion-pairs of other kinds of cationic dyes will be studied in later work.

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AN EVALUATION OF THE ALKALINE *p*-HYDROXYBENZOIC ACID HYDRAZIDE PROCEDURE FOR THE DETERMINATION OF REDUCING SUGARS

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SUMMARY

The use of alkaline *p*-hydroxybenzoic acid hydrazide (HBAH) for the determination of reducing sugars in extracts of plant tissues offers an attractive alternative method to those currently employed. The procedure has good selectivity for reducing sugars, a broad temperature range for maximal colour development, tolerance for ethanol concentrations in the sample up to 40% (v/v), and interferences limited to chloroform and to high protein and calcium concentrations. Further, the range of the method is easily adjusted so that reducing sugar determinations may be performed on a macro- or micro-scale.

Various methods have been developed for the determination of reducing sugars in samples of biological material. Gas—liquid chromatography (g.l.c.) provides by far the most sensitive method for qualitative and quantitative work: concise reviews of the techniques involved have been offered by Holligan [1] and Holligan and Drew [2]. Total reducing sugar content of the sample must then be determined by summation of the individual sugars detected, however. Although providing the most information on an extract of soluble carbohydrates, the g.l.c. procedures are unsuitable when a rapid determination of reducing sugar content is desired on a crude extract.

Spectrophotometric techniques, however, provide suitable rapid determinations. As the determinations are in most cases done on fairly unpurified extracts, reaction of the chromogen with the sugars may be hindered by the presence of interfering contaminants. In addition, attention must be paid to reaction conditions (heating periods and temperatures) and to the stability of both the chromogen and coloured product.

The procedure for the determination of reducing sugars based on alkaline *p*-hydroxybenzoic acid hydrazide (HBAH) introduced by Lever [3] is a potentially valuable assay because of its apparent selectivity for reducing sugars, and the limited number of reported interfering substances. The method utilizes the instability of sugars in hot alkaline solution to produce the yellow anionic species of the *bis*(4-hydroxybenzoylhydrazones) of glyoxal and methylglyoxal in the presence of HBAH. The selectivity of the reaction for reducing sugars was demonstrated by the absence of any signifi-

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cant reaction from compounds such as pyruvate, ascorbate, or α-oxoglutarate [3]. Non-carbohydrate interference was found to be limited to high protein concentrations [3] and to calcium(II) and cadmium(II) ions [4]. The sensitivity of the method can be increased four- to five-fold, and the colorimetric reaction carried out rapidly at lower temperatures if a bismuth catalyst is used [5]. The major liability of the method is the instability of HBAH in alkaline solution. Lever [3] had reported some loss of colour after heating the sample–chromogen mixture for 20 min, but no information has been presented for the stability of the chromogen nor of the sample–chromogen mixture before and after heating. For this evaluation, it was therefore necessary to supplement the existing information of the HBAH method with the results of further investigations into the stability of the chromogen and coloured product, the extent of interference from protein and divalent cations, and the effects of various sample purification procedures on colour development.

EXPERIMENTAL

Chemicals and equipment

The *p*-hydroxybenzoic acid hydrazide (HBAH) was obtained from Aldrich Chemical Company. All other chemicals used were of analytical-reagent quality. Sodium hydroxide pellets, calcium chloride dihydrate and magnesium chloride hexahydrate were used in the preparation of the relevant solutions. Solutions of protein were prepared from crystallized bovine serum albumin (B.D.H.). Standard solutions of glucose were prepared fresh daily from a stock solution of 1 mg glucose ml^{-1}.

Absorption spectra were obtained using a Unicam SP 800A spectrophotometer (1-cm path length); absorbance values at 410 nm were obtained using a Beckman DB spectrophotometer at narrow slitwidth (1-cm path length).

Preparation of chromogenic reagents

The sodium bismuth tartrate catalyst recommended by Lever [5] is now difficult to obtain commercially, and must be prepared in the laboratory. As this preparation is somewhat time-consuming, an expedient alternative was sought to bismuth catalysis. Consequently, the effect of alkali concentration on colour development and stability was investigated. The reagents were prepared as follows.

Reagent A. Lever's procedure [3] was modified: a 5% (w/v) HBAH solution in 0.5 M HCl was adjusted to 1% HBAH with 0.5 M NaOH.

Reagent B. This is Lever's improved analytical reagent [4] containing 0.1 M HBAH (equivalent to 1.5% HBAH), 0.1 M Na_2SO_3 (anhydrous), and 0.05 M trisodium citrate, all dissolved in 0.5 M NaOH. Calcium chloride was not added as recommended [4].

Reagent C. This is the reagent for the bismuth-catalyzed reaction [5]. Stock alkali bismuth was prepared from bismuth nitrate and sodium potas-

sium tartrate [5] in 3 M NaOH to provide a bismuth concentration of 6 mmol l^{-1}. Dissolution of bismuth was facilitated by using a stronger alkali solution than recommended. Alternatively, the stock reagent may be more easily prepared according to the prescribed mole ratios if the components are initially dissolved in a minimum volume of water (Lever, 1977, personal communication). To prepare the chromogen, HBAH was added to 1 part of alkaline bismuth stock solution and diluted with 3 parts of distilled water. Addition of 2 ml of chromogen to 1 ml of aqueous sample gave final concentrations of 1 mmol l^{-1} bismuth and 0.05 M HBAH in 0.5 M NaOH.

Reagent D. A 4% HBAH solution in 0.5 M HCl was adjusted to 1% HBAH with 0.5 M NaOH.

Reagents E—K. A 2% HBAH solution in 0.5 M HCl was mixed with an equal volume of sodium hydroxide solution with a concentration according to the schedule: E, 1 M NaOH; F, 2 M NaOH: G, 3 M NaOH; H, 4 M NaOH; I, 5 M NaOH; J, 6 M NaOH; K, 8 M NaOH.

General test procedure

To 1 ml of sample dispensed from a 1-ml fast-flow (Westergren) pipette, 2 ml of the reagent was added from a burette. The solutions were mixed on a vortex mixer, heated for 5 min in a boiling water bath, cooled from 15—20 s in an ice-water bath, and transferred to disposable cuvettes: absorbance values were read at 410 nm. This basic procedure was modified as necessary in the course of the investigations.

Recommended procedure for the determination of reducing sugars

For samples extracted from plant materials and containing reducing sugars at concentrations up to 16 μg ml^{-1} of solution, the following procedure is recommended. The best chromogenic reagent is a 1% (w/v) HBAH solution prepared by mixing equal volumes of 2% HBAH in 0.5 M HCl and 3 M NaOH; this should be stored refrigerated.

In the procedure, 2 ml of the reagent is added to 1 ml of sample and mixed thoroughly. The solution is heated in a boiling water bath for 5 min and treated further as described above.

A linear regression analysis on a typical standard graph for glucose concentrations in the range 1—16 μg ml^{-1} gave the equation $y = 0.0285x + 0.0037$, with a coefficient of determination (r^2) of 0.9992.

RESULTS AND DISCUSSION

The effects of alkali concentration on colour development and stability are summarized in Table 1. Absorbance was read after 5, 10, 30, and 60 min had elapsed from the time at which the samples were removed from the boiling water bath. The cuvettes containing the samples were left on the open bench between readings, either under fluorescent light or in daylight, but never in direct sunlight: ambient temperatures varied from 18 to 23°C.

TABLE 1

Effect of sodium hydroxide concentration on colour intensity and stability of the coloured product

Colour reagent	Glucose (μg ml^{-1})	Absorbance (410 nm)[a]			
		Time elapsed from removal from water bath (min)			
		5	10	30	60
A	0	0.052 ± 0.007	0.053	0.058	0.062
	2	0.142 ± 0.007	0.145	0.152	0.156
	8	0.404 ± 0.009	0.408	0.423	0.433
B	0	0.089 ± 0.007	0.093	0.107	0.122
	2	0.172 ± 0.009	0.178	0.195	0.211
	8	0.427 ± 0.009	0.433	0.453	0.465
C	0	0.107 ± 0.016	0.108	0.112	0.113
	2	0.266 ± 0.010	0.267	0.267	0.261
	8	0.710 ± 0.013	0.710	0.702	0.692
D	0	0.065 ± 0.009	0.066	0.073	0.079
	2	0.152 ± 0.013	0.155	0.162	0.169
	8	0.392 ± 0.017	0.395	0.409	0.423
E	0	0.057 ± 0.009	0.059	0.063	0.068
	2	0.134 ± 0.009	0.136	0.142	0.148
	8	0.354 ± 0.015	0.359	0.375	0.390
F	0	0.070 ± 0.010	0.073	0.077	0.080
	2	0.161 ± 0.013	0.163	0.169	0.171
	8	0.383 ± 0.015	0.387	0.392	0.393
G	0	0.061 ± 0.010	0.062	0.064	0.067
	2	0.129 ± 0.012	0.130	0.134	0.135
	8	0.274 ± 0.013	0.276	0.277	0.276
H	0	0.067 ± 0.009	0.069	0.072	0.074
	2	0.129 ± 0.012	0.131	0.134	0.135
	8	0.274 ± 0.013	0.276	0.277	0.276
I	0	0.060 ± 0.009	0.061	0.062	0.066
	2	0.131 ± 0.011	0.133	0.136	0.138
	8	0.314 ± 0.013	0.315	0.317	0.318
J	0	0.067 ± 0.010	0.069	0.072	0.075
	2	0.112 ± 0.012	0.114	0.117	0.118
	8	0.216 ± 0.014	0.217	0.218	0.217
K	0	0.065 ± 0.010			
	2	0.090 ± 0.015			
	8	0.154 ± 0.009			

[a]Absorbance read against distilled water blanks: values listed are the means of 16—30 determinations ± standard deviations.

Deviations from the initial values greater than the calculated standard deviations were taken as an indication of instability of the coloured product.

The stability of the coloured product apparently improves with increasing alkali concentration, but the colour intensity was attenuated at concentrations of 6 M NaOH and greater. Colour reagents F—I yielded reaction products with glucose that had absorbance values determined at 60 min within the standard deviations of values determined at 5 min without appreciable attenuation of colour, and were therefore considered to yield stable products. The enhanced absorbance values for the coloured product formed with the highest glucose standard with reagent I is a curious anomaly without an immediately obvious explanation. Reagent C (containing the bismuth catalyst) also produced stable coloured products. The laboratory preparation of the catalyst involved heating and stirring bismuth nitrate and sodium potassium tartrate in 3 M NaOH for 3 h; after this period, the amount of undissolved bismuth was assayed. Bismuth concentrations in the catalytic reagent were then calculated to be between 0.9 and 1.0 mmol l^{-1}. On the basis of the kinetic data presented by Lever ([5], his Fig. 2), deviations in bismuth concentrations of this order are unlikely to introduce any significant variability between determinations.

Increasing the concentration of alkali in the colour reagent altered the absorption curves as illustrated in Fig. 1. Glucose standards read against reagent blanks continued to exhibit an absorption maximum at 410 nm, however. Of the reagents designated as yielding stable coloured products with glucose, reagent G, prepared from equal volumes of 3 M NaOH and 2% HBAH in 0.5 M HCl, was selected for further study as it represented the reagent with the lowest concentration of alkali whilst providing good stability of the coloured product.

Reagent stability

Davies et al. [6] reported the crystallization of the 5% HBAH solutions in 0.5 M HCl suggested by Lever [3], and recommended 2% solutions instead. Solutions of 2% HBAH were prepared and allowed to age for varying periods

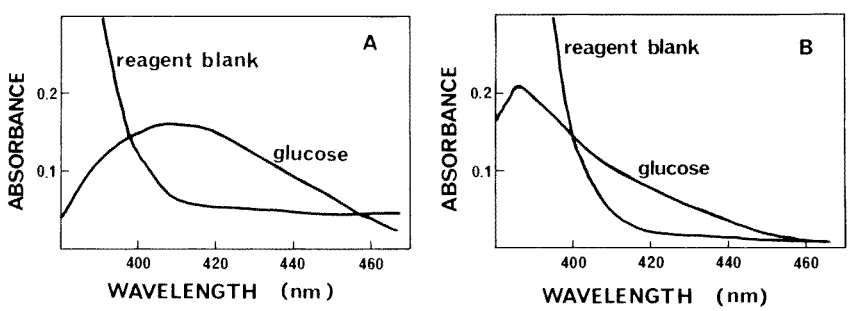

Fig. 1. Effect of sodium hydroxide concentration on the absorption spectra of the reagent blank and coloured product with glucose (4 μg glucose ml^{-1}). (A) spectra obtained with colour reagent E; (B) spectra obtained with colour reagent G.

of time before mixing with 3 M NaOH. These solutions were stable: a 212-day old solution kept refrigerated and away from light gave absorbance values for standard glucose solutions identical with those of freshly-prepared HBAH solution.

To determine the stability of the alkaline reagent, 2% solutions of HBAH in 0.5 M HCl were mixed with equal volumes of 3 M NaOH and allowed to stand on the open bench in clear or tinted bottles: other samples were placed in dark cupboards or refrigerated. After definite times had elapsed, aliquots of the alkaline reagents were dispensed and reacted with glucose solutions: aliquots were also taken at hourly intervals up to 4 h from preparation. Table 2 shows the effects of ageing of the colour reagent kept in a clear bottle on the open bench: after more than 30 min, absorbance values deviated from the initial values by more than the calculated standard deviations. This was found to be the case for all methods of storage except refrigeration, which prolonged the useful life of the reagent to 1 h. Although the variability in absorbance values as the reagent ages suggests that it is best used within 10 min of preparation, it does have a usable life of 30 min. This time constraint becomes important only when large numbers of determinations are done manually.

When the freshly-prepared alkaline reagent was added to sample solutions and the mixtures were left to stand on the open bench before the recommended heating, the time elapsed became more important. These mixtures began to deteriorate after only 10 min on the bench; the loss in final absorbance for solutions left for 15—20 min before heating was about 10% compared to solutions heated immediately after mixing.

Dependence of colour development on heating temperature

Maximal conversion of reducing sugars to coloured product occurs throughout the temperature range 85—100°C (Fig. 2). When bismuth is used

TABLE 2

Stability of freshly-prepared alkaline reagent (reagent G)

Sample		Absorbance (410 nm)[a]				
		Time elapsed before use (min)				
		0	10	15	20	30
Reagent blank	$\bar{x}$	0.062	0.064	0.051	0.051	0.061
	σ	0.011	0.007	0.007	0.009	0.012
	n	22	23	23	23	23
Glucose (4 μg ml^{-1})	$\bar{x}$	0.180	0.179	0.169	0.166	0.169
	σ	0.015	0.010	0.014	0.013	0.015
	n	23	22	23	24	23

[a]Absorbance read against distilled water blanks: $\bar{x}$ is the mean of n determinations with standard deviation σ.

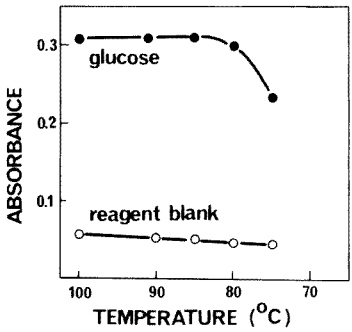

Fig. 2. Effect of temperature on maximum colour development: (○) reagent blank; (●) glucose stock (8 μg ml^{-1}). Absorbance read at 410 nm against distilled water.

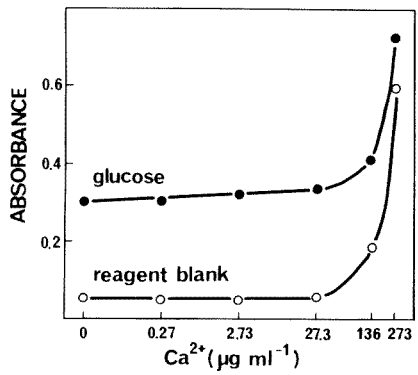

Fig. 3. Effect of calcium ion concentration on colour development: (○) reagent blank; (●) glucose stock (8 μg ml^{-1}). Absorbance read at 410 nm against distilled water.

as a catalyst the reaction may be carried out at 70°C [5]. In this respect the HBAH method is superior to the standard Nelson-Somogyi method, where reduction of the copper reagent is highly sensitive to small deviations in heating temperature [7]. Considering the time spent in preparing the bismuth catalyst and the broad temperature range for colour development when the chromogen is prepared with 3 M NaOH, this lowering of the heating temperature for maximal colour development by 15°C is of questionable advantage.

Effects of divalent cations on colour development

Calcium interference [4] is an important consideration when reducing sugars are determined in extracts of plant material. Solutions of reagent blanks and glucose standards were prepared containing 0, 0.27, 2.73, 27.3, 136, and 273 μg Ca^{2+} ml^{-1}. Reducing sugar determinations were affected by Ca^{2+} concentrations above 27.3 μg ml^{-1} (Fig. 3). The effect of Ca^{2+} on the absorbance of the coloured product may be compensated by the addition of a small quantity of calcium to the HBAH reagent [3], or of trisodium citrate to inhibit the chelation of calcium with the *bis*(4-hydroxybenzoylhydrazones) of glyoxal and methyl glyoxal [8]. If calcium concentrations in the sample extract are high, interference in the determination may be removed by the addition of oxalic acid to form an insoluble calcium salt, so that the calcium-free supernatant solution can be used.

Interference by magnesium ions occurred only at concentrations approaching the limits of solubility of $Mg(OH)_2$, where it was characterized by an increase in the turbidity of the solution.

Effects of bovine serum albumin with and without added calcium

Reagent blanks and glucose standards were prepared containing 0, 0.01, 0.10, 1.00, and 10.00 mg BSA ml^{-1} without Ca^{2+}, and with either 27.3 or 136 μg Ca^{2+} ml^{-1} added. The BSA had little effect on the absorbance of either the reagent blank or the glucose standard up to a concentration of 0.10 mg BSA ml^{-1} (Fig. 4). In this respect, the sensitivity of the HBAH method to protein interference parallels that of the alkaline copper method proposed by Dygert et al. [9]. The coloured product of glucose was much more sensitive to the combination of BSA and Ca^{2+} than the reagent blank, so that samples suspected of containing high protein concentrations are best deproteinized.

Effects of sample preparation on determinations of reducing sugar

Ethanolic extracts of plant material may have been subjected to various purification treatments prior to the determination of reducing sugar concentrations. These treatments may have included extraction with chloroform or petroleum ether to remove lipophilic substances, deproteinization, or ion-exchange chromatography.

To determine the effects of such procedures, a stock solution of 6 μg glucose ml^{-1} was divided into three aliquots: one was left untreated, the second extracted with chloroform, and the third extracted with petroleum ether (b.p. 40—60°C). These three aliquots were further subdivided into four parts which were, respectively, untreated, deproteinized, deionized, and deproteinized and deionized. Deproteinization was done by the zinc sulphate precipitation method of Somogyi [10, 11], and deionization by shaking 10 ml of sample first with 4 g of Dowex 50W-X8 (H^+) resin, and then with 4 g of Dowex 21K (OH^-) resin, allowing 5 min of agitation with each.

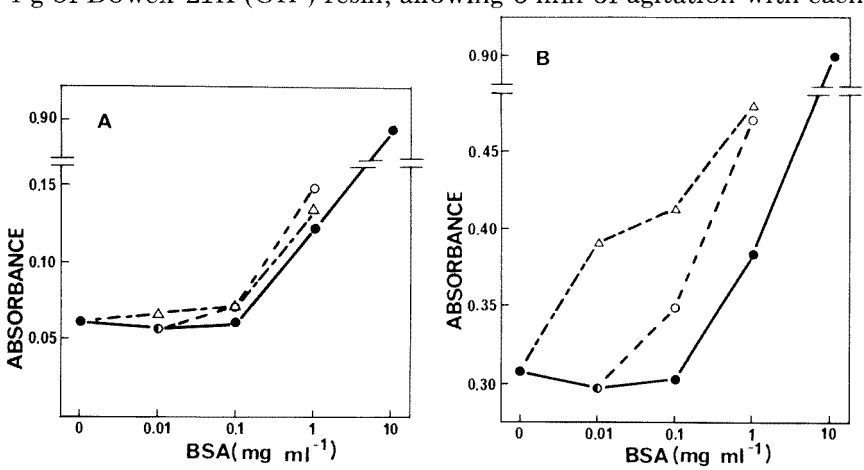

Fig. 4. Effect of BSA with or without added calcium on colour development. (A) Effects on reagent blank; (B) effects on glucose stock (8 μg ml^{-1}): (•) without Ca^{2+}; (○) with 27.3 μg Ca^{2+} ml^{-1}; (△) with 136 μg Ca^{2+} ml^{-1}. Absorbance read at 410 nm against distilled water.

Two samples (0.02 g) of freeze-dried and pulverized leaf tissue from soya bean (*Glycine max* [L.] Merrill var. "Wayne") were extracted three times with 10-ml portions of 80% ethanol. The extracts were combined and diluted to a final volume of 200 ml with distilled water, giving a final ethanol concentration of about 10%. A separate series of investigations showed ethanol concentrations only in excess of 40% (v/v) to interfere with the reducing sugar determination. As plant extracts were normally treated with ion-exchange resins, it was not considered necessary to remove any potential calcium interference by the chemical means mentioned earlier. The results of these tests are shown in Table 3, where both the untreated stock glucose solution and the untreated plant extract are assigned values of 100. Chloroform was found to interfere severely with the determination whereas petroleum ether offered little interference. As Table 3 suggests, laborious deproteinization and deionization procedures need not necessarily be done on extracts of plant tissues should a rapid estimate of reducing sugar concentration be desired.

Comparison of reducing sugar methods

A comparison of selected methods for the determination of reducing sugars is presented in summarized form in Table 4. Both the sensitivity and number of interferences in the HBAH method compare favourably with other methods currently in use: the limited stability of the alkaline colour reagent (30 min) and variations in absorbance values likely to result if the colour reagent—sample mixture age more than 10 min before heating are cause for concern only in manual determinations. Several authors have demonstrated that the HBAH method is easily adapted to semi-automatic techniques of sugar analysis [6, 8, 12—14], thereby obviating this liability of instability.

TABLE 3

Effects of various purification treatments on colour development

Sample	Relative response[a]			
	Untreated	Deproteinized	Deionized	Deproteinized and deionized
Glucose stock soln. (6 μg ml^{-1})	100	103	82	91
Glucose stock, chloroform-extracted	569	200	199	169
Glucose stock, pet. ether-extracted	106	—	—	—
80% Ethanol-extracted sample of leaf tissue	100	97	73	89

[a]Glucose stock solution (6 μg ml^{-1}) and untreated plant extract assigned a value of 100 for comparison with other treatments.

TABLE 4

Comparison of reducing sugar methods

Method [ref.]	Conc. of glucose ($\mu g\ ml^{-1}$)	Sample size (ml)	Effective sugar conc. range (μg)	Interferences
Alkaline copper Nelson-Somogyi [7]	0—100	3.00	0—300	125 mM citrate [15]; tartrate [9, 16]; glycine, cystine, glutamic and aspartic acids [16]; ammonium salts [17]; reducing contaminants and reoxidation by air [18]
Folin-Wu [22]	6—44	10.00	60—440	Reducing contaminants and reoxidation by air
Dygert et al. [9]	5—125	1.00	5—125	0.2 M citrate, phosphate, and Tris, 1 M acetate and glycine, BSA > 0.1 mg [9]
o-Toluidine Dubowski [20]	250—3000	0.02	5—60	Protein, dextrans [20]
Hexacyanoferrate(III) Fuller [21][a]	2—18	1.00	2—18	Amino acids, aldehydes, reducing contaminants, reoxidation by air [18]
HBAH Lever [3]	1000—5000	0.01	10—50	BSA, calcium [3]
	1—10	0.50	0.5—5	$Ca^{2+} > 27.3\ \mu g\ ml^{-1}$, BSA > 0.1
	1—16	1.00	1—16	$mg\ ml^{-1}$, chloroform

[a]Manual adaptation of method cited in [21].

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DETERMINATION OF VANADIUM, MOLYBDENUM AND TUNGSTEN IN CHLORALKALI ELECTROLYSIS BRINES BY X-RAY FLUORESCENCE SPECTROMETRY

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SUMMARY

The levels of vanadium, molybdenum and tungsten are critical in chloralkali electrolysis brines because these elements catalyze the hydrogen-producing side-reaction. Preconcentration by chelation with 8-quinolinol and subsequent adsorption on activated carbon, can be combined with energy- or wavelength-dispersive x-ray fluorescence. Under optimized conditions, the detection limits are in the $\mu g\ l^{-1}$ range, and below the critical levels. Up to 100 $\mu g\ l^{-1}$, reliable results are obtained for V, Mo and W. The coefficient of variation is typically 15% in the 25—100 $\mu g\ l^{-1}$ range.

Electrolysis of saturated sodium chloride brines on mercury electrodes is being used on a large scale for chlorine production in industry. Some impurities in the brines can affect the electrolysis to the extent that hydrogen is released at the cathodes, implying a significant explosion risk. Vanadium, molybdenum and tungsten are normally the most critical impurities: they are reduced to lower valency states, giving oxides that are insoluble in mercury, and catalyze the hydrogen-producing side-reaction from concentration levels of or above 30 μg V l^{-1}, 10 μg Mo l^{-1} and 100 μg W l^{-1} [1]. The chromium impurity level is also very critical. Other elements (e.g., Co, Ge, Ti, Zr) can normally be tolerated at higher levels [1].

To examine whether or not a chloralkali brine can be further recycled safely, micro-electrolysis as described by Hauck and Durr [1] is often carried out and the hydrogen bubble formation at the mercury cathode is checked visually. In view of the $\mu g\ l^{-1}$ concentrations of metal impurities to be measured in the concentrated (350 g l^{-1}) brines, quantitative analytical procedures are seldomly applied. One elaborate procedure involves successive extractions with cupferron, pyrrolidine dithiocarbamate and 8-quinolinol (oxine) and 8-hydroxyquinaldine in combination with d.c.-arc emission spectrography [1].

Leyden et al. [2] have described a procedure based on x-ray fluorescence (x.r.f.), to determine molybdenum and tungsten at 20—30 $\mu g\ l^{-1}$ levels in

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aqueous concentrated sodium chloride solutions after extraction on a column with controlled-pore glass beads treated with N-β-aminoethyl-γ-aminopropylsilane. Methods based on chelation by oxine and adsorption onto activated carbon have been proposed for the determination of dissolved trace ions in water by x.r.f. [3–5]. This general method is insensitive to high sodium chloride concentrations, and was expected to be applicable for highly sensitive determinations of vanadium, molybdenum and tungsten. In the present paper, the potential of the oxine chelation/activated carbon adsorption procedure combined with x.r.f. in the analysis of chloralkali brines for these critical impurities is described.

EXPERIMENTAL

Apparatus

The energy-dispersive x-ray fluorescence instrumentation included a Siemens Kristalloflex-2 high-voltage power supply and an x-ray tube with a tungsten anode, several secondary fluorescers and filters (e.g., Ti, Ge, Mo, Ag, Sn and Nd), a 16-position sample holder in vacuum, a Si(Li) detector with related electronics, a 4096-channel analyzer and a magnetic tape recorder for off-line spectrum evaluation by a PDP 11/45 computer. The operating conditions were always 40 mA/40 kV. A tin secondary fluorescer was usually preferred.

The wavelength-dispersive x-ray fluorescence system consisted of a Siemens SRS-unit with a chromium tube. For the V—K_α measurement, a PET crystal was used, and a gas-flow proportional counter placed at 33.387°. The Mo—K_α and W—L_α x-rays, diffracted by a LiF crystal, were measured by a scintillation detector at 20.359° and 43.026°, respectively. The backgrounds were taken at +0.5° and −0.5°. All samples were measured under vacuum at 40 mA/50 kV. A series of thin-film Micromatter standards was used for calibration in both x.r.f. techniques.

Radiotracer experiments were carried out by means of a Ge(Li) detector coupled to a multichannel analyzer and tape recorder. The neutron activation analyses were based on irradiations at 10^{12} n s^{-1} cm^{-2} in the Thetis reactor of Ghent State University.

The filtration unit was a polycarbonate magnetic filter funnel (Gelman 4200) with 9.6-cm^2 active area.

Reagents and samples

The activated carbon (Baker Analyzed) was previously treated with hydrochloric and hydrofluoric acids to reduce its metal contamination [6]. Apart from 8-quinolinol (oxine; Union Chimique Belge), all reagents were of analytical grade, and the hydrochloric acid and sodium hydroxide were of ultrapure quality. Standard solutions of V, Mo and W were made up from Merck Titrisol standards. The radioactive tracers used were ^{48}V ($t_{1/2}$ = 16.0 d; E_γ = 983 keV; as carrier-free VOCl solution) and ^{185}W ($t_{1/2}$ = 75 d; E_γ = 125 keV; as a Na_2WO_4 solution), both from Amersham.

The brines used in all experiments were taken from an industrial chlor-alkali electrolysis plant at different times.

Procedure

Based on earlier experience with the oxine/activated carbon preconcentration procedure [3—5] and on the further investigation outlined below, the following optimal procedure is recommended.

Heat a 200-ml aliquot of the brine (of pH 5—6) to 60°C, add 30 mg of oxine and allow to cool for 2 h. If a precipitate forms, filter off on a 47-mm diameter Nuclepore 0.4-μm pore-size membrane. Add 120 mg of activated carbon to the solution, stir for 1 h and filter off again on a membrane filter.

Suspend the dried activated carbon-loaded membrane (or both membranes separately) on a teflon ring and measure by energy-dispersive x.r.f. for 2000 s. The resulting spectra are analyzed by a non-linear least-squares fitting computer routine [7].

Alternatively, measure by wavelength-dispersive x.r.f. for 60 s. In this case, a simple background subtraction is applied to the integral peak window count rate.

For an activated carbon load of 120 mg per 9.6-cm^2 filter area, the x-ray absorption correction factor, as determined via transmission experiments, amounts to approximately 1.47 for V—K_α and 1.14 for W—L_α, but it is negligible for the Mo—K_α rays.

RESULTS AND DISCUSSION

Influence of experimental conditions on the preconcentration yield

According to the literature [8], V, Mo and W are quantitatively precipitated by oxine in the pH ranges 2—5, 3—7 and 5—6.5, respectively. Stary [9] reported quantitative solvent extractions of vanadium and molybdenum oxinates in the ranges 2—6 and 1—6, respectively. Since oxine is adsorbed efficiently onto activated carbon between pH 2 and 10 [3], the optimal pH level for simultaneous V, Mo and W preconcentrations as oxinates was expected to be near or below 6, without being very critical. Experiments on brines doped with 100 μg l^{-1} concentrations of V, Mo and W showed no significant variation of the collection efficiencies between pH 3 and 7. Further work was therefore done at pH 5—6, the normal pH level of the brines.

Excess of oxine does not hamper the recovery of the transition metals tested earlier [3] but if one wants to restrict the collection of alkaline earth ions in the precipitate (in order to use minimal amounts of activated carbon and obtain optimal detection limits in x.r.f.), it is best to ensure that only a 5-ppm excess of free oxine is left in the solution after the chelation step. Taking into account the normal composition of the chloralkali cell brines under study (Ca $\leqslant$20 ppm; Mg $\leqslant$5 ppm; Fe $\leqslant$1 ppm; Hg $\leqslant$10 ppm, Cu, Ni, Co, and Cr $\leqslant$0.2 ppm), and the stability products of the metal oxinates (cf. [1]), it can be calculated that an oxine concentration of 30 mg per 200 ml

is preferable. Experiments with brines doped with as much as 500 $\mu g\ l^{-1}$ concentrations of V, Mo and W indicated a constant recovery for added oxine amounts from 20 to 40 mg, except for tungsten at 40 mg of oxine.

The quantity of activated carbon should be sufficient to adsorb all transition metal oxinates, with its capacity of typically 0.5 mmol g^{-1}, but should not be excessively large, in order to achieve satisfactory detection limits in x.r.f. It was found that constant recoveries were obtained for 100 $\mu g\ l^{-1}$ spikes of V, Mo and W only when the amount of activated carbon exceeded 80 mg per 200 ml of sample, while the oxine color in the solution disappeared completely. A 120-mg quantity was chosen as a safe compromise. Although equilibrium is reached within a few minutes for the chelation of most metals with oxine, it can be significantly slower for molybdenum and tungsten [9]; therefore provision was made for a total equilibration time of 3 h.

Simultaneous filtration of the precipitated metal oxinates and the activated carbon phase would shorten the manipulation and counting times, but for all three elements, the recoveries were found to be consistently lower by ca. 25%; therefore this approach was abandoned.

Detection limits

The detection limits, defined as three times the square root of the blank count rate (i.e., assuming only counting statistics variations on the scatter background and blank) are listed in Table 1 for energy-dispersive and wavelength-dispersive x.r.f. However, the blank levels measured by the wavelength-dispersive method corresponded to $1.5 \pm 1.6\ \mu g\ V\ l^{-1}$, $12 \pm 11\ \mu g\ Mo\ l^{-1}$ and $37 \pm 16\ \mu g\ W\ l^{-1}$. For molybdenum and tungsten these indicated standard deviations per measurement are clearly larger than those expected from counting statistics. In practice, the detection limits will be somewhat less favourable than indicated in Table 1, mainly because of inaccuracies in the background subtraction in wavelength-dispersive x.r.f. Yet, it seems that the proposed method is sensitive enough to determine vanadium and tungsten well below the critical levels that can be tolerated for safe recycling of the chloralkali brines. Tin excitation, although less sensitive for vanadium and tungsten, was used in the energy-dispersive method for simultaneous detection of the three elements.

TABLE 1

Detection limits (given as $\mu g\ l^{-1}$) for energy-dispersive (2000-s counting) and wavelength-dispersive (100-s counting, Cr-tube) x-ray fluorescence

Element	Energy-dispersive with secondary target of			Wavelength-dispersive
	Ge	Mo	Sn	
V	0.9	3.3	12	5
Mo	—	—	2.0	4.2
W	—	4.2	11	14

TABLE 2

Results obtained by energy-dispersive and wavelength-dispersive x-ray fluorescence, and by neutron activation analysis, on synthetic saturated NaCl solutions (single determinations) and chloralkali brines (4—6 determinations)

Concentrations added to ($\mu g\ l^{-1}$)		V found ($\mu g\ l^{-1}$)			Mo found ($\mu g\ l^{-1}$)		W found ($\mu g\ l^{-1}$)	
Saturated NaCl solution	Chloralkali brine	A[a]	B[b]	C[c]	A	B	A	B
0		<12	<5	<0.1	<2	<4	<11	<14
4		<12	<5	2.7	3	5	<11	<14
10		16	10	13	13	13	12	<14
20		24	17	17	19	19	27	17
	25	21 ± 3[d]	25 ± 7		18 ± 3	16 ± 1	27 ± 4	27 ± 7
50		50	59	43	48	50	54	37
	50	50 ± 10	49 ± 9		41 ± 12	41 ± 5	49 ± 13	35 ± 7
100		116	—	90	81	—	65	—
	125	108 ± 25	124 ± 14		83 ± 7	75 ± 8	94 ± 6	90 ± 10

[a]A, energy-dispersive x.r.f. [b]B, wavelength-dispersive x.r.f. [c]Neutron activation. [d]Standard deviation per measurement.

Accuracy and precision

Table 2 lists the results obtained for sodium chloride solutions and synthetic brines spiked with V, Mo and W. The agreement between the energy-dispersive and wavelength-dispersive x.r.f. methods is satisfactory in most cases. The agreement with the vanadium results obtained by neutron activation analysis (using ^{52}V, $t_{1/2}$ = 3.75 min, E_γ = 1443 keV) is reasonable, in view of the precisions involved. Radioactive tracer experiments, with ^{48}V tracer as VOCl and 25 or 125 $\mu g\ l^{-1}$ vanadium carrier, indicated that only 3% or 0.6% of the activity, respectively, was not removed from the solution by the preconcentration step; this again indicates practically quantitative recoveries. At the high concentration end, the recoveries of molybdenum and tungsten are significantly depressed, and this could be linked to the polymerization of molybdate and tungstate. Stary [9] has attributed the erratic extraction behavior of tungsten oxinate to insoluble polytungstic acid formation. Also, tracer experiments with ^{185}W (not carrier-free, corresponding to 500 $\mu g\ W\ l^{-1}$) showed recoveries around 30% only.

The scatter of the multiple determinations indicated in Table 2, together with pooled results of nearly 20 other experiments carried out under optimal conditions on brines spiked with 50—100 $\mu g\ l^{-1}$ V, Mo and W, yielded a relative standard deviation per measurement of 14—16% for vanadium and molybdenum as well as tungsten. Neither of the x.r.f. modes proved clearly superior. In view of the relative constancy of the standard deviations, neither spectral interferences from variable other impurities (e.g., from the Zn—K lines on the W—L lines) nor possible uncontrolled variations in the chemical speciation of the elements, are believed to contribute significantly

to the overall uncertainty. Rather inhomogeneities of the loading of the activated carbon filter and uncertainties in the background subtractions are undoubtedly the most significant sources of variability.

Applicability for control of chloralkali brines

Although the reliability of the determinations of molybdenum and tungsten becomes questionable above 100 $\mu g\ l^{-1}$, the procedure appears applicable for checking chloralkali brines because the critical levels of V, Mo and W are in the 10–100 $\mu g\ l^{-1}$ range.

The proposed method with wavelength-dispersive x.r.f. was applied to various industrial chloralkali brines that had shown negative micro-analysis tests [1]; the concentrations found were $<5\ \mu g\ V\ l^{-1}$, $<25\ \mu g\ Mo\ l^{-1}$ and $20–60\ \mu g\ W\ l^{-1}$.

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THE DETERMINATION OF ZIRCONIUM IN ANIMAL TISSUES BY NEUTRON ACTIVATION AND γ-SPECTROMETRY

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SUMMARY

A neutron activation method has been developed for determining microgram and submicrogram quantities of zirconium in animal tissue. The procedure uses phosphate to aid in the separation of zirconium from interfering radio-isotopes. Tissues are dried, sealed in quartz vials, irradiated at a high flux, and wet-ashed; the zirconium is precipitated as $ZrO(H_2PO_4)$, redissolved, and precipitated as $BaZrF_6$. This procedure effectively removed interfering radioactive elements. Chemical oxidation and the simple separation procedure were checked by radiotracer experiments, and by processing tissues to which known quantities of zirconium had been added. The detection limit of the method for a 100-h irradiation at 1×10^{14} n cm^{-2} s^{-1} followed by radiochemical processing is 10 ng, based on 2 σ counting statistics. The error is estimated to be ±5—10%.

A method was needed to measure the absorption and distribution of microgram and submicrogram quantities of zirconium in the tissues of animals exposed to zirconium-containing compounds. Neutron activation was chosen as the analytical technique because it is extremely sensitive and specific. Other methods considered for zirconium determination included atomic absorption, x-ray fluorescence, and colorimetry. These methods were not used because they are susceptible to interferences or they are not sensitive enough for determining low levels of zirconium in very small tissues. Velandia and Perkons [1] used neutron activation to detect trace quantities of zirconium in rabbit livers, but were unable to quantify their results. Budinger et al. [2] detected zirconium by neutron activation in human tissue but reported no quantitative data. Some neutron activation procedures for zirconium are available for geological samples [3, 4], but they were not suited for processing rapidly the many samples generated in animal experiments.

The nuclear reactions [5] suitable for the determination of zirconium are ^{96}Zn (n,γ) ^{97}Zr ($t_{1/2}$ = 17 h) and ^{94}Zr (n,γ) ^{95}Zr ($t_{1/2}$ = 65 d). The best isotope for the present use was ^{95}Zr. Its long half-life allowed ample time for shipping the samples from the distant nuclear reactor site to the laboratory and for the decay of intense short-lived radioisotopes.

EXPERIMENTAL

Preparation of samples and standards

Procedures were developed by using bovine tissues and verified with tissues from albino rats, guinea pigs, and New Zealand rabbits. Tissues were freeze-dried and then homogenized; up to 200 mg of dried soft tissue or 100 mg of bone was sealed in Suprasil quartz vials for neutron irradiation. Standards were prepared by spotting 10 μl (10 μg Zr) and 50 μl (50 μg Zr) of a Harleco zirconium standard on ashless filter paper. The filters were carefully dried, and placed in quartz vials which were then heat-sealed. One 10-μg Zr standard and one 50-μg Zr standard were irradiated with each set of 20 samples. Small iron wires were taped to each of the vials as flux monitors.

Irradiation

The vials that contained 50—200 mg of dried tissue were shipped to Georgia Institute of Technology Research Reactor for neutron irradiation. The samples and standards were irradiated for 40 h at a flux of 1.3×10^{13} n cm^{-2} s^{-1}. Vials that contained 1—50 mg of tissue were sent to the University of Missouri Research Reactor for irradiation at a higher flux, 1×10^{14} n cm^{-2} s^{-1}, for 100 h. Five days after the end of neutron irradiation, the samples were returned to this laboratory for radiochemical processing and γ-ray spectroscopy.

Sample oxidation and radiochemical separation

When the samples were returned to this laboratory, the flux monitors were removed, and the vials were washed off with dilute hydrofluoric acid, rinsed and dried. The quartz irradiation vials were cooled in liquid nitrogen and fractured in a small sealed stainless steel box to prevent sample loss resulting from rapid pressure release upon opening. The vials were fractured by a screw-operated wedge manually operated from outside the box. Then the box was opened, large quartz fragments were removed, and the contents were transferred to a Pyrex tube for wet-ashing.

A 1-ml portion of solution containing 10 μg Eu, 10 μg Sb, 200 μg Zn, 1 mg Fe, and 7 mg Sc as chlorides was added to the wet-ashing tubes. Carrier solution (1 ml) containing 7.5 mg Zr in the same chemical form as the dose to the animals was also added. Fuming nitric acid (10 ml) was added to the tube along with boiling chips. Air-cooled condensers were used to prevent the acid from evaporating during sample oxidation. The oxidation tubes were then placed in an aluminum block for heating. The aluminum contained 50 holes to accommodate the wet-ashing tubes and was placed on a Precision Scientific Thermo-plate. A thermocouple in the aluminum block was connected to a temperature programmer that controlled the block temperature by regulating the power supplied to the Thermo-plate. The programmer increased the block temperature to 50°C at a rate of 2.1°C

min^{-1}, held it constant for 1.0 h, and then increased it to 130°C at a rate of 2.1°C min^{-1}. After 14 h the block was allowed to cool.

After wet-ashing, the reflux condensers were rinsed with 10—15 ml of distilled water, and the contents of the tubes were transferred to Nalgene centrifuge tubes. The wet-ashing tubes were rinsed twice with 5 ml of water, and the rinsings were added to the centrifuge tubes. Two 1-ml portions of sodium dihydrogen phosphate solution (10.0 mg P ml^{-1}) were added to the centrifuge tube and thoroughly mixed. The mixture was allowed to stand for 5 min to ensure complete precipitation, then it was centrifuged and the supernatant liquid was discarded. The precipitate was washed with 10 ml of distilled water and centrifuged, and the supernatant liquid was discarded.

Thirteen drops (ca. 0.6 ml) of 50% hydrofluoric acid and 4 ml of water were added to the centrifuge tubes and mixed thoroughly with the zirconium phosphate with a plastic stirring rod; 4 ml of water and 1 ml of concentrated hydrochloric acid were added. The zirconium phosphate dissolved on stirring. The solution was transferred to counting tubes and the zirconium was reprecipitated as $BaZrF_6$ by adding 0.25 ml of saturated barium chloride solution. The suspension was centrifuged, and the supernatant liquid was discarded; the precipitate was then redissolved in 3 ml of saturated boric acid, 2 ml of concentrated hydrochloric acid, and 2 ml of water. The solutions were then ready for measurement on the γ-ray spectrometer.

Spectrometry

A Searle Analytic sample changer (Model 1185) was interfaced to a Nuclear Data 2200 multichannel analyzer to facilitate the acquisition of data. The sample changer, originally designed for a small NaI(Tl) detector, was rebuilt to accommodate an Ortec Ge(Li) detector (30 cm^3, 2.5 keV FWHM) and its Dewar flask. The Ge(Li) detector assembly slid into a lead shield 10 cm thick, lined with cadmium and copper. The assembly was bolted to the frame of the sample changer to prevent geometry changes by inadvertent movement of the detector assembly or sample changer. A sample was lowered from the sample changer belt through a 2.5-cm diameter hole in the top of the lead shield. A Lucite guide was designed to ensure that samples were reproducibly positioned over the detector face. Measurements of 30 identical samples containing a radioisotope mixture had a relative standard deviation of ±1.5%. Subsequent work indicates that a well-type Ge(Li) detector (Princeton Gamma-Tech, 58 cm^3, 2.2 keV FWHM, 23 mm well diameter) is also compatible with this sample changer system. The well-type detector has the advantage of enhanced detection efficiency and minimizes geometry effects. Contributions from samples on the changer containing 100 times more ^{95}Zr than typical samples were not observable in background spectra.

Radiochemical yields

The radiochemical yield for each sample was determined by x-ray fluore-

scence (x.r.f.) determination of zirconium in the solutions. Care was taken to match the composition of the x.r.f. standards with the composition of the sample solutions. The x.r.f. standards were prepared from neutron-irradiated carrier material. Quantities of the zirconium carrier equivalent to 100%, 90%, 85% and 75% chemical yields were processed like the samples. The recovery of the carrier was monitored by analyzing the ^{95}Zr γ-ray activity. The chemical yields for the individual samples were determined by comparing the intensity of the zirconium K_α emission line of the sample to the chemical yield calibration curve. The zirconium K_α fluorescence of the standards and samples was measured with an EDAX International Model 707A energy-dispersive x-ray fluorescence system.

Data processing

The γ-ray spectra were stored on magnetic tape and analyzed by a program developed for an IBM 370 computer. The ^{95}Zr 724-keV and 756-keV photopeak areas calculated by Gaussian [6] and Covell [7, 8] methods agreed within 1σ counting statistics. A 2σ upper limit concentration was calculated if no ^{95}Zr photopeaks were identified by the program and if the Covell areas were less than or equal to zero, or if the error associated with the photopeak area was greater than the Covell area. The final values reported were the averages of the concentrations derived from the two photopeaks.

RESULTS AND DISCUSSION

Potential interferences to the determination were assessed before method development began in order to guide the development. Interference from the nuclear reaction ^{235}U (n,f) ^{95}Zr (σ_f = 557 b with a fission yield of 6%) was possible [5]. Calculations based on the given but uncertificated uranium content of NBS standard reference material 1557 (bovine liver) indicated that the interference was negligible at the desired detection limit of 0.1 ppm. Interferences from other radio-isotopes in the irradiated samples were also expected to be present. Interferences from ^{108m}Ag, ^{124}Sb, and ^{114m}In γ-rays were possible on the 724-keV photopeak of ^{95}Zr, and ^{154}Eu with γ-rays of 722 keV (20% absolute intensity) and 757 keV (4% absolute intensity) were potential interferences with both of the ^{95}Zr photopeaks. In addition, high Compton continuum from the short-lived isotopes ^{24}Na, ^{42}K, and ^{82}Br and the long-lived radionuclides ^{46}Sc, ^{59}Fe, and ^{65}Zn interfered with the determination of zirconium. These potential interferences indicated that separations were required.

Several methods of sample destruction were investigated in this study. A chemical oxidation procedure was preferred to dry ashing because it facilitated subsequent separations. Methods for wet decomposition of the tissues investigated included various combinations of nitric, perchloric, and sulfuric acids. Perchloric acid made the separations difficult because it precipitated $ZrOCl_2$, which clung to the walls of the oxidation tubes. Sulfuric

acid interfered with the $BaZrF_6$ precipitation. Fuming nitric acid produced satisfactory sample destruction and facilitated subsequent separations. A white precipitate occasionally formed during the oxidation of the tissues. X-ray fluorescence showed that the precipitate contained zirconium and phosphorus. It was believed to be zirconium phosphate with the phosphorus coming from the tissues. The precipitate rather than being a nuisance, was used as the basis to facilitate the separation of zirconium.

Figure 1 presents γ-ray spectra, before and after radiochemical separation, of a tissue to which ^{46}Sc, ^{60}Co, ^{59}Fe, ^{65}Zn, ^{124}Sb, $^{152,154}Eu$, and ^{95}Zr had been added. The ^{46}Sc activity is the only observable interfering radioactivity remaining in the spectrum and it has been reduced 100-fold. The decontamination factors of the radionuclides that interfere with the zirconium photopeaks or mask them by producing large Compton continuums are presented in Table 1. The decontamination factors of ^{114m}In and ^{108m}Ag were not investigated because these isotopes were not identified in the tissues.

Colloid formation and polymerization of zirconium in solution are well known [9—11] and can prevent the complete exchange of the radioactive zirconium with the carrier. A test was designed to determine whether the proposed method was influenced by this effect. Microgram quantities of a ^{95}Zr test compound were added to bovine liver and bovine bone. These tissues were dried out and then processed by the procedure recommended above. The data in Table 2 show that the recoveries of tracer and carrier are equal within the experimental error. This indicates that the zirconium carrier and the radioactive zirconium exchanged.

Another verification of the procedure was also used because the chemical form of zirconium in tissue may not be the same as the carrier and because

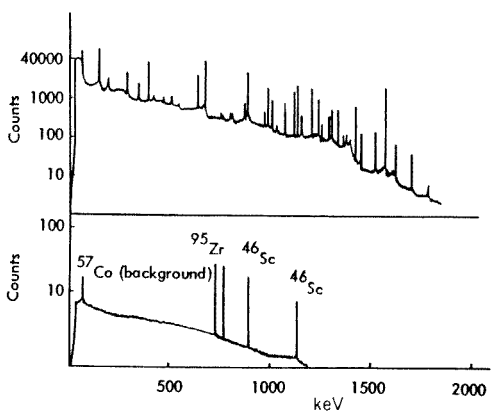

Fig. 1. γ-Ray spectra spiked with ^{46}Sc, ^{60}Co, ^{59}Fe, ^{65}Zn, ^{124}Sb, ^{152}Eu, ^{154}Eu, and ^{95}Zr before and after separation. Both spectra were accumulated for 4000 s in a 30 cm^3 Ge(Li) detector.

TABLE 1

Decontamination factors for interfering radionuclides

Element	Added (mg)	Decontamination factor[a] after:	
		Phosphate precipitation	$BaZrF_6$ precipitation
Sc	7	80	90
Fe	1	250	>500
Zn	0.2	>1000	>1000
Sb	0.01	4	25
Eu	0.01	>500	>500

[a]Decontamination factor is defined as the initial activity divided by the activity following radiochemical separation.

TABLE 2

Comparison of radiotracer recoveries and chemical recovery based on zirconium carrier

Matrix	Sample weight (mg)	Radiotracer recovered (%)	Carrier recovered (%)	Matrix	Sample weight (mg)	Radiotracer recovered (%)	Carrier recovered (%)
Liver	100	92 ± 2	96 ± 4	Bone	100	94 ± 2	93 ± 4
	100	91 ± 2	94 ± 4		100	92 ± 4	97 ± 4
	100	91 ± 4	90 ± 4		100	92 ± 4	94 ± 4
	200	91 ± 4	90 ± 4				
	200	93 ± 5	93 ± 4			Av. 93 ± 1	95 ± 2
	200	92 ± 3	89 ± 4				
		Av. 92 ± 1	92 ± 3				

of difficulties of complete exchange among different forms of zirconium. A known quantity of a commercial zirconium atomic absorption standard was added to bone and liver samples. The tissues were then dried and processed as described above. The results (Table 3) indicate that the analytical results are accurate to within 5—10%. Measurements carried out with the well-type detector on the $BaZrF_6$ precipitate without subsequent radiochemical yield determination of individual samples have an accuracy better than 20%. Measurement without radiochemical yield determination on individual samples is particularly useful for processing a large number of samples. Interferences from ^{124}Sb, $^{154,152}Eu$, ^{108m}Ag and ^{114m}In were not detected.

The zirconium concentration found in various tissues from untreated guinea pigs ranged from <0.1 ppm to 4.0 ppm zirconium on a dry-weight basis. Calcified tissues contained more zirconium than soft tissues. The concentration of zirconium in calcified tissues was expected on the basis

TABLE 3

Recovery of zirconium from spiking experiments

Matrix	Weight (mg)	Number of samples	Spiked (μg)	Recovered[a] (μg)	Recovery (%)
Liver	100	3	0.5	0.53 ± 0.05	106
	100	3	2.0	2.00 ± 0.21	100
	100	3	10.0	9.9 ± 0.1	99
	200	3	1.0	1.05 ± 0.05	105
	200	3	4.0	3.93 ± 0.22	98
	200	3	20.0	19.9 ± 1.1	100
Bone	100	3	2.0	1.94 ± 0.03	97
	100	3	10.0	10.1 ± 0.1	101
					Av. 101

[a]Background levels of zirconium in these tissues were below the 2 σ detection limit of 0.05 μg for this set of data and, therefore, no background levels were subtracted from the reported results.

of ^{95}Zr tracer studies [12]. The results obtained by this method for guinea pigs are considerably lower than those reported by Schroeder et al. [13], who reported a mean zirconium concentration of 26.6 ppm on a wet-weight basis for mice. Although species and dietary effects may be present, the present values are more consistent with the range of 0.2—3.9 ppm zirconium found in human hair by Chattopadhyay and Jervis [14] by photon activation. The low concentration of zirconium found in guinea pig tissues by the method reported here is also consistent with the known chemical behavior of zirconium [15].

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Short Communication

ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRIC DETERMINATION OF ALUMINUM IN BLOOD SERUM

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Summary. An aerosol-generating device is used for sample application. Precision ranges from 2.5 to 8.0% and recoveries of 30 and 100 $\mu g\ l^{-1}$ added to each of five samples ranged from 95 to 105%. Correlation with a slightly different method gave a least-squares fit of $y = 1.02x - 1.35\ \mu g\ l^{-1}$. The technique described is satisfactory for studying aluminum toxicity in dialysis encephalopathy and Alzheimer's Disease.

Aluminum toxicity has been implicated in the pathogenesis of a number of clinical disorders in patients with chronic renal failure on long-term intermittent hemodialysis treatment [1—4]. The predominant disorders have been those involving either bone (osteomalacic dialysis osteodystrophy) or brain (dialysis encephalopathy).

Several methods used to determine aluminum concentrations in serum include neutron activation, x-ray fluorescence, flame atomic emission and atomic absorption spectrometry. Neutron activation suffers from the short half-life of ^{28}Al which is 2.27 min and, also, it is difficult to separate the aluminum from interfering isotopes such as sodium [5] with longer half-lives. In their present state, neither x-ray fluorescence nor flame atomic emission methods are sensitive enough to measure trace levels of aluminum in biological samples.

Atomic absorption spectrometry is the technique of choice for routine clinical chemistry laboratories. The best methods use electrothermal atomization and in the method described here an aerosol generation system [6] is used to apply to the graphite cuvette.

A fine, uniform specimen mist is sprayed directly into a hot (175°C) furnace cuvette which allows for a uniform coating of the interior surface of the cuvette during sample application. Because the sample mist dries on hitting the surface of the cuvette, drying problems such as sample spattering and spreading are eliminated. The uniform coating and the elimination of sample drying problems give improved precision over manual pipetting and simplify the sample application procedure.

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Experimental

Apparatus. Atomic absorption measurements were performed with an Instrumentation Laboratory Model 751 atomic absorption spectrometer equipped with a Model 555 furnace and a Model 254 FASTAC, mechanized sample deposition system (Instrumentation Laboratory, Lexington, MA 02173). A mechanized dilutor (Hamilton, Reno, NV 09510) was used to dilute all standards and samples.

Reagents. A diluent solution was prepared by transferring 10 ml of ultrapure nitric acid (G. Frederick Smith Chemical, Columbus, OH 43223) and 20 ml of Triton X-100 (Fisher Scientific, Fair Lawn, NJ 07410) to a 1-l volumetric flask containing 900 ml of deionized water followed by gentle mixing and dilution to the calibrated volume. The solution was stable for two months stored at room temperature in an acid-washed polyethylene bottle.

Standard solutions were prepared as follows using a 1000 mg l^{-1} aluminum stock standard (Fisher Scientific). Aluminum working standard (10 mg l^{-1}) was prepared by transferring 1.0 ml of stock standard and 1.0 ml of ultrapure nitric acid to a 100-ml volumetric flask followed by dilution with deionized water. This solution was stable for one week stored at room temperature in a polyethylene container. Pooled serum from specimens submitted for routine clinical chemistry determinations was collected and stored frozen. Serum-based aluminum standards then were prepared by adding 900 μl of pooled serum and 100 μl of the aluminum working standard to a polypropylene tube. This solution (1000 μg Al l^{-1}) was then added to further aliquots of the pooled serum to make the serum-based standards. The concentrations of these standards were 0, 10, 20, 50, 80, 100, 200, 300, and 400 μg l^{-1}. All of these standards contained the endogenous aluminum present in the serum pool.

Blood processing. Blood was collected using a syringe and needle, transferred to a polypropylene tube and allowed to clot. After centrifugation, the serum was removed using an Eppendorf pipet (Brinkman Instruments, Westbury, NJ 11590) and placed in a polypropylene tube. If necessary, the samples were frozen for several weeks before atomic absorption measurements were done.

Measurements. The atomic absorption spectrometer was operated at 309.3 nm with no background correction. The graphite furnace times and temperatures were adjusted using the ramp mode as follows: sample deposition, 175°C; drying, 30 s at 300°C; ashing, 30 s at 500°C, 30 s at 1300°C; and atomization, 5 s at 2500°C, hold for 5 s. The sample introduction system was set for two replicates with a 5-s delay cycle and a 10-s deposition cycle at a nebulizer flow rate of 2.6 l min^{-1}. The Hamilton dilutor was used to prepare 1:25 dilutions of the standards and samples in polypropylene tubes. The diluent blank, standards, and samples were placed in the sample changer; the absorbance of the diluent blank was adjusted automatically to read zero. Zero and 50 μg l^{-1} standards were run after every 10 samples to check for

standard curve drift. After the sample introduction system had been started, the measurements were completed automatically and the absorbances were recorded on an electrostatic printer. A standard curve was constructed and the absorbances of the unknown samples were compared to the standard curve and the concentration of the unknowns determined. The absorbances used to construct the standard curve were corrected for endogenous aluminum in the serum pool by subtraction of the absorbance of the zero standard.

Results and discussion

Background correction is often needed to correct for broad band absorption that can occur during the atomization cycle and cause falsely increased values. In order to study the need for background correction, aluminum (0, 125, 250, 500 and 750 $\mu g\ l^{-1}$) was added to aliquots of pooled serum. The specimens were processed with and without using background correction. The two curves were parallel and the differences between the curves were within the analytical variation of the method which indicated that background correction was not necessary in the proposed method. The method presented here employs serum-based standards to avoid matrix effects in the sample deposition procedure and in the electrothermal atomization step. The necessity of using a serum pool for preparing the standards complicated the determination to the extent that the "zero standard" contained a substantial amount of aluminum from the serum pool. The absorbance from this aluminum had to be subtracted from all of the other standard absorbances in order to construct a standard curve.

The sensitivity of the method is such that 13.2 μg Al l^{-1} in serum gives an absorbance of 0.0044. The response is linear to at least 400 μg Al l^{-1}. Precision data are presented in Table 1. The aluminum concentrations of the replicates were determined from a single standard curve. The within-day and day-to-day precision data are based on a serum pool which was aliquoted and kept frozen until absorbance measurements were made. The within-day precision is the result of determining the aluminum concentration in the serum pool using four different standard curves. The day-to-day precision represents the aluminum concentration of the serum pool determined on 11 days over a 5-month period.

Recovery studies were performed by adding two known quantities of aluminum to separate aliquots of several serum samples. The original serum and the two spiked aliquots were processed and the calculated recovery data are given in Table 2.

In order to compare this method with another method based on electrothermal atomic absorption spectrometry, samples were obtained which had been processed by the method of Alderman and Gitelman [7] in those investigators' laboratory. Twelve specimens examined ranged in concentrations from 120—800 μg Al l^{-1}. The correlation between the two methods is expressed by the equation $y = (1.02 \pm 0.042)x + (-1.35 \pm 14.71)$ with $S_{yx} = 23.304$ and $r = 0.99$ where y is the present method and x is the Alderman and Gitelman method [7].

TABLE 1

Precision data for aluminum is sera

	Within-run	Within-day	Day-to-day
n	20	4	11
Mean (μg Al l^{-1})	116	88	79
SD (μg Al l^{-1})	±2.9	±4.5	±6.3
R.s.d. (%)	2.5	5.1	8.1

TABLE 2

Determination of aluminum added to pooled sera[a]

Pool (μg l^{-1})	Added (μg l^{-1})	Found (μg l^{-1})	Recovery (%)	Pool (μg l^{-1})	Added (μg l^{-1})	Found (μg l^{-1})	Recovery (%)
210	30	240	100	117	30	148	103
	100	305	95		100	221	104
89	30	120	102	83	30	114	103
	100	194	105		100	184	101
92	30	123	103				
	100	187	95				

[a]For x = Pool + Added, and y = Found: $y = (0.979 \pm 0.017)x + (4.3 \pm 3.3)\mu g\ l^{-1}$; $S_{yx} = 3.1$; $r = 0.999$.

The method proposed here for the determination of aluminum in serum has the sensitivity, precision, and apparent accuracy needed for studies of patients on long-term hemodialysis and in patients with Alzheimer's Disease.

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Short Communication

MATRIX MODIFICATION WITH SILVER FOR THE ELECTROTHERMAL ATOMIZATION OF ARSENIC AND SELENIUM

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Summary. Silver as a matrix modifier is shown to improve the carbon-rod atomization of both arsenic and selenium for atomic absorption spectrometry. Compared to nickel, the efficiency of silver is greater for arsenic and about the same for selenium. Silver fulfils two functions in its reaction, namely stabilization during the ashing stage and enhancement of absorbance in the final atomization.

Matrix modification is often used in determinations of various volatile analytes by electrothermal atomic absorption spectrometry (a.a.s.). A chemical reagent is added directly to the analyte solution, or to the atomizer before addition of the analyte solution, to allow a chemical modification of the matrix so that possible interfering materials may be removed during drying and ashing in the atomization cycle without volatilizing the analyte [1]. Nickel is most frequently used in the graphite-furnace a.a.s. determination of arsenic [1—4]. Among metals that have been employed to stabilize selenium in the electrothermal atomization are nickel [1, 3, 5—8], copper [9, 10], molybdenum [11], chromium, and manganese [8].

This communication presents data to show the enhancement effect of silver as a matrix modifier on the carbon-rod atomization of arsenic and selenium in comparison with the effect of the commonly used nickel. After this work had been completed, a report by Kirkbright et al. [8] was published describing the stabilizing effect of silver and other metals on selenium in the graphite furnace.

Experimental

Apparatus and operating conditions. A Varian atomic-absorption spectrometer equipped with a Model 63 carbon-rod atomizer, a simultaneous background corrector, an electrodeless discharge lamp power supply, and a Model 53 automatic sample dispenser, was used. Instrument settings for arsenic were: wavelength, 193.7 nm; EDL. 7.5 W; slit width, 1.0 nm; nitrogen flow meter, 8.0; drying voltage, 7 (ca. 150°C for 30 s); ashing voltage, variable (400—1400°C for 30 s); and ramp cutoff voltage for atomization (ca. 2400°C at rate 2). Instrument settings for selenium were: wavelength, 196.0 nm;

settings for EDL, slit width and nitrogen flow meter as for arsenic; drying voltage, 6.5 (ca. 100°C for 30 s); ashing voltage, variable (200—1400°C for 30 s); and atomizing voltage, 8.5 (ca. 2500°C for 2 s).

Reagents and standards. For the arsenic stock solution (1000 μg ml^{-1} in 2% HCl), 0.132 g of As_2O_3 was dissolved in 2 ml of 1 M NaOH, and the solution was acidified with 20 ml of 10% (v/v) HCl, and diluted to 100 ml with water. For the selenium stock solution (1000 μg ml^{-1} in 1% HCl), 2.190 g of Na_2SeO_3 was dissolved in 50 ml of water, 10 ml of 11 M HCl, was added, and the solution was diluted to 1 l with water. Arsenic or selenium working standard solutions (0.1—2.0 μg ml^{-1}) were prepared from the stock solutions by serial dilution with 1% nitric acid.

Nickel or silver solution (4000 μg ml^{-1}) was prepared from nickel nitrate hexahydrate or silver nitrate in 1% nitric acid.

Procedure. Portions (5 μl) of the silver or nickel solution (equivalent to 20 μg Ag or Ni) were pipetted manually into the carbon-rod atomizer just prior to the injection of 5 μl of the arsenic or selenium standards by the automatic sample dispenser. Duplicate or triplicate readings were recorded.

Results and discussion

Figure 1(a) illustrates the enhancement effect of silver and nickel on the absorbance of arsenic as the ashing temperature was varied. A decrease can be seen in the absorbance of arsenic atomized from 1% nitric acid as the ashing temperature reaches 700°C (curve A). The decrease continues steadily up to 1300°C and then drops abruptly at the maximum setting of 1400°C, indicating a loss of arsenic by volatilization. Addition of silver stabilizes arsenic during the ashing step and no decrease in arsenic is apparent until 1150°C (curve C). Silver also significantly enhances (by about 100%) the final atomization absorbance values within the ashing temperature range 400—600°C. Nickel shows an enhancement effect similar to that of silver, but the improvement on the final absorbance of arsenic is about 10% less (curve B).

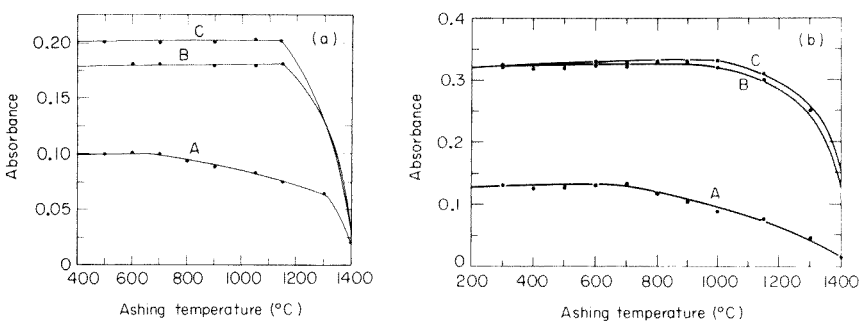

Fig. 1. Enhancement effects of silver and nickel on the absorbances for (a) arsenic and (b) selenium at different ashing temperatures: (A) 2 ng As or Se in 1% HNO_3; (B) 2 ng As or Se + 20 μg Ni; (C) 2 ng As or Se + 20 μg Ag.

Silver and nickel exhibit a similar stabilizing effect on the absorbance of selenium (Fig. 1b). Without either additive, the decrease of the final selenium absorbance caused by volatilization during the ashing step starts at 700°C. When silver or nickel is present, loss of selenium does not occur until 1000°C. The slightly better enhancement effect of silver than nickel may not be significant statistically. The average improvement of the atomization absorbance of selenium by addition of silver or nickel amounts to 140%.

Calibration graphs prepared from the working standards of arsenic or selenium were linear within the range 0.5—10 ng. Amounts of each element required to produce a 1% absorption (0.0044 absorbance) for conditions A—C in Fig. 1 were 90, 48, and 43 pg, respectively, for As (193.7 nm); and 67, 23, and 23 pg, respectively, for Se (196.0 nm).

Thus, silver as a matrix modifier for the electrothermal atomization of arsenic and selenium fulfils two functions; it stabilizes both during the ashing stage and it enhances absorbance in the final atomization.

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Short Communication

DETERMINATION OF ANTIMONY DEPTH PROFILES IN SEMICONDUCTOR SILICON BY CHEMICAL ETCHING AND NON-DISPERSIVE ATOMIC FLUORESCENCE SPECTROMETRY WITH HYDRIDE GENERATION

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Summary. After the silicon has been anodized, the silica film formed is removed by dilute hydrofluoric acid, and the antimony in the etching solution determined by non-dispersive atomic fluorescence spectrometry. The amount of silicon removed is measured in the etching solution by inductively-coupled plasma atomic emission spectrometry. Down to 10^{18} atoms Sb cm^{-3} can be determined at sectioning intervals of 50 nm.

Semiconductor characteristics are known to depend critically on the depth concentration profiles of dopant elements. To date, the electro-active impurity concentration has been determined by differential sheet resistivity methods using a four-point probe [1—4] or the Hall effect [4—6]. However, these methods give no information on electroinactive precipitates and produce distorted values when there are electrically compensating impurities. The net impurity concentration profiles have been determined by sensitive analytical techniques combined with suitable chemical sectioning [4, 7—11].

The depth profile of arsenic in semiconductor silicon has been determined [12] by non-dispersive atomic fluorescence spectrometry (a.f.s.) based on a sodium tetrahydroborate reduction technique and a small argon—hydrogen—entrained air flame. Antimony is also a useful doping donor in the semiconductor silicon industry. It too can be determined sensitively by non-dispersive a.f.s. after tetrahydroborate reduction [13—15]. This communication extends the application of the non-dispersive a.f.s. system [12] to the determination of antimony depth profiles in semiconductor silicon.

Experimental

Apparatus. The non-dispersive atomic fluorescence spectrometer, the inductively-coupled plasma (i.c.p.) atomic emission spectrometer used for silicon determination, and the equipment for silicon anodization and stibine evolution were similar to those reported previously [12].

Reagents. A commercially available antimony(III) 1000-ppm standard solution (Kanto Chemicals Corp.) made from antimony trichloride was used. An antimony(V) 100-ppm standard solution was prepared by oxidizing the

standard antimony(III) solution. For this purpose, 5 ml of the 1000-ppm antimony(III) solution was mixed with 5 ml of 12 M hydrochloric acid and 5 ml of a 2% cerium(IV) sulfate solution in 0.5 M sulfuric acid; 1% hydrazine sulfate solution was added to remove the excess of oxidant, and the solution was diluted to 50 ml with 4 M hydrochloric acid. The sodium tetrahydroborate solution (1% w/v) was prepared just before use by dissolving pellets of the reagent (98%, Alfa Inorganics) in water. A potassium iodide solution (2.5 M) was used to reduce antimony(V) to antimony(III). The methanol, trichloroethylene and hydrofluoric acid used were of electronic grade. All other reagents were of analytical-reagent grade.

Procedure. In the previous paper [12] silicon slices were fixed to a copper electrode as shown in Fig. 1(A). However, this slice setting method was only applicable to depth profile determinations to about 300 nm below the surface, because the dissolution of dopant elements from the wax-coated slice surface area (where they were in high concentration) became significant and contributed to positive errors. Antimony doped on silicon slices diffuses to far deeper levels from the surface than arsenic. Therefore, the dissolution problem is more critical in the depth profile determination of antimony. An improved sample pretreatment procedure was employed to overcome this disadvantage. Apiezon wax was applied to the silicon slice surfaces as shown in Fig. 1(B), leaving an uncoated area about 3 mm from the slice edge. The slice was dipped in a mixed acid solution (1 part of hydrofluoric acid plus 10 parts of nitric acid) for 1 min, etching off the uncoated part of the slice surface as shown in Fig. 1(C). The wax was dissolved by trichloroethylene (see Fig. 1(D)), and the slice was washed with methanol, trichloroethylene, aqua regia, hydrofluoric acid and running deionized water as described previously [12]. Silver paint (Silbest, Tokuriki Chemical Research Co. Ltd.) was applied to the back of the silicon slice to ensure good electrical contact

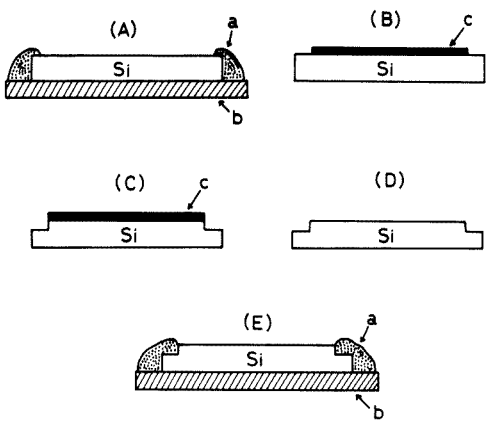

Fig. 1. Improved sample pretreatment procedure for the determination of antimony profiles in silicon slices. (a) Electronic wax; (b) copper electrode; (c) apiezon wax. For explanation, see text.

between the slice and a copper electrode, and the slice was fixed to a copper electrode as shown in Fig. 1(E).

The silicon anodization procedure was similar to that reported previously [12], except that the constant current (50 mA) anodization was carried out for 12 min to achieve thicker sectioning of the slice. The silica film formed was dissolved by 1 ml of 0.1 M hydrofluoric acid, and the etching solution was diluted to 5 ml.

A 2.5-ml portion of each etching solution was taken, 1 ml of potassium iodide solution and 0.42 ml of hydrochloric acid solution were added, and the mixture was diluted to exactly 5 ml. This solution was used for atomic fluorescence measurements. A 2.5-ml portion of each etching solution was diluted to 25 ml for i.c.p. atomic emission spectrometric measurements.

Atomic fluorescence measurements were carried out under conditions shown in Table 1. The i.c.p. measurements were made under the same conditions employed previously [12].

TABLE 1

Experimental conditions for non-dispersive atomic fluorescence spectrometry of antimony

Lamp operating power	9 W	Distance between the beam center and burner top	10 mm
Chopping frequency	27 Hz	Time constant of lock-in amplifier	1.0 s
Photomultiplier voltage	400 V	Acid (HCl) concentration	1.0 M
Argon flow rate	0.7 l min^{-1}	Reductant volume	2.0 ml
Hydrogen flow rate	0.15 l min^{-1}	Sample volume	200 µl
Distance between the flame center and photo-cathode	ca. 45 mm		

Results and discussion

Effect of acid. The effects of type and concentration of acid on antimony fluorescence intensity were measured for antimony(III) and antimony(V). The results are shown in Fig. 2. Subsequent measurements were made with 1 M hydrochloric acid to suppress the pressure increase in the stibine evolution cell observed at higher acidities that caused the small argon–hydrogen–entrained air flame to extinguish.

Effect of argon and hydrogen flow rates. The noise level dependence on hydrogen flow rate was described previously [12]. The effects of argon and hydrogen flow rates on antimony fluorescence intensity were measured. The fluorescence intensity gradually increased with increasing argon flow rate and decreasing hydrogen flow rate. Optimal gas flow rates were found to be 0.7 l Ar min^{-1} and 0.15 l H_2 min^{-1}, at which the visible portion of the flame was about 1 cm high.

Detection limit, calibration and repeatability. The detection limit for antimony(s/n = 2) was 20 pg and the linear analytical range covered four concentration decades (up to 200 ng) above the detection limit. Relative standard deviations obtained from ten signal measurements were 4.9% (0.01 ppm), 3.1% (0.05 ppm), 1.6% (0.1 ppm) and 1.9% (0.5 ppm).

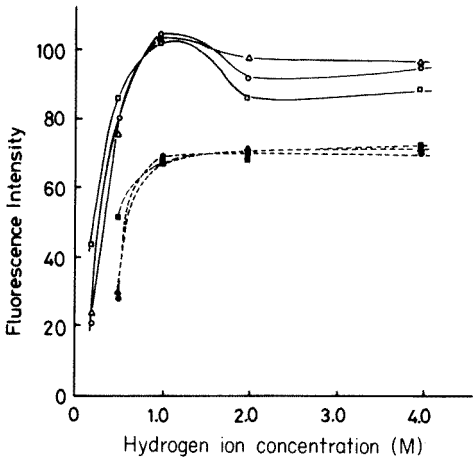

Fig. 2. Effect of acids at various concentrations on antimony fluorescence intensity (in arbitrary units). (—) Sb(III); (---) Sb(V); (○ ●) HCl; (□ ■) H_2SO_4; (△ ▲) HNO_3.

Effects of silicon and hydrofluoric acid. The possible interfering effects of up to 10^5-fold amounts of silicon (by weight) and 0.01—0.1 M hydrofluoric acid were investigated. No interference was observed from silicon. Hydrofluoric acid also gave no interference for antimony(III), but it inhibited stibine evolution from antimony(V) completely in the investigated concentration range, as reported elsewhere [16]. This inhibition is probably caused by the formation of a stable fluoro complex.

Reduction of antimony(V) to antimony(III). To apply the present technique to practical analysis the following problems had to be solved. First, the antimony must be converted to a single oxidation state prior to stibine evolution, because the fluorescence intensity from antimony(V) is about 65% of that from an equivalent amount of antimony(III), when the hydrochloric acid concentration is 1 M (Fig. 2). Secondly, the interference of hydrofluoric acid on antimony(V) must be eliminated. Potassium iodide reduction overcame these problems. The hydrofluoric acid concentration in the sample solution for a.f.s. measurements is estimated to be 0.01 M. Therefore, the presence of 0.5 M potassium iodide is sufficient to reduce antimony(V) to antimony(III), and eliminate the interference of hydrofluoric acid (Fig. 3).

Determination of antimony in synthesized samples. A silicon slice, undoped with antimony, was anodized. Then the silica film formed was dissolved by 1 ml of 0.1 M hydrofluoric acid. Next, known amounts of antimony(III), antimony(V), 1 ml of potassium iodide solution and 0.42 ml of 12 M hydrochloric acid were added. Finally, the solution was diluted to 5 ml. This solution was used for non-dispersive a.f.s. measurements. Recovery values were satisfactory, as shown in Table 2.

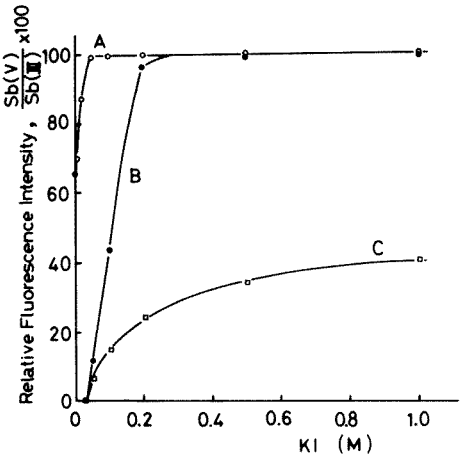

Fig. 3. Effect of potassium iodide concentration on antimony(V) reduction to antimony(III). Total antimony = 100 ng. HF concentration: (A) 0; (B) 0.01 M; (C) 0.05 M.

Depth profiles of antimony in a silicon slice. The proposed technique was applied to establish depth profiles in silicon slices. A typical depth profile is shown in Fig. 4, which also demonstrates the good agreement with results obtained by a four-point probe resistivity method.

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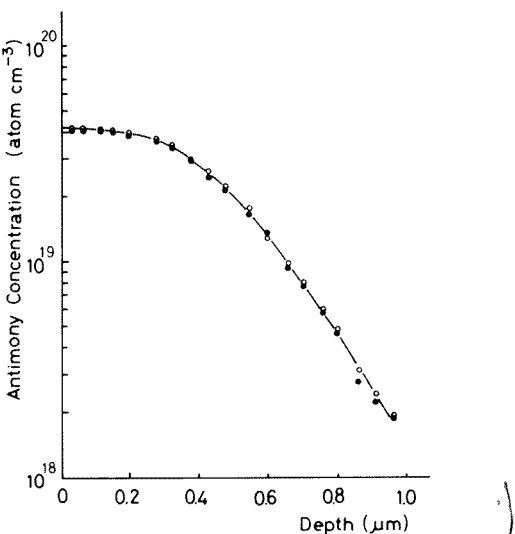

Fig. 4. Typical depth profile of antimony in a silicon slice with a surface area of 13.9 cm^2. (•) Present method; (○) Four-point probe resistivity method.

TABLE 2

Determination of antimony in synthesized solutions

Sb added (μg)	Sb(III)	0.0	1.0	0.5	0.0
	Sb(V)	0.0	0.0	0.5	1.0
Sb found[a] (μg)		0.0	1.00	0.97	1.10

[a]Average of 5 replicate determinations.

slices, to Mr. H. Usui for his guidance in the anodization and resistivity measurements and for providing a computer program for calculating concentration profiles from resistivity data.

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Short Communication

THE DETERMINATION OF PHENOLIC ANTI-OXIDANTS IN EDIBLE OILS AND FATS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Summary. A simple procedure for the extraction of phenolic anti-oxidants with methanol is described. The extracted compounds are determined by gradient elution with retention times of 4.0—13.2 min; an internal standard technique is used for quantification.

Phenolic compounds are added to edible oils and fats to delay or prevent the development of rancidity or other flavour deterioration caused by oxidation. The most commonly used compounds are the n-propyl, octyl and dodecyl esters of gallic acid (3,4,5-trihydroxybenzoic acid), butylated hydroxyanisole [BHA; a mixture of 2-(1,1-dimethylethyl)-4-methoxyphenol and 3-(1,1-dimethylethyl)-4-methoxyphenol], butylated hydroxytoluene [BHT; 2,6-bis(1,1-dimethylethyl)-4-methylphenol] and t-butylhydroquinone [TBHQ; 2-(1,1-dimethylethyl-1,4-dihydroxybenzene]. The maximum concentrations permitted are usually 100 mg kg^{-1} for the gallate esters and 200 mg kg^{-1} for the other compounds. The anti-oxidants have been isolated by various methods [1]; this communication describes the direct extraction of the compounds with methanol containing an internal standard, thus reducing the number of manipulative steps to a minimum.

Experimental

Apparatus and chromatographic conditions. The apparatus consisted of an Altex model 321 liquid chromatograph with an Altex—Hitachi variable wavelength detector set at 280 nm and 0.05 absorbance units; a μBondapak C_{18} column (300 × 3.9 mm i.d.; Waters Associates), with a pre-column (30 × 3.0 mm i.d.) packed with Co-Pell ODS (30—38 μm; Whatman) was used with a mobile-phase flow rate of 1.5 ml min^{-1}. The mobile phase required to separate the six anti-oxidants consisted of a mixture of 1% (v/v) acetic acid in water (solvent A) and 1% (v/v) acetic acid in methanol (solvent B), programmed linearly from 50% B to 90% B in 10 min and then isocratically at 90% B for 10 min. Samples (20 μl) were injected through a loop injector, and peak areas were measured and concentrations calculated with a Hewlett-Packard 3354A Laboratory Data System, using an internal standard procedure.

Standard solution. Dissolve 200 mg of 2,4,6-trimethylphenol (internal standard), 50 mg of propyl gallate and 100 mg each of octyl gallate, dodecyl gallate, BHA, BHT and TBHQ in methanol and dilute to 100 ml with methanol; dilute 2 ml of this solution to 100 ml with methanol.

Internal standard solution. Dissolve 200 mg of 2,4,6-trimethylphenol in methanol and dilute to 100 ml with methanol; dilute 2 ml of this solution to 100 ml with methanol.

Procedure. Inject 20 μl of standard solution and determine the areas under the peaks; determine the retention times of the anti-oxidants and the response factors of each relative to the internal standard. BHA gives only one peak under the chromatographic conditions described above.

For edible oils, weigh accurately about 1 g of sample into a glass-stoppered test tube (capacity 15—20 ml), add 10.0 ml of internal standard solution and mix. Shake vigorously for 30 s, cool in ice-water and centrifuge for 5 min at 2000 rpm. Inject 20 μl of the clear upper layer and measure the areas under the peaks as before. Calculate the concentration of anti-oxidants in the sample by reference to the response factors relative to the internal standard. For solid samples, weigh accurately about 1 g of sample into a glass-stoppered test tube, add 10 ml of internal standard solution and warm gently until the fat has just melted. Shake vigorously for 30 s and proceed as described for edible oils.

If only one known anti-oxidant is to be determined, the following mobile phases may be used isocratically: for BHA, TBHQ or propyl gallate, 70% solvent B; for octyl gallate, 80% solvent B; for BHT or dodecyl gallate, 90% solvent B. The three gallate esters may be separated by programming a mixture of 70% B to a mixture of 90% B linearly in 10 min and then isocratically at 90% B for 5 min. An appropriate standard solution should be prepared in each case.

Results and discussion

Samples of maize oil, safflower oil, sunflower oil, soybean oil, grape seed oil, olive oil, peanut oil, sesame oil, a blended vegetable oil, polyunsaturated margarine (separated fat) and lard, each containing no added anti-oxidant, were extracted with methanol by the procedure described above; no interfering peaks were found when the extracts were examined under the chromatographic conditions required to separate the six anti-oxidants. The internal standard solution was extracted with a blended vegetable oil as described above; ten injections of the methanol solution were made before and after extraction and no significant difference in the mean concentrations of the internal standard was found. Typical retention times found for the anti-oxidants and the internal standard when separated under the above conditions were: propyl gallate 4.0 min, TBHQ 5.0 min, 2,4,6-trimethylphenol 6.8 min, BHA 8.1 min, octyl gallate 10.0 min, BHT 12.4 min and dodecyl gallate 13.2 min.

TABLE 1

Recovery of phenolic anti-oxidants added to edible oils

Anti-oxidant	Oil	Added (mg kg^{-1})	Mean recovery[a] (%)	Relative standard deviation (%)
Propyl gallate	Blended vegetable	96	98.0	3.3
Octyl gallate	Safflower	84	101.7	2.6
Dodecyl gallate	Blended vegetable	91	102.8	1.9
BHA	Safflower	130	99.9	2.1
BHT	Blended vegetable	81	90.0	2.7
TBHQ	Safflower	148	102.1	1.8

[a]5 Determinations, except propyl gallate (10 determinations).

TABLE 2

Concentrations of anti-oxidants found in edible oils and fats

Sample	Anti-oxidant	Concentration (mg kg^{-1})
Sunflower oil	BHA	81
Blended vegetable oil A	Propyl gallate	27
Blended vegetable oil B	BHA	89
Lard A	BHA	65
Lard B	BHA	185
Maize oil	BHA	113

Samples of edible oils containing known concentrations of anti-oxidants were prepared and the prepared oils were analysed as described above; the results obtained are shown in Table 1. The mean recovery for BHT was 90.0% and for the remaining compounds, 98.0—102.1%. These values compare favourably with those reported by Page [2], viz., 85.4% for BHT and 99.5—101.9% for the remaining compounds at the 100 mg kg^{-1} level in oil, and those reported by Hammond [1], viz., 92—106% for the three gallate esters, 68% for BHA and 33% for BHT at the same concentrations. Van Niekerk and Du Plessis [3] reported a recovery of 98.5% for TBHQ by direct injection of oil samples. The coefficients of variation are similar to those reported earlier [1—3]. A selection of results found by the method is shown in Table 2.

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Short Communication

CONSTRUCTION AND ANALYTICAL APPLICATIONS OF LIQUID-MEMBRANE ELECTRODES FOR ATROPINE AND NOVATROPINE

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Summary. Liquid-membrane electrodes sensitive to atropinium and novatropinium cations are described. The atropinium electrode exhibits rapid and near-Nernstian response in the 10^{-2}—3×10^{-5} M range over the pH range 2—8.5; the novatropinium electrode shows near-Nernstian response in the 10^{-2}—3×10^{-6} M range at pH 2—10. Other alkaloids interfere. Direct potentiometry and potentiometric titrations are used to determine atropine and novatropine in pharmaceutical preparations with satisfactory results.

Several sensitive potentiometric sensors for some organic bases of pharmaceutical importance have recently been reported. Electrodes have been constructed that are sensitive to methacholine, neostigmine [1], ephedrine [2], novocaine [3], codeine, morphine, ethylmorphine [4], strychnine [5], and nicotine [6]. Also, a potentiometric method [7] for the determination of various alkaloids with picrate ions using a picrate-selective electrode [8] has been reported.

In this communication, the performance characteristics of new electrodes sensitive to atropinium and novatropinium (methylhomatropinium) cations are described. The liquid ion-exchangers are atropinium tetraphenylboron and novatropinium tetraphenylboron, respectively, dissolved in 2-nitrotoluene. The electrodes proved useful in direct potentiometric determinations of atropine and novatropine in pharmaceutical preparations. The atropinium sensitive electrode was also used to estimate the pK_a value of atropine and in potentiometric titrations of atropine.

Experimental

Instrumentation. The reference electrode was an Orion 90-01-00 Ag/AgCl single-junction electrode, filled with Orion 90-00-01 solution. E.m.f. values were measured with a Corning Model 12 Research pH/mV meter and recorded on a Heath-Schlumberger Model SR-255 B potentiometric recorder. All solutions were measured at ambient temperature with constant magnetic stirring.

Reagents. All solutions were prepared with deionized distilled water from

reagent-grade materials, unless otherwise stated. The chemicals were atropine sulfate and sodium tetraphenylboron (E. Merck, Darmstadt), novatropine (a gift from Chifar Chemical and Pharmaceutical Co., Athens); sodium tetra(*m*-chlorophenyl)boron was prepared as described by Jarzembowski et al. [9]. Pharmaceutical preparations were obtained from local drugstores. Standard 0.1000 M solutions of atropine, novatropine and sodium tetraphenylboron were prepared by dissolving the appropriate amount of substance in water. Working solutions were prepared by appropriate dilutions. Ionic strength adjustment solutions of 0.10 M and 0.50 M sodium sulfate and 0.10 or 0.20 M phosphate buffer, pH 6.5, were prepared in the usual manner.

Preparation of the liquid ion-exchangers. Atropinium tetraphenylboron was precipitated by mixing 1.0 ml each of aqueous 0.1 M solutions of atropine sulfate and sodium tetraphenylboron. The salt was extracted with 10.0 ml of 2-nitrotoluene, and the organic phase was washed twice with distilled water and dried thoroughly with anhydrous sodium sulfate. For the atropinium tetra(*m*-chlorophenyl)boron, sodium tetra(*m*-chlorophenyl)boron was substituted for sodium tetraphenylboron. Novatropine tetraphenylboron was prepared by mixing 0.5 ml of 0.01 M novatropine solution with 0.5 ml of 0.1 M sodium tetraphenylboron solution. The precipitate was extracted and treated as indicated above for the atropinium salt.

Construction of the electrodes. An Orion liquid-membrane electrode body (model 92) was used as the electrode assembly with a Millipore LCWPO 1300 teflon membrane; the teflon membranes were cut to the appropriate size and a stack of four was used to avoid any leakage of the liquid exchanger. The electrode was assembled in the usual way; the internal reference solution was 0.01 M atropine sulfate—0.1 M NaCl for the atropinium electrode and 0.01 M novatropine—0.1 M NaCl for the novatropinium electrode. The electrodes were conditioned by soaking in 10^{-2} M atropine or novatropine solution, as appropriate, for 24 h before use, and were stored in the same solution when not in use. Their operative lifetime was about one month.

Preparation of the calibration curve. A portion (30.00 ml) of 0.10 M phosphate buffer was pipetted into a 100-ml beaker, the electrodes were immersed, constant stirring (without air bubbles) was started, and 0.001 ml of 0.1 M atropine or novatropine standard solution was added. Readings were made after stabilization to ±0.1 mV. Further increments of 0.1 M standard solution were added to cover the 3.3×10^{-6}—1.2×10^{-2} M range, and the calibration graphs were plotted in the usual way.

Potentiometric titration of atropine. A 30.00-ml aliquot of the sample was pipetted into the reaction cell, and the stirred solution was titrated with a standard sodium tetraphenylboron solution. For atropine samples in the ranges 3×10^{-2}—5×10^{-3} and 3×10^{-3}—5×10^{-4} M, the sodium tetraphenylboron solutions used were 0.1000 and 0.01000 M, respectively. The inflection point of the titration curve was taken as the end-point.

Determination of atropine or novatropine in pharmaceutical preparations.

Atropine sulfate eye-drops (0.5 or 1%) were diluted 100-fold with water. Atropine sulfate injections (1 mg ml^{-1}) were diluted three-fold. For direct potentiometry of atropine a 15.00-ml aliquot of the diluted sample and 15.00 ml of 0.20 M phosphate buffer, pH 6.5, were pipetted into the reaction cell. The atropine concentration was found from a calibration graph for 0.1 M phosphate buffer, pH 6.5.

The novatropine (4 mg ml^{-1}) and novalumine (1.3 mg ml^{-1}) solutions were diluted 20-fold with water. Novatropine was determined as described above for the atropine. For the determination of novatropine in tablets, fifteen 2.5-mg tablets were powdered and extracted with 300 ml of water. The extract was filtered and diluted to 500.0 ml, and a 15.00-ml aliquot was used for the measurement, as described above for the diluted atropine sample.

Results and discussion

Characteristics of the electrodes. Typical calibration curves for the electrodes under various experimental conditions are shown in Figs. 1 and 2. The response of the atropinium electrode is linear in the 10^{-2}—3×10^{-5} M range with a slope of about 59 mV/concentration decade, at 27°C. Similar calibration curves were obtained when an atropinium electrode with atropinium tetra(*m*-chlorophenyl)boron in 2-nitrotoluene as liquid ion-exchanger was used. Atropinium tetraphenylboron was preferred because sodium tetraphenylboron is readily available. The response of the novatropinium electrode is linear in the 10^{-2}—3×10^{-6} M range with a slope of 61 mV/concentration decade, at 27°C. Considerable deviation from linearity was observed with unbuffered novatropine solution (Fig. 2, curve A).

The electrodes reached stable potential readings (±0.1 mV) within 15 s to 3 min depending on the alkaloid concentration, the buffer, etc. Generally, fast response (less than 1 min) was observed with solutions $>10^{-4}$ M. For

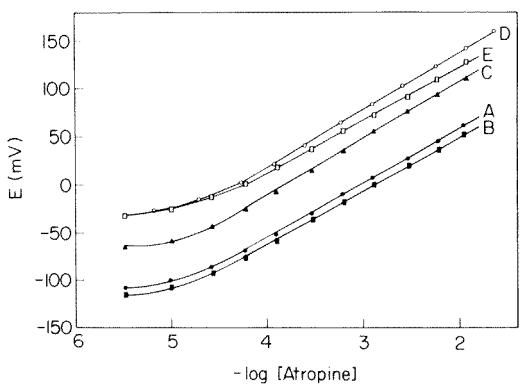


Fig. 1. Calibration curves for the atropinium-selective electrode. (A) In pure atropine sulfate solutions; (B) in 0.10 M Na_2SO_4 solution; (C) in 0.50 M Na_2SO_4 solution; (D) in 0.10 M phosphate buffer, pH 6.5. Curve E shows the response of the atropinium electrode to codeine, in 0.10 M phosphate buffer pH 6.5.

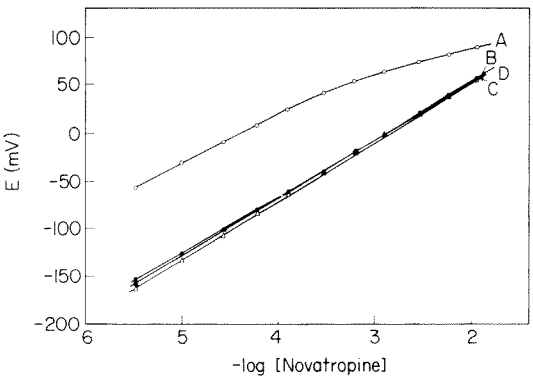


Fig. 2. Calibration curves for the novatropinium-selective electrode. (A) In pure novatropine solutions; (B) in 0.10 M phosphate buffer, pH 6.5; (C) in 0.10 M Na_2SO_4 solution; (D) in 0.50 M Na_2SO_4 solution.

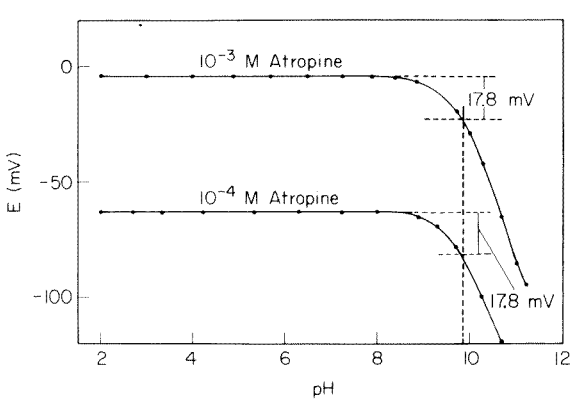
solutions $<10^{-4}$ M the response time increased with increasing ionic strength.

To check the pH-dependence of the potential of the atropinium electrode, potential—pH curves were constructed. The initial solution was made acidic by adding concentrated sulfuric acid in 30.00 ml of atropine solution (10^{-3} or 10^{-4} M), and then the pH was adjusted (pH meter) by addition of small volumes of 10 M NaOH solution. The plots (Fig. 3) show that at pH 2—8.5, the potentials are constant. At higher pH values, the potentials gradually decrease because of the decreased concentration of the atropinium ion which is converted to atropine. Similarly, the potential of the novatropinium electrode was practically independent of pH in the range 2—10. At higher pH values, the electrode potential was unstable. The acidity constant K_a of atropine can be calculated from Fig. 3, as pK_a is equal to the pH value where the initial atropinium concentration, $[AH^+]$, is halved, i.e., when the potential decreases by 17.8 mV (Fig. 3). The pK_a value thus calculated is 9.80 at 27°C, which is in good agreement with the literature value of 9.65 [10].

Potentiometric selectivity coefficients for the atropinium electrode were measured by the mixed solution method and calculated as previously described [11]. The results are presented in Table 1. The atropinium electrode was also found to respond to various other alkaloids, e.g., codeine, papaverine, cocaine, and morphine. A response curve for codeine given by the atropinium electrode is shown in Fig. 1 (curve E).

Analytical applications. The atropinium electrode proved useful in the potentiometric titration of atropine with sodium tetraphenylboron. Typical titration curves are shown in Fig. 4. Amounts of atropine in the range 15—900 μmol were determined with an average error of about 2%. Direct potentiometric determinations on aqueous 10^{-3} M atropine and novatropine solutions from 3-point calibration curves showed imprecision of about 1.5%.

Comparative results for the determination of atropine and novatropine in some pharmaceutical preparations are shown in Table 2. The eye-drops con-



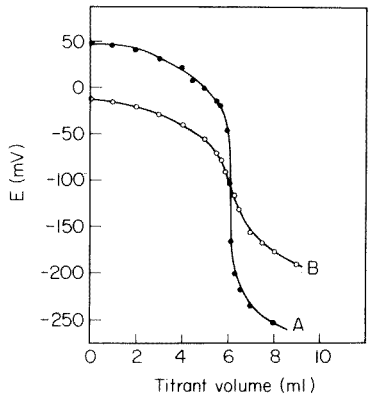


Fig. 3. Effect of pH on the potential of the atropinium electrode.

Fig. 4. Titration curves for the potentiometric titration of 30 ml of atropine sulfate with sodium tetraphenylboron, at pH 6. (A) 2×10^{-2} M atropine with 10^{-1} M sodium tetraphenylboron; (B) 2×10^{-3} M atropine with 10^{-2} M sodium tetraphenylboron.

TABLE 1

Potentiometric selectivity coefficients for the atropine electrode[a]

Possible interference	a_j, ($\times 10^{-2}$ M)	a'_{AH^+} ($\times 10^{-4}$ M)	$k^{pot}_{AH^+,j}$	Possible interference	a_j, ($\times 10^{-2}$ M)	a'_{AH^+} ($\times 10^{-4}$ M)	$k^{pot}_{AH^+,j}$
Na^+	6.8	7.6	$<10^{-4}$	Pb^{2+}	1.8	6.7	7×10^{-4}
K^+	6.8	7.6	1×10^{-4}	Mg^{2+}	1.8	6.7	8×10^{-4}
NH_4^+	6.8	7.6	2×10^{-4}	Cd^{2+}	1.8	6.7	2×10^{-3}
Ni^{2+}	1.8	6.7	5×10^{-4}	Sr^{2+}	1.8	6.7	3×10^{-3}
Ca^{2+}	1.8	6.7	6×10^{-4}				

[a] a_j, a_{AH^+} and a'_{AH^+} are the activities of the possible interference, atropinium in pure solutions, and atropinium in mixed atropine—interfering ion solutions, respectively. For full details, see [11]. Solutions of all possible interfering ions were prepared from their corresponding nitrate salts. a_{AH^+} was 10^{-3} M in all cases.

tained unspecified additives, in order to be isotonic with tears. There is satisfactory agreement between the results obtained by the proposed method and the official methods. In contrast to most of the common methods used for the determination of atropine and novatropine in pharmaceutical preparations, which are time-consuming and require large samples, the proposed method is simple, fast and sensitive.

TABLE 2

Determination of atropine and novatropine in pharmaceutical preparations

Pharmaceutical preparation	Atropine sulfate or novatropine (mg ml^{-1})		
	Present method	Reference method	Nominal
Atropine sulfate injection (aqueous)	0.92	0.93[a]	1
0.5% Atropine sulfate (eye-drops)	6.06	5.64[a]	5
1% Atropine sulfate (eye-drops)	9.8	9.3[a]	10
Novatropine solution (aqueous)	4.08	3.97[b]	4
Novalumine solution[d]	1.27	1.29[c]	1.3
Novatropine tablets[e]	2.27	2.35[c]	2.5

[a]U.S.P. (titrimetric) [13]. [b]Gravimetric (AgBr) [12, 14]. [c]The reference gravimetric method was not applicable to these samples. The bromide content was found by potentiometric titration with $AgNO_3$. [d]Oily solution also containing phenobarbital (16 mg ml^{-1}). [e]mg/tablet; unspecified excipients.

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Short Communication

ELECTROSORPTION OF VITAMIN K_1 AT MERCURY AND ITS DETERMINATION AT SUBMICROGRAM LEVELS BY DIFFERENTIAL PULSE VOLTAMMETRY AT A HANGING MERCURY ELECTRODE

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Summary. The electrochemical behaviour of vitamin K_1 has been studied in ethanolic and methanolic acetate buffers by cyclic voltammetry and differential pulse voltammetry (d.p.v.) at the h.m.d.e. and differential pulse polarography at the d.m.e. Increased adsorption occurs at the mercury electrode as the percentage of water is increased. The most sensitive signal was obtained by d.p.v. with acetate-buffered 60% methanolic solutions; vitamin K_1 could then be measured down to 10 ng ml^{-1}.

Vitamin K_1 is important in biological systems, particularly in bone formation, and a differential pulse polarographic (d.p.p.) method for monitoring the substance in plasma has been reported [1]. Although this method proved useful for the study of clearance rates of vitamin K_1 from plasma after an intravenous injection, basic information that might allow improvement of the sensitivity to enable basal circulating levels to be measured was lacking. Flanagan et al. [2] have reported the possibility of improving the sensitivity of the (d.p.p.) technique by the use of a hanging mercury drop electrode when the substance under test undergoes electrosorption at the mercury surface. Recently, Vire and Patriarche [3] discussed the adsorption of vitamin K_1 at a mercury electrode from methanolic solutions; similar observations have been made for the structurally related compounds vitamin K_2 [4] and ubiquinone [5]. Therefore, the possibility of enhancing the sensitivity of the d.p.p. method for vitamin K_1 by using adsorption phenomena was considered worthy of investigation. In order to understand these phenomena more fully, it was decided to study various solutions of the vitamin with several electrochemical techniques.

This communication presents a systematic study of the adsorption of vitamin K_1 at a mercury electrode by cyclic voltammetry (c.v.), differential pulse polarography (d.p.p.) and differential pulse voltammetry (d.p.v.). Optimisation of conditions for determining submicrogram levels, by varying the composition of the solution and by conditioning the electrode, is discussed.

Experimental

Chemicals and equipment. Vitamin K_1 (phylloquinone; Sigma Chemical Company) was used as received. All other reagents were of analytical reagent grade (B.D.H.).

A Princeton Applied Research (PAR) Model 174A Polarographic Analyzer equipped with a PAR Model 172A drop timer assembly and a Servoscribe 1 s potentiometric recorder was used for all measurements. A three-electrode system was used in all cases. For d.p.p., this consisted of a saturated calomel electrode (s.c.e.) reference electrode, platinum counter electrode and dropping mercury electrode (d.m.e.) which had a natural drop time of 8.0 s (flow rate 0.69 mg s^{-1}) in 1 M potassium chloride solution. For d.p.v. and c.v., the working electrode was a PAR Model 9323 hanging mercury drop electrode (h.m.d.e.).

Procedures. The 70 and 90% (v/v) methanolic and ethanolic acetate buffers were prepared by mixing a 1 M sodium acetate/acetic buffer solution (pH 6.0), alcohol and distilled water to give a final electrolyte concentration of 0.05 M. Stock solutions of vitamin K_1 were prepared in ethanol or methanol and aliquots of these solutions were diluted with the necessary quantities of buffer components to give the desired concentration. All glassware was protected from light throughout the course of the studies. Current—voltage curves were recorded on solutions which had been deaerated for 15 min with oxygen-free nitrogen, which had been passed through vanadium(II) chloride solution and the appropriate alcoholic buffer solution.

Cyclic voltammetry was done by dialling a six-division drop on the h.m.d.e. and applying an initial potential of —0.1 V for 15 s prior to scanning. Voltage scan rates of 10, 20, 50 and 100 mV s^{-1} were employed and at a potential about 200 mV beyond the peak the potential was scanned back to the initial value.

Differential pulse voltammetry was done under the following conditions: initial potential —0.1 V, scan rate 2 mV s^{-1}, modulation amplitude 50 mV, pulse time 2 s, and low pass filter 0.3 s. A six-division drop was dialled on the h.m.d.e. and the initial potential held for 60 s before scanning. At a potential approximately 200 mV beyond the peak the voltage was reversed back to the initial value. Differential pulse polarography was done under similar conditions.

Results and discussion

Cyclic voltammetry at the h.m.d.e. Cyclic voltammograms of vitamin K_1 in 70% and 90% ethanolic and methanolic buffers showed one peak on both the forward and reverse scans. This is consistent with a two-electron reduction to produce the corresponding hydroquinone on the cathodic scan and re-oxidation to the quinone on the anodic scan. Typical cyclic voltammograms for a 4 μM solution of vitamin K_1 in various alcoholic buffer solutions at a fixed scan rate are shown in Fig. 1. The peaks obtained in 70% ethanolic and methanolic acetate buffers show a high degree of symmetry. This indicates strong adsorption in both cases, but from the magnitude of the peak current,

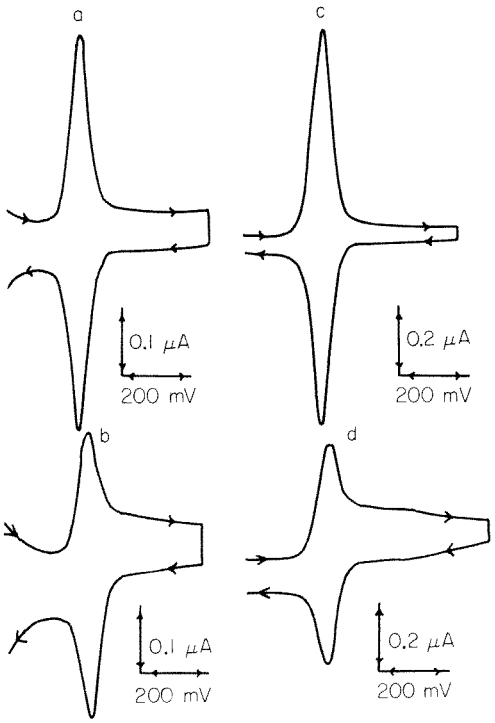

Fig. 1. Cyclic voltammograms for acetate-buffered 4 μM vitamin K_1 solutions; (a) 70% ethanol; (b) 90% ethanol; (c) 70% methanol; (d) 90% methanol. Initial potential −0.1 V, scan rate 100 mV s^{-1}.

adsorption appears greatest in 70% methanol—0.05 M acetate buffer pH 6.0. In 90% alcoholic buffers the peaks obtained for vitamin K_1 were smaller and were considerably less symmetrical than those obtained in 70% alcoholic buffers. This suggests that adsorption occurs to a lesser extent at the higher alcohol concentration. The separation between the cathodic and anodic E_p values indicates some degree of adsorption in all cases.

Graphs of the current function $i_p/cv^{1/2}$ vs. $v^{1/2}$ (where i_p is the peak current in μA, c is the concentration in mmol l^{-1}, and v is the scan rate in mV s^{-1}) were constructed for vitamin K_1 dissolved in the four solutions, at a constant concentration. As can be seen in Fig. 2, the current function increased with scan rate in all cases for both cathodic and anodic currents. This again is evidence for the magnitude of peak current being dependent on adsorption of both reactant and product. If the current had been totally diffusion-controlled, the $i_p/cv^{1/2}$ vs. $v^{1/2}$ function would not have increased.

The above data indicate that the largest peak current is to be expected in an acetate buffer containing 70% methanol. This is considered to be the result of adsorption at the mercury surface of the hydrophobic side-chain of vitamin K_1, and this is greatest at the lower concentration of methanol.

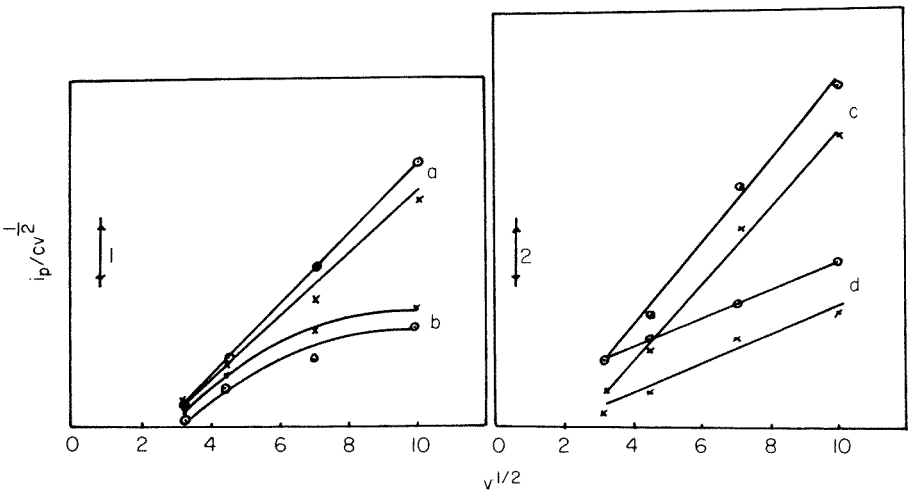

Fig. 2. Graphs of $i_p/cv^{1/2}$ vs. $v^{1/2}$ for acetate-buffered 4 μM vitamin K_1 solutions: (a) 70% ethanol; (b) 90% ethanol; (c) 70% methanol; (d) 90% methanol. (○) Cathodic scan; (×) anodic scan.

This behaviour has also been reported in the case of the two structurally related compounds vitamin K_2 [4] and ubiquinone [5].

Differential pulse polarography at the d.m.e. and differential pulse voltammetry at the h.m.d.e. Differential pulse voltammograms at the h.m.d.e. were recorded on vitamin K_1 dissolved in ethanolic and methanolic acetate buffers, at a concentration of 4 μM, by scanning cathodically then anodically. As was observed in cyclic voltammetry, the largest peak current was obtained in an acetate buffer containing 70% methanol. Again the cathodic peak currents were greater than the anodic peak currents, except for the solution containing 90% ethanol where presumably adsorption was greater for the reduced form.

In contrast to the above, d.p.p. peak currents at the d.m.e. were found to increase as the percentage of alcohol was increased. In addition, the peak current obtained for a solution containing vitamin K_1 and a hydrotropic agent (pentadecylbenzene sulphonate) in 70% methanolic 0.05 M acetate buffer was larger than that obtained for an identical solution without the hydrotropic agent (Fig. 3). This type of behaviour has been reported for several aromatic compounds by Franklin and Iwunze [6], who suggested that micelle formation was partly responsible for the increase in current. Therefore, increasing the solubility of vitamin K_1 apparently causes an increase in d.p.p. peak current, but decreases the d.p.v. peak current. This might be explained if lowering the solubility of the substance caused an aggregation of molecules which resulted in lowering the magnitude of the diffusion coefficient. Since the d.p.p. peak current is predominantly diffusion-controlled a decrease in current would be expected as the aggregation increased. The opposite effect would be expected with the d.p.v. technique because the current is predominantly due to reduction of adsorbed vitamin K_1.

The most sensitive signal observed for vitamin K_1 at a concentration of 4 μM was obtained by using the d.p.v. technique with a 70% methanolic acetate buffered solution (Fig. 4). This shows a marked increase in sensitivity over the d.p.p. technique.

Calibration graphs. Attempts were then made to develop a d.p.v. procedure, based on the above solution conditions, for the determination of vitamin K_1 at low levels at the h.m.d.e. In a preliminary investigation the 70% methanolic medium was used with a vitamin K_1 level of 20 ng ml^{-1}, but only small ill-defined peaks were obtained, indicating that the original procedure was unsuitable. An improvement in sensitivity was achieved by carrying out repetitive runs on the same hanging mercury drop with a 1-min plating period between runs, and a further increase was obtained by decreasing the percentage of methanol to 60%. Differential pulse voltammograms of a solution containing vitamin K_1 (10 ng ml^{-1}) in 60% methanolic acetate buffer are shown in Fig. 5. Clearly five successive scans on the same drop yield a well-defined and sensitive signal suitable for quantitative purposes. A calibra-

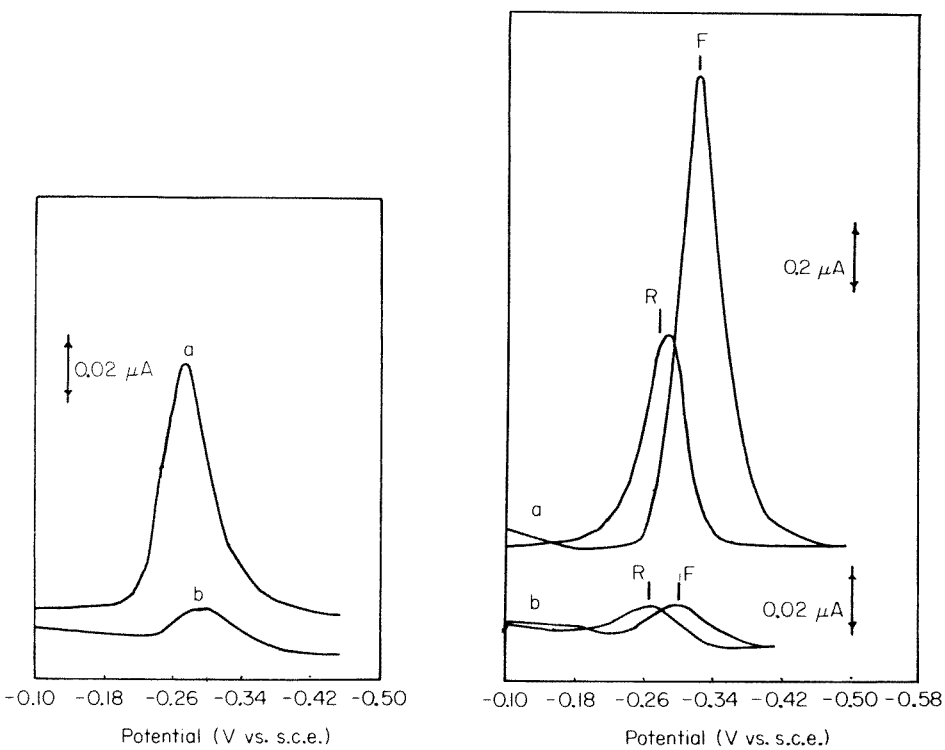

Fig. 3. Differential pulse polarograms of 4 μM vitamin K_1 in 70% methanolic acetate buffer-pH 6.0 (a) with and (b) without pentadecylbenzene sulphonate.

Fig. 4. (a) Differential pulse voltammograms and (b) differential pulse polarograms, of a 4 μM vitamin K_1 solution in 70% methanolic acetate buffer. F = forward scan and R = reverse scan.

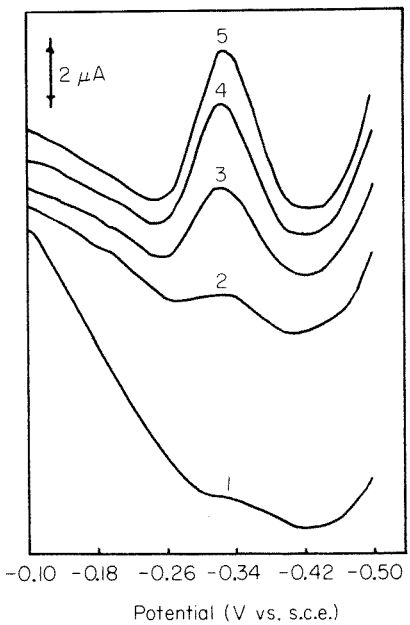

Fig. 5. Differential pulse voltammograms of a 10 ng ml^{-1} solution of vitamin K_1 obtained by repetitive scans on the same drop with 1-min plating times between scans: the numbers on the curves indicate the number of repetitive scans.

tion graph obtained over the range 10—100 ng ml^{-1} by using the five-scan procedure was found to be rectilinear. The coefficient of variation for ten determinations at a concentration of 20 ng ml^{-1} was 8.95%. The standards were prepared by evaporating methanolic solutions of vitamin K_1 in 15 ml conical centrifuge tubes which had been silanized with a 5% solution of dimethyldichlorosilane in carbon tetrachloride. This coating procedure was found to be necessary to prevent loss of vitamin K_1 by adsorption onto the glass walls of the evaporating vessels. Aliquots of methanol were added to the residues and after vigorous shaking a portion of the resulting solutions was transferred to the polarographic cell containing the methanolic acetate buffer.

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Short Communication

DETERMINATION OF PHENOL IN THE PRESENCE OF SULFITE (SULFUR DIOXIDE) BY THE 4-AMINOANTIPYRINE SPECTROPHOTOMETRIC METHOD

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Summary. The interference of sulfite (sulfur dioxide) with the 4-aminoantipyrine (4-AAP) spectrophotometric method for phenol is discussed for procedures without distillation, and with distillation in the absence and presence of copper(II) sulfate. Sulfite represses the color development in all these procedures. Without chloroform extraction, the maximum tolerable amounts of sulfite in the procedures without distillation, distillation without copper(II) sulfate, and distillation with copper(II) sulfate are 15, 10, and 20 mg/100 ml, respectively. For the extraction method, the limits are 4.0, 4.0, and 10 mg/100 ml, respectively. Copper(II) sulfate catalyzes the air-oxidation of sulfite. Phenol can be determined in the presence of large amounts of sulfite by treating with sulfide to form polythionates and thiosulfate; excess of sulfide is removed with copper(II) sulfate, sulfuric acid is added, the phenol is distilled, and the 4-AAP method is applied. Improvements are made in the overall accuracy of the 4-AAP method.

Sulfite (sulfur dioxide) interferes with the determination of phenol by the 4-aminoantipyrine (4-AAP) spectrophotometric method but there is little information on the extent or nature of the interference. Brief mention is made that phenol, in the presence of the relatively small amounts of sulfite encountered in water, can be determined by the 4-AAP method after distillation in the presence of copper(II) sulfate [1—3]; however, the limitations of the method insofar as the interference of sulfite is concerned have not been established. Prior aeration has been used to remove sulfite [1—3]. Phenol has been determined titrimetrically [4—6] or spectrophotometrically using diazotized *p*-nitroaniline [7] in the presence of relatively large amounts of sulfite encountered in connection with the sulfonation process for manufacturing phenol [8], the paper industry, and syntans; however, the methods are not readily applicable to the determination of small amounts of phenol in the presence of large amounts of sulfite.

The present communication reports a study of the effect of sulfite on the 4-AAP method (with and without extraction), and the limitations of the copper(II) sulfate distillation procedure with regard to sulfite interference. Methods for the removal of large amounts of sulfite are described, and the accuracy of the 4-AAP method is improved.

Experimental

Apparatus and reagents. The distillation apparatus has been described [9]. The 90-ml delivery pipet was obtained on special order from Ace Glass Co., Vineland, NJ.

Phenol solutions no. 1 (1.00 mg ml^{-1}), no. 2 (0.10 mg ml^{-1}), and no. 3 (0.005 mg ml^{-1}) were prepared as described earlier [9]. Potassium hexacyanoferrate(III) solution (16%) and 4-AAP solution (4%) were stored in brown bottles and were prepared every 4 and 2 weeks, respectively. Copper(II) sulfate pentahydrate solution (25%) was prepared by dissolution of the salt in hot water and cooling. Sodium sulfide nonahydrate solution (8%) was prepared fresh every 2 weeks. Sodium sulfite solution no. 1 (10 mg SO_3^{2-} ml^{-1}) was prepared fresh every 3 days by dissolving 7.90 g of the salt in water and diluting to 500 ml in a volumetric flask. Sodium sulfite solution no. 2 (1.0 mg SO_3^{2-} ml^{-1}) was prepared fresh daily by appropriate dilution.

Preparation of calibration curve without extraction. Transfer 0.00—4.00 ml of phenol solution no. 2 to 150-ml beakers and dilute to 80—90 ml. Add 1.0 ml of ammonium chloride solution (10%) and adjust to pH 10.0 ± 0.2 with (1 + 1) ammonia solution. Add 1.0 ml of 4-AAP solution (4%) and 1.0 ml of potassium hexacyanoferrate(III) solution (16%) and mix. Wash into 100-ml volumetric flasks and dilute to the mark with water. After 5 min, measure the absorbance at 510 nm against distilled water. Deduct the blank and plot absorbance against mg of phenol (per 100 ml).

Calibration curve for the extraction method. Transfer 0.00—10.00 ml of phenol solution no. 3 to 600-ml beakers and dilute to about 500 ml with water. Add 5 ml of ammonium chloride solution (10%) and adjust to pH 10.0 ± 0.2 with (1 + 1) ammonia solution. Add 1.5 ml of 4-AAP solution (4%) and 1.5 ml of potassium hexacyanoferrate(III) solution (16%), mix, and allow to stand for 5 min. Wash into 1-l separatory funnels, add exactly 25 ml of chloroform, and shake vigorously for 60 s. Allow to settle, swirl vigorously, and allow to settle again. Filter 21—23 ml of the chloroform layers into dry flasks through dry 9-cm no. 40 Whatman filter papers containing about 2 g of anhydrous sodium sulfate. Within 5 min, measure the absorbance at 460 nm against chloroform. Deduct the blank and plot absorbance against mg of phenol (per 500 ml).

Procedures. For samples containing less than 20 mg of SO_3^{2-} per 100 ml for the method without extraction and less than 10 mg of SO_3^{2-} per 100 ml for the extraction method, proceed as follows. Transfer 500 ml of the sample to a 1-l round-bottomed flask that has been calibrated at 550 ml. If the presence of oxidants is suspected, add 10 ml of sodium arsenite solution (10%) [10], followed by methyl orange indicator solution (0.05%). Add sulfuric acid (1 + 9) until the color changes to a definite pink, and then add about 5 drops in excess. If the indicator is destroyed, use indicator paper (to pH 2.5—3). Add 5 ml of the copper(II) solution (25%) and dilute to about 550 ml. Distill 500 ml into a calibrated 500-ml Erlenmeyer flask. For the method without extraction, pipet an aliquot of the distillate (up to 90.0 ml)

containing preferably 0.15—0.35 mg of phenol into a 150-ml beaker, dilute to 80—90 ml (if necessary), and proceed as described in the preparation of the calibration curve. For the extraction method, distill the sample as in the method without extraction. For samples containing up to 0.01 mg of phenol per 100 ml, transfer the entire 500 ml of distillate to a 600-ml beaker; for samples containing 0.01—0.05 mg of phenol per 100 ml, dilute an appropriate aliquot of the distillate to about 500 ml in a 600-ml beaker. Proceed as described for the preparation of the calibration curve for the extraction method.

For samples containing more than 20 mg of sulfite per 100 ml for the sample without extraction, or more than 10 mg of sulfite per 100 ml for the extraction method, transfer 400 ml of the sample to the 1-l round-bottomed flask. Add 10 ml of sodium arsenite catalyst solution (10%) and 50 ml of the sodium sulfide solution (8%) (or 85 ml if 100—200 mg of sulfite is present per 100 ml). Swirl the solution, add 25 ml of the copper(II) solution (25%) (or 35 ml to samples treated with 85 ml of the sulfide solution), and swirl again. Add 10 ml of sulfuric acid (1 + 9), swirl, dilute to about 550 ml, and proceed with the distillation and spectrophotometric measurement. Use the appropriate calibration curve to evaluate phenol concentration.

Results and discussion

Interference of sulfite with the 4-AAP method. The interference of sulfite with the 4-AAP method without distillation, and the distillation procedures with and without copper(II) sulfate, were studied using standard phenol and sulfite solutions (Table 1). For these studies, the phenol was determined by preparing the calibration curve and taking a 100-ml portion of the distillate in the manner used previously for the study of the interference of oxidizing agents, aromatic amines, and formaldehyde [9, 10]. This technique is advantageous because it permits ready comparison of results obtained without and with distillation on the basis of mg of interfering substance and phenol per 100 ml. It can be seen from Table 1 that sulfite interferes by repressing the color development. It is believed that the mechanism of the repression involves a reaction between the sulfite and the 4-AAP—phenol complex. The addition of a larger excess of 4-AAP or potassium hexacyanoferrate(III) solution did not reduce the extent of the interference to an appreciable extent. Distillation did not necessarily decrease the interference of sulfite; this was surprising because it was expected that the interference would decrease considerably because of oxidation of the sulfite (to sulfate) and volatilization of sulfur dioxide. Apparently, the extent of the oxidation of the sulfite is restricted by the limited amounts of oxygen in the distillation flask, while the loss of sulfur dioxide by volatilization is small because of the large solubility of sulfur dioxide in water (1 volume of water dissolves 45 volumes of sulfur dioxide at 15°C [11]). Distillation in the presence of copper(II) sulfate decreases the interference of sulfite to a limited extent. The amount of the copper(II) solution (25%) used for the data in Table 1 was 5

TABLE 1

Interference of sulfite with the 4-AAP method for phenol

SO_3^{2-} (mg/100 ml)	Phenol found (mg/100 ml)			
	Without distillation		With distillation	
	Direct[a]	Extraction[b]	Direct[a]	Extraction[b]
0	0.301	0.0060		
1	0.288			
2		0.0062	0.295[c]	
			0.295[d]	
4		0.0058		0.0056[c]
				0.0058[d]
5	0.288	0.0043		0.0045[c]
				0.0058[d]
10	0.288	0.0029	0.288[c]	0.0021[c]
				0.0056[d]
15	0.282	0.0005		0.0030[d]
20	0.255	0.0000	0.194[c]	0.0000[d]
			0.282[d]	
25	0.097		0.210[c]	
			0.255[d]	
30	0.074		0.228[d]	
50			0.038[c]	
			0.157[d]	
100			0.019[c]	
			0.012[d]	

[a]Phenol added: 0.300 mg/100 ml. [b]Phenol added: 0.0060 mg/100 ml. [c]No Cu(II) added. [d]Cu(II) added.

ml, but it is not critical. The use of sodium arsenite and copper(II) sulfate together had no greater effect than copper(II) sulfate alone. The effectiveness of copper(II) sulfate in reducing the interference from sulfite is probably due to the catalytic behavior of Cu^{2+} in promoting the air-oxidation of sulfite [11].

From the data in Table 1, it is concluded that in the method without extraction, the maximum permissible amounts of sulfite that can be present without causing significant interference in the procedures without distillation, distillation without copper(II) sulfate, and distillation with copper(II) sulfate are 15, 10, and 20 mg/100 ml, respectively. For the extraction method, the figures are 4.0, 4.0, and 10.0 mg/100 ml, respectively.

Some experiments indicated that the interference from sulfite during distillation (without and with copper(II) sulfate) tended to increase at high acidity. For this reason, it is recommended that when smaller amounts of sulfite are present, the distillation be conducted at pH 2.5—3.

It was found that the interference of sulfite in the copper(II) sulfate distillation procedure decreased when sulfide was also present, because of a reaction between sulfite and sulfide. This observation led to the develop-

ment of the method described below for eliminating the interference of sulfite.

The effect of aeration in eliminating the interference of sulfite was investigated by passing a fairly rapid stream of air for 10 min through solutions containing phenol and sulfite at pH 7, 4, and 2 and determining phenol by the method without distillation. The results obtained by this method were satisfactory in the presence of up to 25 mg SO_3^{2-}/100 ml at any pH examined, but were low for 50 and 100 mg SO_3^{2-}/100 ml. In any case, aeration is not recommended because it can cause oxidation of phenol in samples containing diverse inorganic or organic substances, as in sewage.

Elimination of the interference from larger amounts of sulfite. The method for the elimination of the interference of larger amounts of sulfite with the 4-AAP method is based on the destruction of sulfite by treatment with sodium sulfide, as noted above. The mechanism of the reaction of sulfite and sulfide is complex and not well understood. Under the conditions used here, it would be expected that a mixture of polythionates ($S_3O_6^{2-}$, $S_4O_6^{2-}$, $S_5O_6^{2-}$, and $S_6O_6^{2-}$) and thiosulfate would be produced [12, 13]. It was found that the reaction proceeds more smoothly in the presence of sodium arsenite, which apparently acts as a catalyst. Polythionates and thiosulfate do not interfere with the 4-AAP method, probably because they are not strong reducing agents. The formation of elemental sulfur, as in Wackenroder's solution, was never observed under the conditions used. Wackenroder's solution, which is primarily a mixture of colloidal sulfur and polythionates, is ordinarily made by passing hydrogen sulfide into a sulfur dioxide solution until all the sulfur dioxide is destroyed [11, 12]. After treatment with the sulfide in the proposed method, the excess of sulfide is removed by precipitation with copper(II) sulfate. Sulfuric acid is then added and the solution is distilled. The acid must be added after the copper(II) sulfate; otherwise, elemental sulfur (which distills over and interferes with the 4-AAP method) will be produced. The amounts of sodium sulfide (and copper(II) sulfate) used in the proposed method were established experimentally, because the stoichiometry of the reaction between sulfite and sulfide is uncertain. It was found that 85 ml of the recommended sulfide solution could handle up to 200 mg of SO_3^{2-} per 100 ml, which is an amount greater than would ordinarily be encountered. Larger amounts of sulfite could probably be handled by adding a larger amount of sulfide, but this was not investigated. This larger amount of sulfide would have to be added as the solid in order not to exceed the 550-ml volume for the distillation.

The 12 results obtained for the method without extraction averaged 0.291 ± 0.004 mg/100 ml for 0.300 mg/100 ml phenol determined in the presence of 0—200 mg SO_3^{2-}/100 ml. The 11 results obtained for the method with extraction averaged 0.0058 ± 0.0002 mg/100 ml for 0.0060 mg/100 ml phenol determined in the presence of 0—200 mg SO_3^{2-}/100 ml.

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Short Communication

SPECTROPHOTOMETRIC DETERMINATION OF INORGANIC PHOSPHATE IN SOIL EXTRACTS BY A SINGLE-SOLUTION NON-REDUCTIVE METHOD

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Summary. A previous method involving crystal violet—12-molybdophosphate formation is modified by including all reagents into a single solution. The apparent molar absorptivity is 0.84×10^5 l mol^{-1} cm^{-1}. The results obtained for a series of soil extracts correlate well with those obtained by a reductive method.

Most of the methods proposed for the spectrophotometric determination of inorganic phosphate in soil extracts and in other aqueous solutions are based on the formation of a 12-molybdophosphate in an acidic medium and its subsequent reduction to a molybdenum blue [1—6]. Many alternative methods are available [4], but in connection with a proposed soil survey a simple reliable procedure with a minimum number of manipulations was required. A method [7] based on crystal violet was therefore re-examined and optimised in a single-reagent solution mode, and this was found to be superior to single-solution reductive methods [5, 6]. The basis of the procedure, similar to that for equivalent reactions with other basic dyes [8, 9], is the formation of an insoluble blue—violet dye salt, crystal violet—12-molybdophosphate, which is kept in colloidal solution by a surface-active agent, and is determined spectrophotometrically. The excess of dye is protonated to form a doubly charged green cation.

Experimental

Apparatus. Single wavelength spectrophotometric measurements were made using a Pye Unicam SP6500 and 1-cm silica cells at room temperature.

Samples. A range of soil samples (pH 4.8—7.6) was obtained from Knocklayd Mountain. Co. Antrim, Northern Ireland. This area is deficient in available phosphorus and has a long slope with a range of soil parent materials and hence a wide range of soil pH (Table 1). The samples, M1—M12, were air dried overnight at 30—35°C, homogenised and reduced in bulk, ground and passed through a 2-mm sieve, collected and stored in screw-cap glass jars. The samples were subjected to characterisation analysis; pH [10], organic carbon [11], exchangeable cations [12], total exchangeable bases (TEB)

TABLE 1

Soil sample characterisation data

Sample	pH	Org. C[a]	Ca^{2+b}	Mg^{2+b}	Na^{+b}	K^{+b}	N[a]	TEB[b]	CEC[b]	Description
M1	5.5	4.08	38.8	13.0	1.1	1.4	0.45	53.4	42.6	Basalt
M2	5.5	4.40	40.0	13.2	1.1	0.80	0.42	55.1	42.7	Basalt
M3	6.3	4.68	36.3	14.6	1.1	0.20	0.34	52.5	38.9	Mineral/Basalt
M4	6.9	6.78	52.5	0.30	0.20	1.4	0.66	54.4	42.9	Chalk/Basalt
M5	5.9	4.14	15.0	0.02	0.30	0.20	0.46	15.5	38.8	Chalk/Basalt
M6	7.2	5.65	67.5	0.18	0.30	1.0	0.34	68.9	42.6	Calcareous
M7	7.6	5.64	46.3	0.02	0.20	0.30	0.23	46.8	30.6	Calcareous
M8	6.6	4.26	40.0	0.30	0.20	0.40	0.50	41.0	37.8	Calcareous
M9	6.9	2.52	26.3	0.16	0.30	0.30	0.28	26.8	19.2	Schist
M10	6.9	3.42	31.3	0.28	0.10	0.30	0.38	31.9	20.0	Schist
M11	7.1	4.50	30.0	0.16	0.11	0.30	0.26	30.6	24.9	Schist
M12	4.9	5.94	7.5	2.4	0.10	0.40	0.59	10.5	38.0	Sandy Schist

[a]Weight percentage. [b]Milliequivalent percentage.

[12], cation exchange capacity (CEC) [13] and nitrogen contents [14] were determined by standard procedures. The results are summarized in Table 1.

Extraction. Soil samples were extracted with four commonly used soil extracts, at appropriate soil:solution ratios, by shaking 100—200 ml of extractant and the soil in a 250-ml Erlenmeyer flask on an orbital shaker for 30 min at approximately 200 oscillations/min. The extractants were filtered through Whatman No. 40 paper. The extractants and conditions were as follows. A 0.5 M sodium hydrogencarbonate solution containing 5 ml of a 0.05% solution of polyacrylamide (m.w 5×10^6, B.D.H.) per litre, and adjusted to pH 8.5 with 1 M sodium hydroxide [15, 16], was used at a soil: solution ratio of 1:20. An ammonium acetate solution (pH 4.4) [17] was prepared by mixing 575 ml of glacial acetic acid carefully with 175 ml of ammonia liquor, adjusting the pH to 4.4 with glacial acetic acid and diluting to 5 l; this was used at a soil:solution ratio of 1:25. For the acetic—sodium acetate extractant [18], 0.5 M acetic acid was adjusted to pH 4.8 with 0.5 M sodium acetate; this was used at a soil:solution ratio of 1:5. For the sulphuric acid extractant [19], 10^{-3} M sulphuric acid was buffered to pH 3 by addition of ammonium sulphate (3 g l^{-1}); this was used at a soil:solution ratio of 1:200.

Reference phosphorus determinations. These were done by a single-solution modification [5] of a molybdenum blue procedure [6].

Reagents. All solutions were prepared in deionised water from Analar-grade reagents unless otherwise stated. For the standard phosphate solution (50 μg P ml^{-1}) dissolve 0.2128 g of potassium dihydrogenphosphate in water and dilute to 1 l. Prepare working standards daily by suitable dilution. For the crystal violet solution (5×10^{-4} M) dissolve 0.51 g of crystal violet (technical dye grade, Hopkin and Williams) in water, add 140 ml of aqueous

1% (w/v) poly(vinyl alcohol) solution and dilute to 250 ml. For the sodium molybdate solution (0.1 M) dissolve 2.42 g of sodium molybdate dihydrate in 80 ml of 24% (w/v) sulphuric acid and dilute to 100 ml with that acid. To prepare the single reagent solution, mix 50 ml of 5×10^{-4} M crystal violet solution with 200 ml of 0.1 M sodium molybdate solution and allow to stand for 1 h before use.

Procedure (0—2 μg phosphorus). Place a 1.00-ml aliquot of sample solution (except for the sulphuric acid extracts, where 5 ml is taken) in a 50-ml beaker, add 3 ml of the single reagent solution, swirl to mix, heat at 35°C for 20 min on a water bath, and then cool. Measure the absorbance at 560 nm in a 1-cm silica cell against a reagent blank. Determine the concentration of the sample from a calibration graph prepared by using the same procedure.

Results and discussion

There are three main forms of phosphate present in soils, designated as non-available, potentially available and immediately available. The last form, known to be soluble orthophosphate, is the most important for plant nutrition. It has not been possible to extract from soils amounts of phosphate truly representative of those taken up by plants, mainly because all plants do not react identically to the available and potentially-available phosphate in a soil solution [2]. The various extractants used in soil examination have been found to correlate with a particular crop response on a specific type of soil. It is desirable that any analytical method for routine use be sensitive, simple and compatible with these common soil extractants.

Preliminary studies were directed to combining the three reagents used in the previous method [7] into a single solution. It was found that 24% (v/v) sulphuric acid was the best acid medium for this single solution and that sodium molybdate was more sensitive than ammonium molybdate in this combined reagent. The method developed herein was four times more sensitive than the reductive method in respect of the slopes of the calibration graphs; both were linear over the range 0—1 μg P ml^{-1}. The improvement in sensitivity is mainly the result of the lower dilution during colour formation in the crystal violet method. The apparent molar absorptivity is 0.84×10^5 l mol^{-1} cm^{-1}.

The results for the determination of available phosphorus in soil samples of a wide range of pH by the reductive method and by the non-reductive crystal violet single solution method, given in Table 2, correlate well [20].

No significant interferences were found for ions likely to be present in soil extracts at weight ratios of 20:1 to phosphorus (Table 3). The high sensitivity of the method allows the analysis of infertile soils having very low concentrations of available phosphate, and the simplicity of the system should allow ready automation.

TABLE 2

Phosphate contents (μg P ml^{-1}) of soil extracts determined by the crystal violet (CV) and reduction (R) methods

Sample	$NaHCO_3$, pH 8.5[a]		HOAc/NaOAc, pH 4.8[b]		NH_4OAc, pH 4.4[c]		H_2SO_4, pH 3.0[d]	
	CV	R	CV	R	CV	R	CV	R R
M1	0.200	0.130	0.299	0.300	0.141	0.130	0.020	0.030
M2	0.130	0.130	0.311	0.300	0.167	0.160	0.010	0.030
M3	0.030	0.040	0.030	0.000	0.027	0.020	0.056	0.050
M4	0.380	0.350	0.164	0.140	0.300	0.280	0.065	0.060
M5	0.147	0.120	0.114	0.110	0.116	0.110	0.000	0.000
M6	0.546	0.540	0.200	0.170	0.392	0.390	0.094	0.100
M7	0.130	0.130	0.060	0.050	0.270	0.280	0.126	0.120
M8	0.360	0.360	0.154	0.140	0.276	0.200	0.050	0.070
M9	0.760	0.830	1.950	2.090	1.826	1.780	0.367	0.370
M10	1.010	1.040	2.000	2.070	1.995	1.880	0.395	0.410
M11	0.720	0.750	1.560	1.580	1.530	1.380	0.300	0.310
M12	0.680	0.580	0.500	0.530	0.485	0.500	0.020	0.020

Correlation coefficients: [a]0.9691; [b]0.9982; [c]0.9991; [d]0.9904.

TABLE 3

Effect of other ions on the crystal violet method (0.5 μg of phosphorus)

Ions added (10 μg each)	None	Ca + Mg	Zn + Pb	K + Na	Al + Fe	Cu + Si
Absorbance	0.326	0.325	0.319	0.320	0.325	0.329

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Short Communication

STUDIES WITH DITHIZONE
Part 33. Effect of Temperature on the Spectrum in Chloroform†

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Summary. The position and relative intensity of the two peaks in the visible absorption spectrum of dithizone dissolved in chloroform do not change on cooling from room temperature to −48°C. Thus either the two absorption peaks cannot be ascribed to two discrete species (thiol and thione tautomers), or the enthalpy of their interconversion is zero.

Solutions of dithizone (3-mercapto-1,5-diphenylformazan; H_2Dz) in organic solvents exhibit two well-defined absorption maxima in their spectrum in the visible region, one at ca. 610 nm, the second at ca. 450 nm. The positions of these maxima vary slightly on changing the organic solvent but there are more pronounced changes in the magnitude of the "peak ratio", defined as the ratio of the intensities of absorption at the two maxima [1].

Although dithizone could exist in two tautomeric forms (thiol ⇌ thione), each of these could exist in a considerable number of conformations (viz., 16 stereochemical modifications with a further 64 if charge-separated species are included [2]). However, many of these possibilities can be excluded on steric or other grounds. It has generally been assumed that the two pronounced absorption peaks are associated with the presence of an equilibrium mixture of thiol–thione tautomers; the peak at the shorter wavelength has been attributed to the thiol form and that at the longer wavelength to the thione form [1].

Since the customary spectrum of dithizone is immediately obtained when the reagent is released from its sodium salt, NaHDz, by treatment with an acid, or from any of its metal complexes, $M(HDz)_n$, by treatment with the appropriate reversion agent, it must be assumed that the tautomeric equilibrium is established very rapidly. To seek additional information, the effect of temperature on the equilibrium (and hence on the spectrum) has been investigated.

Table 1 reports values for the peak ratio $R = A_{605}/A_{445}$ of solutions in chloroform which had been maintained at −48°C for 0.5—3.0 h and for the same solutions at room temperature (23 ± 1°C). The mean values of R are

†Part 32: P. A. McCallum, H. M. N. H. Irving, A. T. Hutton and L. R. Nassimbeni, Acta Cryst., B36 (1980) 1626.

TABLE 1

Peak ratios ($R = A_{505}/A_{445}$) for solutions of dithizone in dry chloroform

Conc. ($\times 10^{-5}$ mol l^{-1})	0.83	0.83	1.26	0.83	0.83	0.83	0.69
R at 23 ± 1°C	2.67	2.71	2.71	2.73	2.63	2.65	2.54
R at −48°C	2.54	2.73	3.06	2.93	2.76	2.88	2.24

2.66 ± 0.02 at 23°C and 2.73 ± 0.19 at −48°C. The spectra (not reproduced) were unchanged apart from showing the small displacement expected from the increase in concentration consequent on cooling to the freezing point of n-hexanol. There was no statistically significant change in the peak ratio since the mean standard deviations quoted are those calculated for 7 observations at a confidence level of 95%. If the optical absorptions at the peak maxima really reflect the concentrations of the two postulated tautomers, it becomes necessary to accept the surprising conclusion that the conversion from thiol to thione form and the reverse process proceeds with no change in enthalpy. The alternative explanation, viz. that the two bands do not originate from two discrete organic molecules, is supported by theoretical calculations by Spěváček and Spěváčková [3] and by a variety of physical methods involving nuclear magnetic resonance spectroscopy on dithizone, its homologues and ^{13}C- and ^{15}N-substituted derivatives which will be reported elsewhere [4, 5].

Experimental

Measurements were made with an Optica spectrophotometer (modified by and placed at our disposal by Professor E. Caldin). The low-temperature bath was provided by a mush of solid n-hexanol (b.p. 155—156°C) in contact with its liquid. Pure chloroform and redistilled dry chloroform were prepared by established methods [1] and all glassware was rigorously purified from metal contaminants. A complete spectrum could be recorded in 3—4 min. Absorbance readings on this modified Optica appear to be high, because spectra for all the solutions used when measured on a Unicam SP500 gave R = 2.57 ± 0.03. This instrumental bias could have no influence on the present results which rest only on the ratio of absorbances measured on the same instrument at the same wavelengths.

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Short Communication

A REDUCTION—SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF SULPHATE IN WATERS

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Summary. The sulphate is reduced by tin(II) chloride in hydriodic acid to hydrogen sulphide which is swept by nitrogen into a buffered solution of bis(2,9-dimethyl-1,10-phenanthroline)copper(II) ion. The resulting copper(I) complex is measured spectrophotometrically. With sample volumes between 10 and 1000 μl, the limit of detection is 0.5 μg of sulphate. Formation of the stable complex is immediate and the reduction system can be used repeatedly. The method is applicable to fresh, estuarine and marine waters.

Spectrophotometry following reduction to hydrogen sulphide is an attractive approach to the determination of sulphate in water. Hydrogen sulphide is easily separated from interfering species and can be measured sensitively, for example, by means of methylene blue formation [1]. Published methods based on this approach, however, have a number of shortcomings. These include intolerance of the reduction systems to water (which necessitates prior evaporation of water samples or frequent replacement of the reduction mixture) and possible generation of phosphine from some mixtures containing phosphorus. In the various methylene blue procedures, colour formation is rather slow, and strict control of conditions is required for reproducible results.

The reducing mixture for the method described below includes tin(II) and hydriodic acid; this mixture is relatively insensitive to water content. Colour is developed by reaction of the hydrogen sulphide with the bis(2,9-dimethyl-1,10-phenanthroline)copper(II) ion. The colour forms rapidly in the trapping reagent, and is stable over a wide temperature range for at least 24 h. The method is analogous to that of Davis and Lindstrom [2] who employed hydriodic acid—hypophosphorous acid as the reductant with formation of the tris(1,10-phenanthroline)iron(II) complex for spectrophotometry. The equipment needed for the proposed method is considerably simpler.

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Experimental

Apparatus. The reduction—absorption system comprised a 250-ml three-necked round-bottom flask, to which was attached a vertical Liebig condenser of effective length 150 mm, which was in turn connected to two 125-ml Drechsel bottles with porosity 0 sintered heads; the glassware was connected by glass and tygon tubing. The water samples were injected through a puncture-type silicone rubber seal in one neck of the flask, using syringe pipettes fitted with push-button dispensers and 4-in. Luer-type hypodermic needles. The nitrogen flow was directed to the bottom of the flask through an inlet tube and monitored with a wet-type gas meter at the end of the system. Absorbance measurements were made with a Varian-Techtron model 634 spectrophotometer.

The nitrogen flow rate and the reduction time specified in the procedure are appropriate to this apparatus. If glassware of different capacity was used, it would be necessary to determine an appropriate flow rate and reduction time.

Reduction mixture. Mix 5 g of tin(II) chloride dihydrate (Merck), 5 ml of water and two or three drops of 10 M hydrochloric acid in the three-necked flask, swirling to dissolve the solid. Add 150 ml of hydriodic acid (Merck, 57% w/w) and, with a stream of nitrogen passing through the mixture, boil it gently for 30 min in the open flask. If kept under nitrogen, this reagent is stable for at least two months.

Other reagents. For the buffer solution of pH 4.0, mix 410 ml of 0.2 M acetic acid and 90 ml of 0.2 M sodium acetate and dilute to 1 l with water. For the pH 4.8 buffer, mix 200 ml of 0.2 M acetic acid and 300 ml of 0.2 M sodium acetate and dilute to 1 l with water.

For the copper—DMP reagent, dissolve 0.11 g of 2,9-dimethyl-1,10-phenanthroline (DMP) hydrochloride (neocuproine-HCl, Merck) in water, add 20 ml of a solution containing 1.87 g of copper sulphate pentahydrate per litre, and dilute to 100 ml with water. This reagent should be used within three days.

For the wash solution place 60 ml of buffer solution pH 4.0 in the first Drechsel bottle and add 10 drops of bromophenol blue indicator (0.1% w/v solution in ethanol).

Procedure. Place 5.0 ml of copper—DMP reagent and 5.0 ml of buffer solution pH 4.8, in the second Drechsel bottle and dilute to about 40 ml with water; this is the trapping reagent. Assemble the apparatus as outlined above. Bring the reduction mixture to a gentle boil over a Bunsen burner, with nitrogen passing through the system at 250—300 ml min^{-1}.

Inject a sample of the water under test through the silicone rubber seal. Any volume up to 1 ml containing not more than 45 μg of sulphate (or 15 μg of total inorganic sulphur) can be injected. In this work, a sample volume of 10 μl was used for sea water and 100—1000 μl for fresh waters. Boil the reduction mixture for a further 10 min, and then remove the Drechsel bottle containing the trapping reagent. Flush the sinter of this bottle with 4—5 ml

of acetone, transfer the reagent and washings quantitatively to a 50-ml volumetric flask, and dilute to volume with water. Measure the absorbance at 454 nm with water in the reference cell. The colour is stable between 10 and 30°C for at least 24 h.

Carry out a blank determination with an equal volume of distilled water. The blank absorbance should not exceed 0.02. Calculate the concentration of sulphate in the sample by comparison with sulphate standards taken through the entire procedure.

For repeated determinations, keep the reduction mixture boiling gently between runs. Replace the wash solution only when the colour change indicates that the pH has dropped below 3. The reduction mixture tolerates up to 50 ml of water; with 100-μl sample volumes, each batch may be used for up to 500 determinations if kept under nitrogen.

Results and discussion

Tin(II) salts and hydriodic acid have both been widely used for the reduction of sulphate to hydrogen sulphide, in combination with phosphorus or phosphorus compounds [1, 3], but they do not appear to have been used previously together. The efficiency of sulphate reduction by the proposed mixture was studied as a function of the composition of the mixture and the amount of water present, by comparing the absorbances produced by the generated hydrogen sulphide with those obtained directly from solutions of sodium sulphide. These determinations were completed by the methylene blue procedure of Gustafsson [4]. The sodium sulphide solutions were freshly prepared from the nonahydrate in oxygen-free water and standardised iodimetrically just before use [5]. Sulphate reduction in the proposed method was thereby shown to proceed with better than 97% efficiency in the presence of up to 50 ml of water.

The trapping reagent is based on the system used earlier for the determination of sulphide and sulphite in water [6]. Reaction with hydrogen sulphide produces the bis(DMP)copper(I) ion (ϵ_{max} = 6.15 × 10^3 l mol^{-1} cm^{-1} at 454 nm, with sulphate as the counter ion in water). The slope of the calibration graph corresponds to an 8-electron oxidation of hydrogen sulphide back to sulphate, proceeding with 93% efficiency.

Calibration, precision and limit of detection. Standard sulphate solutions were prepared from sulphuric acid or reagent-grade anhydrous sodium sulphate [7]. In the range 0—45 μg, the amount of sulphate injected (y μg) could be calculated from the absorbance (x) obtained with 1-cm cells and corrected for the blank, by means of the equation $y = 105.3x$.

The precision of the proposed method was assessed by carrying out repeated determinations of standard solutions. The results are shown in Table 1. The limit of detection, taken as the amount of sulphate which gave an absorbance equal to twice the standard deviation of a set of 12 absorbance readings at or near blank level, was 0.5 μg (corresponding to a concentration of 0.5 mg SO_4^{2-} l^{-1} for a sample volume of 1000 μl).

TABLE 1

Precision of proposed method

Sulphate taken (μg)	Mean sulphate found[a] (μg)	s (μg)	s_r (%)
1.00	0.99	0.18	18
3.00	3.06	0.20	7
15.0	15.3	0.3	2
45.0	44.6	0.8	2

[a]Mean of 8 determinations.

Interferences. The common inorganic sulphur-containing anions (sulphide, sulphite, thiosulphate, tetrathionate and dithionate) interfere quantitatively with the proposed method, as they are all reduced to hydrogen sulphide with the same efficiency as sulphate. No interferences were observed with species containing C—S bonds, such as linear alkylbenzene sulphonates. Thus results by the proposed method can be expressed as total inorganic sulphur. Qualifications to this statement are that thiocyanate ions respond to the method with 70% efficiency, and that surface-active alkyl sulphates interfere quantitatively. The latter compounds, however, are not ingredients of household and industrial detergents and are unlikely to be present in natural waters.

The effects of various non-sulphur ions on the recovery of sulphate by the proposed method are reported in Table 2. The major ions of sea water do not interfere seriously, and the method may be used without modification for the determination of sulphate in marine and estuarine waters. Sea water of salinity 34.0‰ was found to contain 2685 (±30) mg SO_4^{2-} l^{-1} (or 2620 (±30) mg kg^{-1}), from 8 determinations. The standard sulphate solutions were prepared in distilled water and the same sample volume (10 μl) was used for both the sea water and the standards.

TABLE 2

Allowable concentrations of foreign ions[a]

Concentration	Ion
0.5 M	F^-, Cl^-, Br^-, I^-
0.1 M	NO_3^-
2000 mg l^{-1}	NO_2^-, $CH_3CO_2^-$
1000 mg l^{-1}	Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+}, Cr^{3+}
500 mg l^{-1}	PO_4^{3-}, HPO_4^{2-}, $H_2HPO_4^-$

[a]100 (±3)% recovery of 37.5 μg of sulphate (in 1000 μl) was obtained in the presence of the stated concentrations.

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Short Communication

SAMPLE PREPARATION IN ROUTINE ANALYSIS FOR QUARTZ IN DUST BY X-RAY DIFFRACTOMETRY

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(Received 18th February 1981)

Summary. To achieve better reproducibility in the x-ray diffractometric determinations of quartz, the particle size of samples and of the internal standard substance, and the pore size of filters, should be set in the ranges <10 μm and 0.5—0.8 μm, respectively with loadings less than 10 mg cm^{-2}.

Considerable attention has been given to the concentration of quartz in atmospheric dust in working areas, because inhalation of quartz dust can cause fatal lung diseases such as silicosis. Though the determination of quartz in air by means of x-ray diffractometry has been described [1—4], practical details suitable for routine analytical applications are not easily available. In connection with standardization of routine procedures, the present communication is concerned with sample preparation so as to achieve better reproducibility in diffraction intensity measurements. The particle size of sample, the pore size of filter on which samples are mounted and the amount of sample loaded are discussed.

Experimental and results

Measurements. A Shimadzu Model GX-III diffractometer was used with a Philips cobalt-anode x-ray tube which was operated at 30 kV and 10 mA. A film of iron oxide served as the x-ray filter throughout the experiments. Nickel oxide, which is fairly stable and gives an intense diffraction pattern, was employed as the internal standard [5]. Some measurements were extended to Fe_2O_3, ZnO and $CaCO_3$, because they are widely distributed in dust.

Particle size. Silica, Fe_2O_3, ZnO and $CaCO_3$ (all reagent grade) were ground to fine powders with a ball mill for 1 h. The portion of particles in the range of 10—40 μm was first separated by sieving. The fraction <10 μm was dispersed in methanol and was allowed to stand for 2 h. Particle sizes of <1 μm and 1—10 μm were collected from the suspension and from the precipitate, respectively. This is based on the sedimentation speed calculated from the Stokes' equation: $D = [18\ H\ \eta/(P_f - P_s)\ T\ g]^{1/2}$, where D is the particle diameter (cm), H the distance of sedimentation (cm), T the time

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required for sedimentation (s), g the acceleration under gravity (980 cm s^{-2}), P_s the density of the medium (g cm^{-3}), P_f the density of the material (g cm^{-3}), and η the viscosity coefficient of the medium (g cm^{-1} s^{-1}).

Samples for diffraction measurements were mounted on a limited area (35-mm diameter) of membrane or glass-fiber filters by suction. The variation in diffraction intensity from SiO_2 of different particle sizes mounted on membrane (0.45- and 1.20-μm pore size) and glass-fiber filters is shown in Fig. 1(a). The diffraction intensity tends to decrease as the particle size becomes smaller and differs significantly on different types of filter.

The powdered internal standard NiO, the particle size distribution of which was arranged in the same manner as for the samples, was mixed with aliquots of the respective sample powders in equal amount. The variation in intensity ratio of the silica and nickel oxide diffraction lines is shown in Fig. 1(b). These data show that smaller particles tend to give smaller intensity ratios, but the ratios for particle sizes of <1 μm and of 1—10 μm on any of the three filters agree well within experimental error (less than 10%), whereas the ratios for the 10—40-μm particles vary significantly on different filters.

Pore size of filter. The diffractive efficiency of quartz is known to be sensitive to the pore size of filter on which samples are mounted (Fig. 2(a)). Intensity ratios of SiO_2, Fe_2O_3 and ZnO to NiO on several membrane filters with pore sizes in the range 0.45—1.20 μm are given in Fig. 2(b); the ratios for these materials are almost unchanged for filters in the pore size range 0.45—0.80 μm.

Amount of sample loaded on filter. When the amount of sample loaded

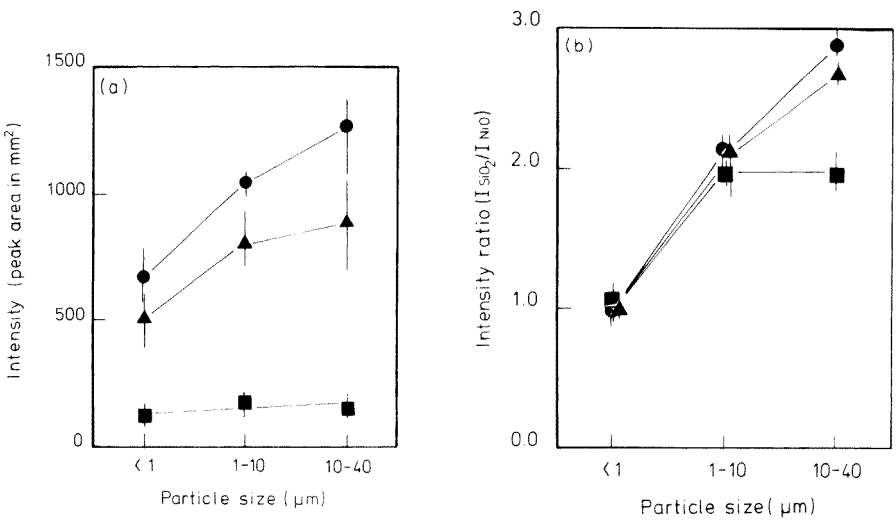

Fig. 1. (a) Variation in diffraction intensity from SiO_2. (b) Intensity ratio of diffraction line from SiO_2 to that from NiO. (•) Membrane filter (0.45-μm pore size); (▲) membrane filter (1.20-μm pore size); (■) glass-fiber filter.

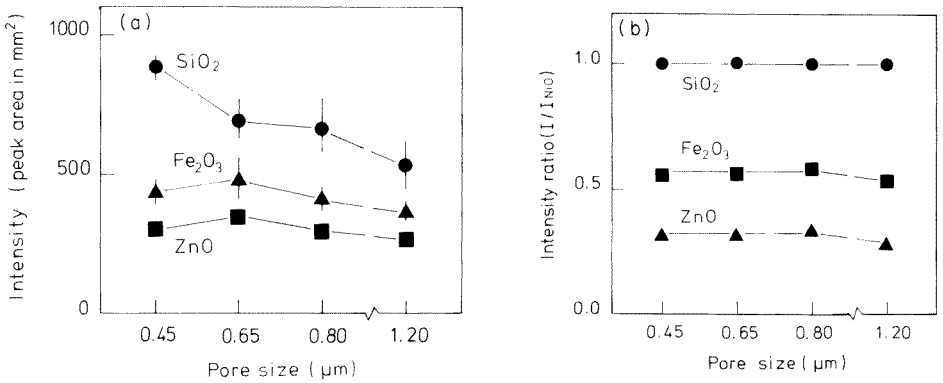

Fig. 2. (a) Variation in diffraction intensities from SiO_2, Fe_2O_3 and ZnO on membrane filters with different pore sizes. (b) Intensity ratios of diffraction lines from SiO_2, Fe_2O_3 and ZnO to that from NiO. Diffraction intensities for SiO_2, Fe_2O_3, ZnO and NiO were deduced from the 101, 104, 101 and 200 lines, respectively.

for measurement exceeds 200 μg cm^{-2}, x-rays are absorbed significantly in the sample. Though this effect is cancelled theoretically when the internal standard methods is applied, it may be necessary to set a margin of loading sample so as to yield a good reproducibility. Figure 3 shows the results obtained for several materials at different loadings. The intensity ratios of Fe_2O_3, ZnO and $CaCO_3$ to NiO are apparently constant up to 3.0 mg cm^{-2} whereas that of SiO_2 decreases significantly when the loading exceeds 1.0 mg cm^{-2}.

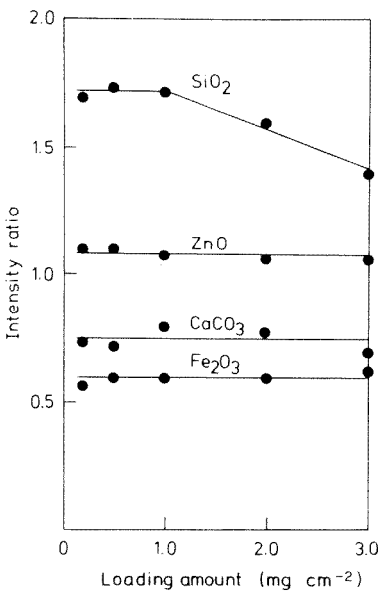

Fig. 3. Variation of intensity ratio of diffraction lines from SiO_2, ZnO, $CaCO_3$ and Fe_2O_3 to that from NiO with loading.

TABLE 1

Quartz contents of some samples

Sample	Matrix	Quartz content (%)	Reproducibility[a] (%)
Sedimentary dust[b]	Carbon, Fe_2O_3	21.6	—
	Carbon, Fe_3O_4	30.8	13.6
Haematite[c]	Fe_2O_3, Fe_3O_4	0.26	19.0
Fluorspar[d]	Fe_2O_3, Fe_3O_4	5.90	—
Volcanic ash[e]	Feldspar	17.1[f]	11.2
		15.7[g]	—

[a]Maximum deviation from the averaged value for ten samples separately prepared. [b]Accumulated in foundries. [c]Rompin haematite, a reference material issued by the Iron and Steel Institute of Japan (JSS 800-3). [d]Fluorspar for iron and steel making, a reference material issued by the Iron and Steel Institute of Japan (JSS 881-2). [e]Ash falls in the 1979 eruption (Oct. 28, 1979) of the Ontake Volcano. Collected on Nov. 17—18, 1979 at Kaida-mura. [f]13.8 km from the crater. [g]13.4 km from the crater.

Discussion

From these observations the following experimental procedure is tentatively recommended in order to achieve better reproducibility in routine analysis for quartz.

The particle size of the samples and the internal standard should be $<10\,\mu m$. The internal standard is mixed with an equal amount of the sample. The resulting mixture is suspended ultrasonically in water or methanol, for instance, and mounted uniformly on a membrane filter. It is preferable to use a filter with a pore size in the range 0.5—0.8 μm. The loading of material on the filter should not exceed 1.0 mg cm^{-2}. The time required for this sample preparation after collection of the required particle size will be less than 30 min.

The proposed method was applied to the determination of quartz in sedimentary dust, iron ores and fresh volcanic ash, with the results given in Table 1.

The authors thank Y. Suzuki, Meiji University, for helpful discussions.

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Short Communication

TITRATIONS OF SULPHONAMIDES IN CATIONIC MICELLAR SYSTEMS

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(Received 20th February 1981)

Summary. A titrimetric determination of some sulphonamides with 0.1 M sodium hydroxide in the presence of hexadecylpyridinium chloride is described. Potentiometric titrations are slow; visual titrations are satisfactory. The pK_a shift can be interpreted in the light of general micellar behaviour.

Sulphonamides have received much attention from the analytical point of view, and various quantitative methods have been reported [1]. Titrations with alkaline solutions involve the use of non-aqueous solvents [2], because the sulphonamides are weak acids and are generally sparingly soluble in water. Recently, there has been considerable interest in the use of micellar systems in analytical procedures [3]. Micelles are colloidal aggregates of amphiphilic molecules with a hydrocarbon-like core and a polar surface. Their role in solubilization processes [4] as well as their ability to influence equilibria [5, 6] and rates of reactions [7], can be useful in a variety of analytical situations. The use of surfactants in some pharmaceutical applications has also been reported [4, 8].

The present communication deals with titrations of sulphonamides in the presence of a cationic surfactant, hexadecylpyridinium chloride (HPC).

Experimental

Reagents. Hexadecylpyridinium chloride (Merck) was recrystallized before use. Sulphonamides (Farmitalia, C. Erba) were used as supplied. Other reagents were C. Erba products.

Procedure. Potentiometric titrations were carried out with a Metrohm E388 instrument with a combined glass electrode (Metrohm E109), at 25.0°C. It was necessary to wait for several minutes for equilibration of the electrode system after each addition of 0.1 M sodium hydroxide. For the visual titrations with phenolphthalein indicator, a weighed quantity of sulphonamide was dissolved in the surfactant solution and titrated with 0.1 M sodium hydroxide. The HPC concentration was 0.20 M, well above the critical micellar concentration [7], in all cases.

Results and discussion

The potentiometric curves for the investigated sulphonamides are reported in Fig. 1. In the presence of the cationic surfactant, the pK_a values relevant to aqueous solutions [9] are lowered for all the investigated sulphonamides. The extent of variation can be probably related to the different hydrophobicity of the derivatives [5, 10]. This behaviour has been clearly explained in terms of association of the acid substrate and its conjugated base with the positive micelles [10, 11].

The effect of anionic surfactant (e.g. sodium dodecyl sulphate) goes in the opposite direction, i.e. anionic micelles increase the apparent pK_a [5, 10], and so the use of cationic systems is preferred in the titration of weak acids [12, 13]. The results of visual titrations are collected in Table 1. The precision and accuracy are reasonable. The micellar systems appear to be an interesting alternative to non-aqueous solvents in some acid—base titrations.

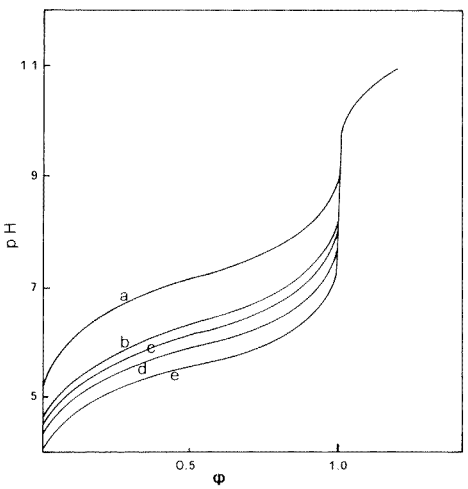

Fig. 1. Potentiometric titrations of sulphonamides (ca. 50 mg) in 0.20 M HPC with 0.1 M NaOH: (a) sulphapyridine; (b) sulphadimidine; (c) sulphamerazine; (d) sulphadiazine; (e) sulphathiazole.

TABLE 1

Results of visual titrations of sulphonamides with 0.1 M NaOH

Sulphonamide	Recovery (%)[a]	Sulphonamide	Recovery (%)[a]
Sulphapyridine	100.3 ± 0.7	Sulphadimidine	100.8 ± 0.6
Sulphadiazine	100.6 ± 0.6	Sulphathiazole	100.2 ± 0.5
Sulphamerazine	100.7 ± 0.7		

[a]Average of 5 experiments with standard deviation, in the range 20—120 mg.

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Erratum

P. L. Buldini, Q. Zini and D. Ferri, Differential Pulse Polarographic Determination of Traces of Molybdenum in Solar-Grade Silicon.
Anal. Chim. Acta, 124 (1981) 233—236.

p. 233. The author "Q. Zini" was accidentally omitted from the title page of this article.

ANALYTICA CHIMICA ACTA, Vol. 128 (1981)

AUTHOR INDEX

EUROPEAN ANALYTICAL COLUMN

WORKING PARTY ON ANALYTICAL CHEMISTRY
FEDERATION OF EUROPEAN CHEMICAL SOCIETIES

In 1980 the Bulgarian Chemical Society joined the Working Party on Analytical Chemistry (WPAC); Prof. Jordanov participated in the 11th meeting of the WPAC on behalf of the official representative Prof. Bliznakov. As of January 1981, 27 national societies of 21 European countries were represented by 31 members in the WPAC. Among the member societies of the Federation of European Chemical Societies (FECS), only the Pancyprian Union of Chemists and the Turkish Chemical Society are not represented in the WPAC.

The 11th meeting of the WPAC was held in Graz on August 26th, 1980, during the 8th International Michochemical Symposium. The following items were discussed.

Euroanalysis IV, August 23rd–28th, 1981, Helsinki. This conference, the 25th event of the FECS, will be organized to cover a wide range of topics as on earlier occasions. The programme will include plenary lectures by Prof. D. Dyrssen (Göteborg), Dr. H. Egan (London), Prof. K. Hadži (Ljubljana) and Prof. Ramendik (Moscow); nine keynote lectures; special oral sessions on: Analytical Chemistry, the Analyst and Society, and Mass Spectrometry in Inorganic Analysis; and a special poster session on Symbolism in Analytical Chemistry. Over 1300 preliminary registrations from 44 countries and more than 600 preregistered papers have been received.

FECS 1981 Lecture. The Advisory Board has given the 1981 FECS lecture to the WPAC on the occasion of the Euroanalysis IV conference, the major FECS event in 1981. The lecture will be given by Prof. Bengt Samuelsson of the Karolinska Institutet, Stockholm, and will deal with prostaglandins and thromboxanes. The lecture will be held at the Finlandia Hall, Helsinki, on August 25th, 1981, and will be open to all participants and accompanying persons.

FECHEM Conference on Education in Analytical Chemistry in a Changing World. This conference was held in Vienna on April 9–11, 1980, and organized under the chairmanship of Prof. H. Malissa. Seven plenary lectures were presented and the following topics were discussed in detail: objectives of teaching analytical chemistry; chemometrics and information theory; teaching of problem solving; textbooks of analytical chemistry; computers in analytical chemistry; and definition of analytical chemistry. The plenary lectures at this conference and summaries of the discussions will be published in a special issue of Fresenius Zeitschrift für Analytische Chemie. The results of this conference together with those of the round-table discussion during Euroanalysis III (Z. Anal. Chem., 4 (1979) 297) will form the basis for the next FECHEM conference on Education in Analytical Chemistry which is being planned in connection with Euroanalysis V 1984 in Krakow.

FECHEM Conferences on Computer-Based Analytical Chemistry, COBAC. It was decided to hold further conferences in 1982 and 1984 following the successful COBAC conference of September, 1979, in Portorož. COBAC II will be organized as a FECHEM conference on April 28 and 29, 1982, in connection with Analytica '82 in Munich by the Fachgruppe Analytische Chemie of the GdCh (conference chairman, E. Fahr, Mainz). COBAC III is planned as a special session of Euroanalysis V in Krakow.

Euroanalysis V, 1984, Krakow, Poland. This conference will be held in the new conference center in Krakow, ready in 1981, and will again cover a broad range of topics, with special sessions.

Joint WPAC/IUPAC venture. A written report on the application of symbolism in analytical chemistry prepared by V. Simeonov, Sofia, was distributed to all WPAC members for consideration and further action. This group is organizing the special poster session on Symbolism in Analytical Chemistry during Euroanalysis IV; the posters will be introduced in keynote lectures by H. Malissa and V. Simeonov. Enquiries should be addressed to Prof. H. Malissa, TU, Vienna, Austria.

Standard Reference Materials. A written report on Sources of Reference Materials was prepared by R. Belcher and distributed to all WPAC members as a basis for further discussion at the 12th WPAC meeting in Helsinki.

Election of the Chairman of WPAC for 1981–84. Two nominations were submitted to the secretariat: Prof. E. Pungor nominated at the 11th WPAC meeting by the GdCh (Prof. Fresenius), and Dr. T.S. West nominated by the French Societies in the WPAC (Prof. Guiochon). Both candidates have agreed to the nomination.

Next (12th) meeting of the WPAC. The Finnish Chemical Society has invited the WPAC to hold its 12th meeting in Helsinki in connection with Euroanalysis IV.

Electrodes of Conductive Metal Oxides: Part A

edited by SERGIO TRASATTI, *Laboratory of Electrochemistry, University of Milan, Italy*

STUDIES IN PHYSICAL AND THEORETICAL CHEMISTRY 11

The discovery by Beer in the second half of the sixties that the performances of anodes made of thermally prepared noble metal oxides were better than those of noble metals provoked something of a technological revolution in the large electrolytic industry. Since then an ever increasing number of fundamental studies have been published but the large amount of data has, until now, not been adequately assimilated.

This two-part work provides a general unifying introduction plus a state-of-the-art review of the physicochemical properties and electrochemical behaviour of conductive oxide electrodes (DSA). The text has been divided into two volumes – Part A dealing mainly with structural and thermodynamic properties and Part B, to be published in due course, dealing with kinetic and electrocatalytic aspects. This division came about due to the large amount of material to be treated and also because, in a rapidly developing field, difficulties arise in collecting all relevant material at one given moment.

The editor approaches the subject from a multidisciplinary angle, for example, the electrochemical behaviour of oxide electrodes is presented and discussed in the context of a variety of physicochemical properties – electronic structure, nonstoichiometry, crystal structure, surface structure, morphology and adsorption properties. For the first time the different groups of oxides are treated together in order to place emphasis on their similarities and differences.

This major reference work is mainly directed to electrochemists and those working on catalysis. It will also be useful to those in the fields of materials science, physical chemistry, surface and colloid chemistry and in areas where oxide surfaces may play a major role as in chromatography and photochemistry.

CONTENTS: Chapters. 1. Electronic Band Structure of Oxides with Metallic or Semiconducting Characteristics *(J. M. Honig)*. 2. Chemisorption and Catalysis on Metal Oxides *(A. Cimino and S. Carrà)*.. 3. Oxide Growth and Oxygen Evolution on Noble Metals *(L. D. Burke)*. 4. Electrochemistry of Lead Dioxide *(J. P. Pohl and H. Rickert)*. 5. Properties of Spinel-Type Oxide Electrodes *(M. R. Tarasevich and B. N. Efremov)*. 6. Physicochemical and Electrochemical Properties of Perovskite Oxides *(H. Tamura, Y. Yoneyama and Y. Matsumoto)*. 7. Properties of Conductive Transition Metal Oxides with Rutile-Type Structure *(S. Trasatti and G. Lodi)*.

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