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EXTRACTION BASED ON THE FLOW-INJECTION PRINCIPLE
Part I. Description of the Extraction System

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(Received 25th November 1977)

SUMMARY

An extraction system has been developed, essentially consisting of a pump, a rotary valve and a spectrophotometer. The sample, 12—25 μl, is introduced via the rotary valve into an aqueous stream (flow injection). The aqueous stream, containing the sample plug, is divided into small segments by an organic phase and led into a Teflon coil so that a regular pattern of the two phases is obtained. No air bubbles should be present. Separation of the two phases is achieved in a specially constructed fitting and the absorbance of the organic phase is measured. The construction and performance of the system are illustrated by analysis of caffeine samples. Up to 100 samples/h can be analysed with a relative precision of better than 1%.

Flow injection analysis has been developed into a very useful and versatile technique [1—9]. Several methods are now applied routinely in many laboratories in Denmark and Sweden. Primarily, it is the small sample volume, the low reagent consumption [10] and the high sampling frequency that attract analytical chemists with heavy working schedules. Since extraction is widely used in pharmaceutical and clinical analysis to separate drugs or drug metabolites, it was logical to attempt to apply the flow-injection principle to this area, the aim being to achieve better economy of extraction methods with respect to time and solvent consumption.

In this first paper, the performance and construction of the extraction system are described. The determination of caffeine in acetylsalicylic acid preparations was selected as an example of a practical application. The preparations contain sodium lauryl sulphate, a surfactant that interferes with the transfer of caffeine from an aqueous to an organic phase, and so tetrapropylammonium bromide was added to the organic phase. The lauryl sulphate and the tetrapropylammonium ions form an ion pair [11] which transfers to the organic phase, but does not contribute to the absorbance measured at 275 nm.

EXPERIMENTAL

Reagents

The caffeine standard substance was of pharmacopoeia quality. The tetrapropylammonium bromide (puriss., Eastman-Kodak) was used as purchased. All other reagents and solvents were of analytical-grade quality. Stock and standard solutions were always freshly prepared.

The organic phase consisted of chloroform containing 1% tetrapropylammonium bromide. The aqueous phase contained 0.16 M sodium hydroxide, and was degassed before use.

Test solutions (always aqueous) of Bamyl-S-Caffeine and Bamyl-Caffeine tablets (Hässle AB, Mölndal, Sweden), were made up; both types of tablet contained 50 mg of caffeine/tablet according to the specifications. The tablets also contained acetylsalicylic acid (500 mg/tablet), potato starch, calcium carbonate, citric acid and sodium lauryl sulphate.

Apparatus and procedures

A five-channel peristaltic pump (model mp-GE; Ismatec, Zürich, Switzerland) with variable speed was provided with Acidflex pump tubes for the organic solvent and Tygon pump tubes for the aqueous solutions. The flow was found to change rather significantly during the first 5 minutes of pumping. No measurements were ever done during this period of time. Apparently, a certain warming-up of the pump tubes is required to obtain a constant flow.

The test solution was introduced into the aqueous stream by a rotary valve (Bifok AB, Sollentuna, Sweden) provided with a by-pass coil. In Fig. 1 the valve is in the filling position and the sample, *S*, can be introduced into the bore while the aqueous phase passes through the coil *b*. When the valve is turned 90°, the aqueous stream starts to pass through the core, thereby bringing the sample into the system. At the same time, the flow in coil *b* ceases completely. The sample volume was either 12 or 25 μl. The construction of the valve has been described in detail [12].

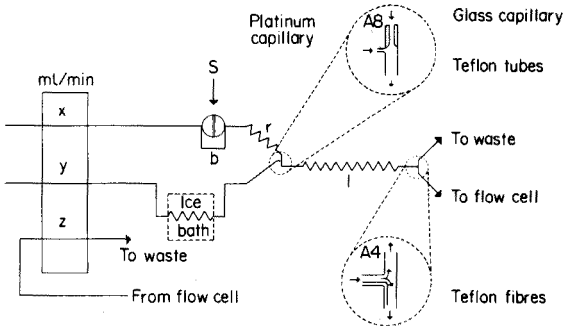

Fig. 1. Manifold for extraction based on the flow-injection principle. The flow-rates are (ml min^{-1}): *x* for the aqueous phase, *y* for the organic phase, and *z* for the fraction of the organic phase passing through the flow cell. *S* denotes the sample filling port of the rotary valve (12 or 25 μl).

The chloroform stream is chilled in an ice bath before being mixed with the aqueous stream, in order to prevent evaporation. The distance between the sample introduction point and the mixing point is denoted by r in Fig. 1. At the mixing point of the phases, a specially constructed fitting makes it possible to obtain a regular pattern of alternate aqueous and organic segments. The fitting is shown in detail in Fig. 1, and consists of a modified standard A8 T-connector (Technicon, Tarrytown, USA). Between the peristaltic pump and this fitting, all tubes for the aqueous phase are of polythene (i.d., 0.8 mm), and all tubes for the organic phase are of Teflon (i.d., 0.8 mm).

The organic stream is led into the platinum capillary while the aqueous stream enters the glass capillary. A Teflon tube is inserted into the outlet of the T-connector. A second Teflon tube is then inserted into the first one, and the positioning of the edge of this tube can easily be adjusted. Appropriate positioning is necessary for a regular mixing pattern. Furthermore, the length of the segments can be adjusted by the inner Teflon tube.

The extraction coils are made by winding Teflon tube on 50-ml beakers (diameter, 3.8 cm). The coils are not thermostatted.

The separating device consists of an A4 T-connector (Technicon, Tarrytown, USA) with Teflon fibres twisted together to a thread and inserted in the bend from the inlet down into the outlet directed towards the flow cell. By differential pumping, the aqueous phase as well as excessive organic phase are forced upwards in the fitting to waste, and no aqueous phase is sucked into the flow cell. Special care must be taken to avoid contaminating the flow cell with aqueous phase since its removal by rinsing was found to be a very tedious procedure. A ratio of 0.7/2.0 of z/y, i.e. the ratio between the flow of organic phase through the flow cell and the total flow of organic phase, was preferred. The separator must be fixed in a perpendicular position as illustrated in Fig. 1. The Teflon thread should be placed rather close to the lower wall of the inlet tube of the T-connector and allowed to bend smoothly and in parallel with the wall downwards.

The u.v. spectrophotometer was a Coleman 55 (Perkin-Elmer, Norwalk, USA), provided with a flow cell (Hellma, Müllheim, Germany) with a volume of 40 μl. The distance between the separator and the flow cell was kept as small as possible. A recorder (W + W 1100, Kontron, Zürich, Switzerland) was connected to the spectrophotometer.

RESULTS AND DISCUSSION

Determination of caffeine

A practical application of the extraction method is exemplified by results from a study of the dissolution rate of caffeine in an acetylsalicylic acid preparation (Bamyl, Hässle, Sweden). In Fig. 2, two calibration sets are shown bracketing samples taken after 1, 2, 3 and 5 min. Each test or calibration solution was injected in duplicate. The sampling rate was about 75/h. The flow rate of the aqueous phase, x, was 2.2 ml min^{-1}; the flow

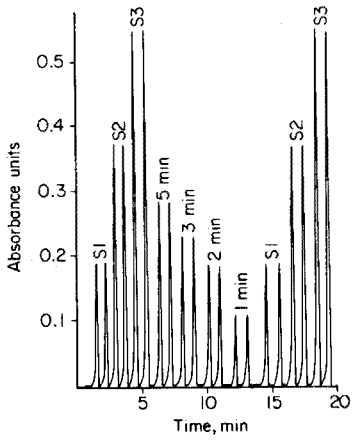

Fig. 2. Determination of caffeine in acetylsalicylic acid tablets (dissolution rate). Samples were taken after 1, 2, 3, and 5 min. Sampling rate 75/h; injected volume 25 μl. Standards: S1, 2.74×10^{-4} M; S2, 5.48×10^{-4} M; S3, 8.22×10^{-4} M. The aqueous flow stream was 0.16 M sodium hydroxide.

rates of the organic phases were y 2.0 ml min^{-1} and z 0.7 ml min^{-1} (see Fig. 1). The coil length, l, was 2 m, and r was 0.15 m. The volume of the bore in the valve was 25 μl. The absorbance was measured at 275 nm.

To obtain a measure of the repeatability of the method, the three different calibration solutions were injected at intervals during a 1-h period, in all ten times for each solution. The results are given in Table 1. The repeatability is excellent, and the values presented are typical for an optimized extraction system. Up to at least 100 samples/h can be analyzed with the present system, and base-line readings can still be made in between samples if the flow rates of the two phases are increased further.

Sample dispersion and coil lengths

Intuitively, the distance between the rotary valve and the mixing point of the two phases should be kept as short as possible to prevent dispersion of the injected sample. However, in the case of caffeine extraction from a solution which also contains acetylsalicylic acid, some mixing with the aqueous alkaline phase is desirable. The acid will then be retained in the

TABLE 1

Repeatability of the extraction method at three different sample concentrations

Caffeine sample solution (M)	Average of maxima of peaks (absorbance units)	Relative standard deviation (%)
2.74×10^{-4}	0.188	0.80
5.48×10^{-4}	0.370	0.63
8.22×10^{-4}	0.546	0.55

aqueous phase because of ionization at the high pH, while caffeine will be easily extracted into the chloroform phase. This means that the distance denoted by r in Fig. 1 must be adjusted empirically, so that the dispersion is kept at a minimum while mixing with the stream of alkali is possible. It might appear easier to make the sample solution alkaline before injection, but caffeine is not stable in a strongly basic solution [13]. The degradation during a residence time of less than 60 s in the extraction system is, however, not significant.

As expected, successive increases of r gave rise to decreases in the peak height (Table 2). Two caffeine solutions were injected; both contained 0.134 mg ml^{-1} (0.69 mM). The second solution also contained 1.34 mg of acetylsalicylic acid per ml (7.45 mM). The experiments were performed with all other parameters constant (x = 2.2, y = 2.0, z = 0.7 ml min^{-1}; l = 2 m; S = 25 μl). It is obvious that coextraction of acetylsalicylic acid does not occur at all, even in the extreme case when r is only 0.04 m.

The length of the mixing coil, l, was found not to be critical for values above 1 m. In one experiment, l was varied from 0.15 to 6 m. The results (Fig. 3) indicate that dispersion of the sample in the mixing coil, as well as degradation of caffeine in alkaline solution, are negligible. For values of l below 0.50 m, the extraction seems to be incomplete.

Extraction efficiency and size of segments

The results in Fig. 3 immediately raise the question of whether the extraction process is complete or not for a certain design of system. In order to investigate this further, the following experiment was performed.

The sodium hydroxide stream in the system was replaced by an aqueous caffeine solution, without introduction of air. The coil length, l, was 1 m. The steady-state value of the absorbance was measured, so that the concentration of caffeine in the chloroform phase could be evaluated; for this purpose, calibration solutions (in chloroform) were pumped separately and directly into the flow cell in a previous experiment. The proportion of the flows of aqueous and organic phases was obtained by measuring

TABLE 2

Variation of r, the distance between the valve and the mixing point of the two phases (see Fig. 1)

r (m)	Peak maximum for caffeine (absorbance units)	
	Caffeine alone	Caffeine + acetylsalicylic acid
0.04	0.46	0.46
0.08	0.46	0.46
0.15	0.47	0.47
0.30	0.46	0.46
0.45	0.43	0.43
0.60	0.42	0.42
1.00	0.37	0.37

the individual flow rates. The efficiency of the extraction was then calculated to be 101 ± 5%, the uncertainty being due to difficulty in measuring the exact flow rates. The extraction efficiency calculated for a coil length of 0.15 m (see Fig. 3) was about 75%, which is a remarkably high value.

The size of the segments of organic and aqueous phases in the mixing coil could be varied by adjusting the inner Teflon tube in the A8 connector (Fig. 1). A variation of the segment size in the range 1—10 mm had no influence on the peak height when a coil length of 2 m was employed. This is probably explained by the rapid and efficient extraction of caffeine. However, for other extractants the size of the segments is a very important parameter, and this will be reported in a later paper in this series.

Ratio of organic to aqueous phase and flow rates

The ratio of the organic phase to the aqueous phase affects the sensitivity of the method. Ideal conditions for a study of this ratio would be a set-up of three different pumps so that the flow of each stream, x, y, and z, could be varied individually (see Fig. 1). In a simplified study, y and z were kept constant while x was varied. The results are presented in Fig. 4. The test solution was a 1.04 mM caffeine solution and the valve volume was 25 µl. The maximum peak height is obtained for a volume ratio of the phases of about 1:1. Above this value the sensitivity drops drastically, probably because of the high total flow through the separator. The residence time of the sample in the separator is then shorter. Furthermore, the pressure in the system increases when the flow increases, and this may influence the

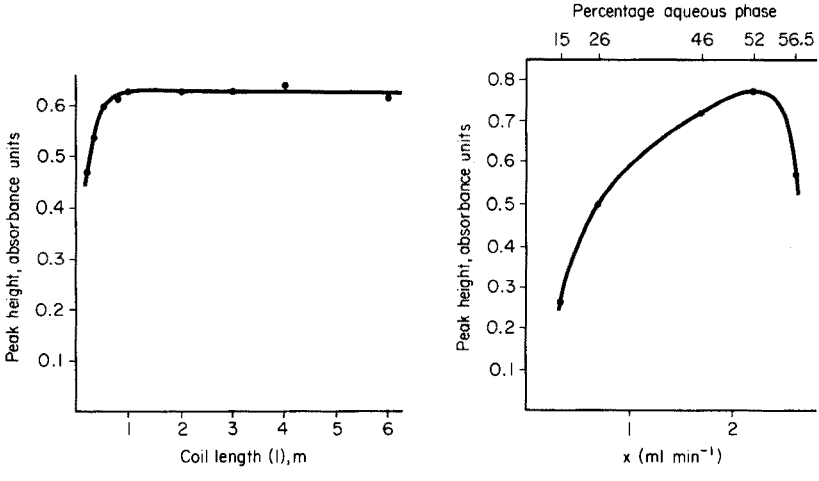

Fig. 3. Absorbance of peak maximum as a function of the length, l, of the mixing coil. Sample solution: ca. 8×10^{-4} M caffeine (aqueous). $x = 2.2$, $y = 2.0$, $z = 0.7$ ml min^{-1}; $S = 25$ µl; $r = 0.15$ m (see Fig. 1).

Fig. 4. Absorbance of peak maximum as a function of the flow of the aqueous phase, x. Sample solution: 1.04×10^{-3} M caffeine (aqueous). $y = 2.0$, $z = 0.7$ ml min^{-1}; $S = 25$ µl; $l = 2$, $r = 0.15$ m (see Fig. 1).

turbulence in the individual segments so that the extraction efficiency decreases. Reconstruction of the present separator will be necessary if the total flow rate is to be allowed to exceed 5 ml min^{-1}; the optimum flow rate seems to be about 4 ml min^{-1}.

Some experiments were done with Teflon tubes with an inner diameter of 0.5 mm. The flow rates of the two phases must then be decreased, otherwise the pressure in the system becomes excessive. The results obtained with this narrower Teflon tube were not completely satisfactory, and the reason for this lies in the separator. A low consumption of organic phase is desirable for economical and environmental reasons, so work is in progress to develop a new separator suitable for low flow rates.

Conclusions

The extraction system developed is very suitable for the simple extraction of caffeine from an aqueous solution. More advanced examples of applications are in progress.

The possibility of performing extractions on very small volumes is extremely valuable in many cases, e.g. in the field of clinical chemistry where this parameter is often the limitation of an application. At present, a sample volume of at least 40—50 μl is required to fill the rotary valve, but this figure can certainly be reduced further, and so can the consumption of reagents. The small volumes of sample and reagent, in combination with the high sampling rate and good precision, are the real attractions of this system.

The authors are indebted to Dr. P.-A. Johansson, Astra Pharmaceuticals, for valuable discussions.

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Analytica Chimica Acta, 98 (1978) 9—15

LIGAND-EXCHANGE CHROMATOGRAPHY OF AMINO SUGARS AND AMINO ACIDS ON COPPER-LOADED SILYLATED CONTROLLED-PORE GLASS

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SUMMARY

The application of silylated controlled-pore glass (CPG) particles as a stationary phase for the liquid chromatographic separation of selected amino acids and amino hexoses is reported. Copper-loaded columns prepared from CPG particles whose surface was silylated to immobilize an ethylenediamine functional group were employed. Copper bleeding from the column occurred but was compensated by 10^{-4} M copper in the ammoniacal eluent. Hydrolysis of the siloxane bonded to the surface limited the practical lifetime of a column to 50 h of continuous operation.

Since the phenomenon of ligand-exchange chromatography was first described by Helfferich [1], the technique has been applied to analytical problems ranging from amino acid determination in sea water, to the separation of stereoisomers and isotopes of nitrogen [2—6]. This versatile technique is essentially a modification of ion-exchange chromatography. In ligand-exchange chromatography, a metal ion is immobilized on a suitable support, most commonly an ion-exchange or chelating resin. The immobilized metal ion must retain available coordination sites such that ligands in the solution phase may bind to the metal ion on the stationary support. Exchange between the mobile and stationary phases then involves solvated ligands and ligands coordinated with the immobilized metal ion. Advantages of ligand-exchange chromatography over conventional chromatographic techniques are the speed, strength and selectivity of complex formation compared to simple physical adsorption or ionic attraction.

Ligand-exchange chromatography is not without shortcomings. The most annoying problem is the slow leaching of metal from the stationary phases. This is generally less of a problem in nonaqueous solvents. A large number of support materials have been proposed for use in ligand exchange chromatography [7, 8]. Chelating resins show less metal ion leaching than ion-exchange resins and cellulosic materials. However, there is a loss in capacity because

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more metal ion coordination sites are involved in the attachment of the metal to the chelating groups of the stationary phase [8].

Previous reports from this laboratory have described silylated controlled-pore glass as a preconcentration material prior to x-ray fluorescence determination of trace elements [9, 10]. The silylated controlled-pore glass acts as an immobilized chelating agent, removing trace metals from aqueous solutions. These materials were found to have capacities similar to chelating resins, yet equilibrated much faster with metal ions in solution. Controlled-pore glass support for the silanes also offers low flow resistance and relatively large surface area. These properties which made the silylated controlled-pore glass ideal for use as a preconcentration agent suggested that it could be used as a stationary phase in ligand-exchange chromatography. This paper describes a feasibility study involving the separation of three amino sugars and eighteen amino acids on copper-loaded silylated controlled-pore glass columns.

EXPERIMENTAL

Apparatus

A simple, low-pressure liquid chromatograph was assembled with a peristaltic pump, septum on-line injection fitting, and 6 mm × 20 cm and 6 mm × 30 cm glass columns. A Bausch-Lomb 505 u.v.—visible spectrometer equipped with a flow cell (250 μl, 1-cm path) and a strip-chart recorder, was used as the detector. The "Masterflex" peristaltic pump (Cole-Parmer Instruments Co.) employed was capable of a maximum pressure of 15 psi. As a result, the maximum obtainable flow rates were 2—4 ml min^{-1}, depending on the packed lengths of the column. A flow rate of 0.5—1.0 ml min^{-1} was used throughout this study. Samples were introduced into the chromatograph with a Hamilton 70-N μl syringe and a Pierce on-line Teflon injection fitting.

Reagents

The silylated controlled-pore glass was prepared by refluxing 10 g of "CPG 10" controlled-pore glass (Electronucleonics Inc.) in 100 ml of a 10% v/v solution of *N*-β-aminoethyl-γ-aminopropyltrimethoxysilane (Dow-Corning Z-6020) in toluene. After refluxing, the excess of Z-6020 and toluene were removed by filtration. The glass was "cured" by drying in an oven at 90°C for 12 h. This process results in a chelating material with a capacity of 0.4 mmol of Cu^{2+} per g. D-Galactosamine, D-mannosamine and D-glucosamine, all amino acids and dextran blue, were obtained from Sigma Chemical Co. (St. Louis, Mo.).

The buffer solutions (0.05 M) used in the pH study were: potassium hydrogenphthalate (pH 3.0), potassium hydrogenphthalate (pH 4.0), sodium acetate (pH 5.0), potassium dihydrogenphosphate (pH 7.0), boric acid (pH 9.0), and dipotassium hydrogenphosphate (pH 11.0).

Procedures

Silylated CPG-10 glass was added to the columns dry. The column was loaded with Cu^{2+} by pumping a 0.05 M copper solution adjusted to pH 10 with ammonia, through the column until breakthrough; all the bed material was dark blue. Zinc and nickel were examined as possible immobilized metal ions. However, the diamine functional silane did not retain these metals strongly enough and metal ion bleeding from the column was a severe problem.

As reported by Navratil et al. [11], the copper—amino sugar complexes absorb in the ultraviolet as do the amino acid complexes. Table 1 shows absorption maxima wavelengths observed here for 0.1% (w/v) solutions of the compounds investigated. The solutions were in ammonia—ammonium chloride buffer at pH 9.5 which was also 10^{-4} M in Cu^{2+}. Consequently, the u.v. detector was adjusted to measure absorbance in a band between 232 and 236 nm. The experimental procedure was essentially that of Navratil et al. [11] who used non-chelating ion-exchange resins for the column support. In the present case, the influent solution was 0.1 M ammonia adjusted to pH 9.5 with hydrochloric acid, and which was 10^{-4} M in copper(II) chloride.

The unit of measure for retention on the columns was the column void volume. This was determined with dextran blue, a high molecular weight polysaccharide whose size precludes its entering the pores (220 Å) of the CPG-10 glass beads. The retention volume of dextran blue represents the excluded or void volume of the column bed.

DISCUSSION

One part of the effort to develop the potential of ligand-exchange chromatography has been to investigate support materials for the metal ion. The most commonly used have been polymer resins with strong or weak acid, or chelating functional groups. The results obtained with those materials have been reviewed [12, 13]. Impregnation of metal salts into silica gel has also been employed [14]. Extensive applications in this

TABLE 1

Wavelengths of maximum absorption for complexes of amino acids and amino sugars with copper(II)

Compound	λ_{max} (nm)	Compound	λ_{max} (nm)	Compound	λ_{max} (nm)
Arginine	234	Tyrosine	280, 260[a]	Glucosamine	239
Aspartic acid	238	Tryptophan	282, 290, 240[a]	Galactosamine	239
Histidine	232[a]	Phenylalanine	238, 230[a]	Mannosamine	234
Lysine	234				

[a]Absorbance cutoff points at indicated wavelengths.

laboratory of covalently bonded chelating functional groups to silica gel or controlled-pore glass for the recovery of trace ions in aqueous solutions [9, 10, 15, 16] suggested that these materials may be suitable as a support material for ligand-exchange chromatography.

The nature of covalently bonded functional groups offer many attractive advantages. The bonding may be easily accomplished on a variety of materials such as glass, silica, metal oxides and other materials. Many suitable supports such as Partisil-10 and controlled-pore glass are commercially available. Siloxanes and chlorosilanes bearing a variety of functional groups are also available. The coupling reactions are simple to execute. The successful application of these materials in chromatography in cases in which non-polar functional groups are employed in non-aqueous media is well known. While this manuscript was in preparation, an application of silylated Partisil-10 to ligand-exchange chromatography in non-aqueous media was reported [17]. However, this attempt used a monofunctional primary amine as the stationary ligand. Chemical capacity and photoacoustic spectroscopic studies in this laboratory [18] and electron spin resonance studies by Alanko and Pinnavaia [19], show that copper is bound by the immobilized 1,2-diamine used in this study as a *bis* complex formed from two adjacent covalently bonded functional groups. It is likely therefore that the primary amine also forms a *bis* complex, although this has not been confirmed. Nonetheless, leaching of the copper ions is a problem in aqueous solutions. This is primarily caused by the fact that a counter ligand is required to displace strongly coordinated ligands from the metal coordination sphere. As shown by Navratil et al. [11], the use of a low concentration of copper in the influent maintains the column loading of copper and in the case of amino acids and amino sugars facilitates detection by ultraviolet absorption.

The major potential disadvantage of silylated materials in protic solvents is that the Si—O bonds are hydrolyzed by acids and bases. Therefore the bound functional groups may be leached from the support material. The overall stability of the copper-loaded silylated glass with respect to pH was studied by pumping each of the six 0.05 M buffers mentioned under Experimental, through separate freshly packed and loaded 10-cm columns. The degree of copper removal was semiquantitatively determined by monitoring the effluent absorption at 580 nm. The effluent for pH 7.0 and 9.0 buffers showed the lowest absorption in this region, followed by the pH 5 buffer. The pH 3, 4 and 11 buffers caused a rapid loss of copper from the columns. Once treated with these latter buffers, the column could not be reloaded with copper by passing fresh 0.05 M copper tetrammine solution as before. The indication is that the functional groups were removed or altered. In the range pH 5—9, a slow leaching occurred. However, an estimated lifetime of a column is 50 h at a continuous flow of 0.5 ml min^{-1} of 0.1 M ammonia at pH 9.

Because of the success previous workers have had with ammonia as an eluting ligand, it was chosen as the counter ligand in this study [8, 11].

The pH of the ammonia eluent was adjusted to 9.5 with 6 M HCl. Another column bed stability study was undertaken in which various concentrations of ammonia—ammonium chloride buffer were used. Ammonia concentrations of 0.01, 0.1, 0.2, 0.3, 0.5, 1.0, 2.0 and 3.0 M were pumped through the column and the effluent was monitored again at 580 nm. The absorption increased with increasing ammonia concentration with copper leaching being lowest at the lower concentrations. To offset even the minimal leaching of copper, Cu^{2+} was added to the eluent solution. The copper was first converted to the tetrammine complex to prevent the Cu^{2+} from being precipitated as the hydroxide.

Aminohexoses

The aminohexoses were injected into the chromatograph as 1% (w/v) solutions in distilled water. Distilled water solutions rather than ammoniacal ones were used to minimize the possibility of oxidation of the amino sugars during storage. The individual retention values of the amino sugars are listed in Table 2 for 20- and 30-cm columns. Mixtures of glucosamine and mannosamine as well as galactosamine and mannosamine could be separated on columns of both lengths. Glucosamine and galactosamine were unresolvable. The elution order of the amino sugars is identical to that reported by Navratil et al. [11]; the magnitudes of retention are slightly less than those reported by Navratil et al. This discrepancy is probably due to the lower capacity of the porous but solid glass beads compared to a thoroughly hydrated resin. In the swollen resin bead, the amino sugar can penetrate and interact with a large percentage of the interior volume of the bead. The interaction of the amino sugar with the equivalent of a resin skeleton structure would be entirely absent in the silylated glass beads.

Amino acids

The retention values of 18 amino acids on 20-cm columns are presented in Table 3. Several binary mixtures of amino acids were injected into the chromatograph. The resulting chromatograms are given in Fig. 1.

Conclusions

This paper has presented a preliminary report of a new material for use in ligand-exchange chromatography in aqueous media. Amino acids and amino sugars were successfully separated on columns filled with copper

TABLE 2

Retention (in void volumes) of amino sugars on copper-loaded Z-6020 silylated CPG-10 columns

Sugar	20-cm column	30-cm column
Glucosamine	2.40	3.03
Galactosamine	2.88	3.42
Mannosamine	3.98	4.55

TABLE 3

Retention (in void volumes) of amino acids on copper-loaded silylated CPG-10 columns (20 cm)

Sample	Retention	Sample	Retention	Sample	Retention
Aspartic acid	2.74	Hydroxy-1-proline	4.21	Glycine	5.30
Glutamic acid	2.75	Tryptophan	7.44	Alanine	4.30
Cystine	3.10	Phenylalanine	4.56	Proline	3.81
Tyrosine	4.18	Valine	4.14	Histidine	10.44
Serine	4.69	Leucine	4.11	Lysine	14.92
Methionine	4.31	Isoleucine	4.14	Arginine	14.96

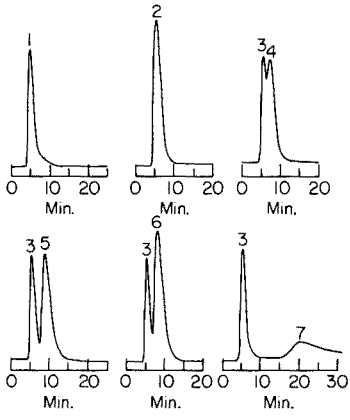

Fig. 1. Separation of amino acids on 20-cm copper-loaded column. Eluent was 0.1 M ammonia buffered to pH 9.5 with hydrochloric acid and was 10^{-4} M in copper tetrammine sulfate. Flow rate was 1 ml min^{-1}, and the samples were binary mixtures of 1% solution (5 μl of each amino acid solution). 1. Aspartic acid and cystine. 2. Glutamic acid and proline. 3. Glutamic acid. 4. Methionine. 5. Serine. 6. Glycine. 7. Histidine.

loaded silylated controlled-pore glass (CPG). The low efficiency of the columns used can be attributed to the large HETP associated with porous glass. Even in exclusion chromatography, columns of CPG have larger HETP values than cross-linked polystyrene gels [20].

The second problem area of silylated glass as a stationary support is the gradual leaching of metal from the copper-loaded bed. This metal loss problem is characteristic of most ligand-exchange supports. However, in addition to leaching of the metal ion from the chelating functional group, the entire covalently bound material may be hydrolyzed from the support surface. This possibility greatly restricts the useful pH range. The columns used in these studies had useful lifetimes of 50 hours at a constant (24 h) flow rate of 0.5 ml min^{-1}.

While silylated glass may not be the ultimate ligand-exchange support, the low flow resistance, possibility of custom-tailoring the immobilized

chelating group, and the added dimension of exclusion interaction make it an interesting and potentially valuable stationary support.

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INORGANIC GAS CHROMATOGRAPHY — THE SEPARATION OF VOLATILE CHLORIDES BY THERMOCHROMATOGRAPHY COMBINED WITH COMPLEX FORMATION

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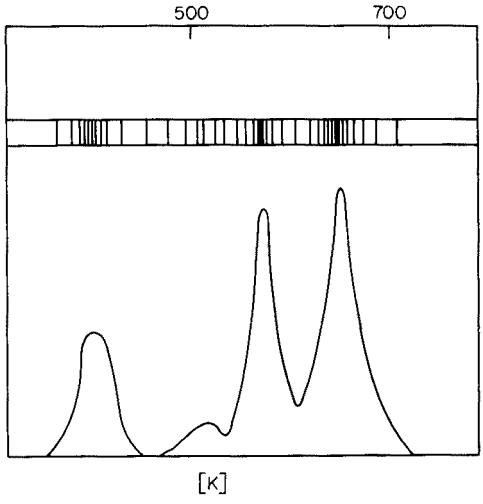
SUMMARY

Separations of the volatile chlorides of I, Te, Sb, Sn, In, Cd, Zr, Hf, Nb, Ta, Mo, Tc, Re, Ru and Os are described. The tube used for the separations has a temperature gradient from 600° C to 25° C, and is coated with KCl, CsCl, NaCl and $BaCl_2$. It is possible to deposit the different compounds at characteristic positions, so that this technique can be used as an on-line chromatographic method.

The detection of very low concentrations of trace elements requires the development of new separation methods. The possibilities and general aspects of inorganic gas chromatography have been discussed by Bächmann and Rudolph [1], and results have been reported on the separation of numerous elements by means of isothermal or temperature-programmed gas chromatography [1, 2]. The main drawback of inorganic gas chromatography is the problem of using detectors with high sensitivities at temperatures high enough to separate, for example, volatile chlorides. However, if the concept of measuring the different elements as a function of time (retention time) is ignored, and if the different elements could be deposited at characteristic positions, then any analytical method could be used for the detection of a certain element. In addition, this procedure could lead to an increase in sensitivity. After adsorption or chemisorption, the element or compound in question can be desorbed or decomposed by heating and led to an analytical detector. Figure 1 shows schematically the distribution of a characteristic separation.

A particular element can be deposited by physical adsorption or by a specific reaction at a characteristic position. In the first case, separation can be achieved by using a tube with a temperature gradient; this method is called thermochromatography.

Thermochromatography has been investigated by several groups using radioactive elements, oxides, fluorides, chlorides and bromides. A good literature survey of thermochromatography has been given by Eichler [3]. Theoretical aspects of the method, with regard to the correlation of deposition



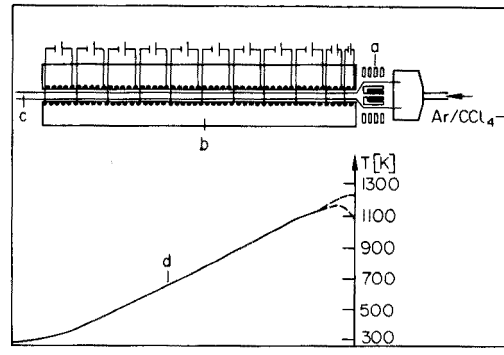


Fig. 1. The distribution of radioactivity for a characteristic separation.

Fig. 2. Thermochromatographic apparatus. (a) Sample heater. (b) Oven. (c) Separation column. (d) Temperature gradient achieved.

temperatures and adsorption enthalpies or sublimation enthalpies, have been discussed [4, 5].

In most of the studies which have been published so far, quartz has served as adsorbent. Zvara et al. [6] used nickel tubes as separation columns in a study of the thermochromatographic separation of bromides, but the deposition temperatures were nearly identical with those obtained with a quartz surface. Eichler and Domanov [7] separated iodine, mercury and astatine by using silver-coated quartz, which led to better separation than was possible with the quartz surface because of chemical reaction with the adsorbent.

In the investigation described here, the method of thermochromatography was combined with specific chemical reaction by coating the wall with different alkali metal chlorides and barium chloride. Zvara and Tarasov [8] used a similar system with a potassium chloride bed to study thermodynamics of some complex formation reactions and later in connection with an investigation of the chemistry of the element 104 (Kurchatovium) [9]. Moreover, the separations can be optimized by using more than one coating in series together with the temperature gradient, as described below.

EXPERIMENTAL

The experimental arrangement and the temperature gradient are shown in Fig. 2. The sample is heated by high-frequency inductive heating, and the oven is fitted with ten different heating coils.

Where specified, the internal tube surface was modified by passing a melt of the alkali metal chloride through the column. This technique was sufficient for the purpose, as demonstrated by the reproducibility of the results. The column was coated only at temperatures up to 600°C because of the high vapour pressure of the alkali metal chlorides at higher temperatures.

Argon saturated with carbon tetrachloride at room temperature was used as the carrier gas and as the reactive mixture. Argon was purified with a molecular sieve at liquid nitrogen temperature and the carbon tetrachloride was pretreated with $CaCl_2$. The flow rate was between 10 cm s^{-1} (at 600°C) and 3.5 cm s^{-1} (at room temperature).

The radioactive nuclides used were produced either by thermal neutron fission of uranium-235 or by high-energy proton-induced nuclear reactions (CERN) with uranium or gold.

The distribution of the different nuclides in the column was measured by γ-spectrometry. Every 2 cm, a γ-spectrum was measured by a germanium—lithium detector which was positioned behind a lead collimator. The lead shielding was only 2 cm thick, so that part of the γ-rays with higher energy contributed to the spectrum; the resolution is therefore actually better than it appears to be from Figs. 3—9.

RESULTS AND DISCUSSION

γ-Spectrometry was used for the identification of the species transported. Consequently, the detection was nuclide-specific but not compound-specific. When macro samples are used, additional analytical methods can give information about the nature of the deposited compounds [10]. However, when carrier-free nuclides are used, experimental identification of the species transported and deposited becomes very difficult. In this case, it is necessary to make some reasonable assumptions on the basis of the experimental conditions and of the possibilities indicated by available literature data.

The experimental conditions for the formation of the volatile compounds can be summarized as follows. An excess of the chlorinating agent (CCl_4) is used at a high temperature (ca. 1500°C), such that CCl_4 is largely decomposed, the main product being Cl_2. A metal foil (uranium or gold) is used as the source for the nuclear reaction products. The volatile chlorides are probably formed by a reaction of the metal with chlorine. The presence of even small amounts of oxygen or water in the reactive gas stream can lead to the formation of oxychlorides. Under the conditions used, the concentration of the radioactive nuclides is so small that only mononuclear compounds can be expected.

Table 1 shows the chlorides and the anionic complexes expected to be formed on the alkali metal chloride coatings. The thermochromatograms which were obtained when KCl or CsCl was used as the column coating are presented in Figs. 3—5. Table 2 summarizes the deposition temperatures of the elements investigated on KCl and CsCl (this work) and on quartz (Eichler [3]). Figure 6(A) shows the results obtained with uranium-235 fission products

TABLE 1

Summary of the volatile chlorides and the reaction products with the solid phase

Elements	Chlorides	Expected complex compounds	Elements	Chlorides	Expected complex compounds
Te	$TeCl_2$	Me_2TeCl_4	Hf	$HfCl_4$	K_2HfCl_6[c]
	$TeCl_4$	Me_2TeCl_6			Cs_2HfCl_6[d]
Sb	$SbCl_3$	$MeSbCl_4$	Nb	$NbCl_5$	$MeNbCl_6$
	$SbCl_5$	$MeSbCl_6$	Ta	$TaCl_5$	$MeTaCl_6$
Sn	$SnCl_2$	Me_2SnCl_4	Ru	$RuCl_4$	Me_2RuCl_6
	$SnCl_4$	Me_2SnCl_6	Os	$OsCl_4$	Me_2OsCl_6
In	$InCl_3$	Me_3InCl_6	Tc	$TcCl_4$	Me_2TcCl_6
Cd	$CdCl_2$	Me_2CdCl_4	Re	$ReCl_4$	Me_2ReCl_6
Zr	$ZrCl_4$	K_2ZrCl_6[a]			$MeReCl_6$
		Cs_2ZrCl_6[b]			

[a] $\Delta H = -51.8$ kcal mol^{-1}, $\Delta S = -48.2$ e.u. mol^{-1} [11]. [b] $\Delta H = -51.9$ kcal mol^{-1}, $\Delta S = -40.8$ e.u. mol^{-1} [11]. [c] $\Delta H = -54.1$ kcal mol^{-1}, $\Delta S = -47.8$ e.u. mol^{-1} [11]. [d] $\Delta H = -54.5$ kcal mol^{-1}, $\Delta S = -33.7$ e.u. mol^{-1} [11].

TABLE 2

Deposition temperatures (K) of chlorides on different solid phases

Element	on SiO_2 (ref. 3)	on KCl (this work)	on CsCl (this work)	Element	on SiO_2 (ref. 3)	on KCl (this work)	on CsCl (this work)
I	—	353	423	Nb	416	513	663
Te	448	473	713	Ta	—	553	653
Sb	333	473/393	553/473	Mo	358	533/453	653/573
Sn	300	473/393	693/643	Tc	408	813	873
In	623	693	873	Re	315	613	643
Cd	—	773	873	Ru	—	873	873
Zr	463	753	873	Os	393	873	873
Hf	453	753	873				

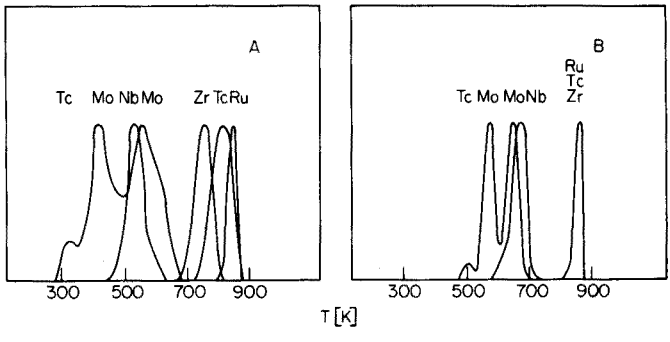


Fig. 3. Separation of 2nd transition row elements on KCl (A) and on CsCl (B).

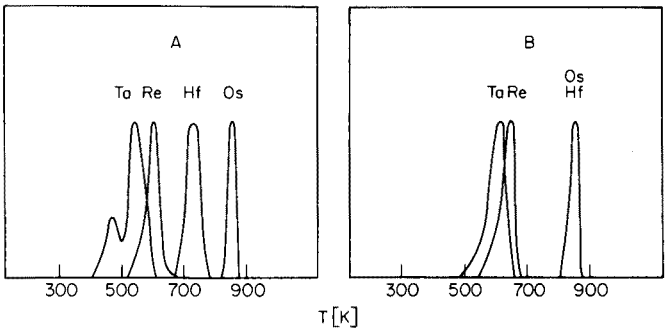


Fig. 4. Separation of 3rd transition row elements on KCl (A) and on CsCl (B).

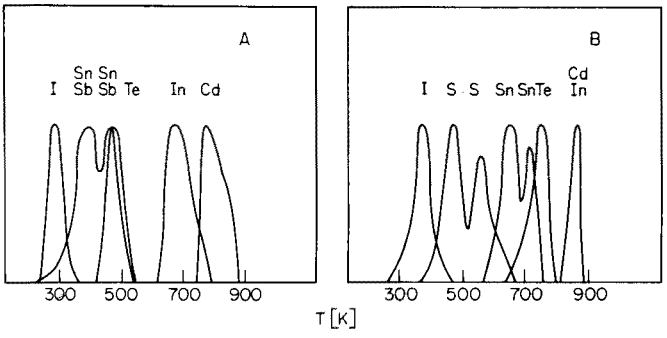


Fig. 5. Separation of 5th main row elements on KCl (A) and on CsCl (B).

on quartz, which are in good agreement with the literature values [3], and Fig. 6(B) shows the thermochromatogram when a $BaCl_2$ coating was used. The use of $BaCl_2$ in the high-temperature part of the column (> 600° C) has proved useful for decontamination of these elements from cerium and lanthanum [12]. Figure 6(B) indicates that the $BaCl_2$ coating offers possibilities for the separation of technetium which is the only element showing a significant deviation in its behaviour compared with the quartz experiments. A further interesting effect, independent of the coating material, was observed when water was not removed completely from the reactive gas stream. This result is demonstrated in Fig. 7, which shows the distribution pattern of the technetium activity in the column when water is absent or present. Peaks 2 and 3 are due to deposition of molybdenum-99 which decays to technetium-99m. Peak 1 corresponds to the deposition of $TcCl_4$ as $Me_2^{I}TcCl_6$. The second technetium peak (4) in the low-temperature region must be due to another volatile technetium compound. A comparison of curves (a) and (b) makes it obvious that peak 4 is influenced by the presence of moisture in the gas stream. The resulting compound is an oxychloride, probably TcO_3Cl.

Molybdenum also appears in two different positions in the thermochromatographic column but no significant dependence on the water concentration of the reactive gas was observed. However, the chlorination product of moly-

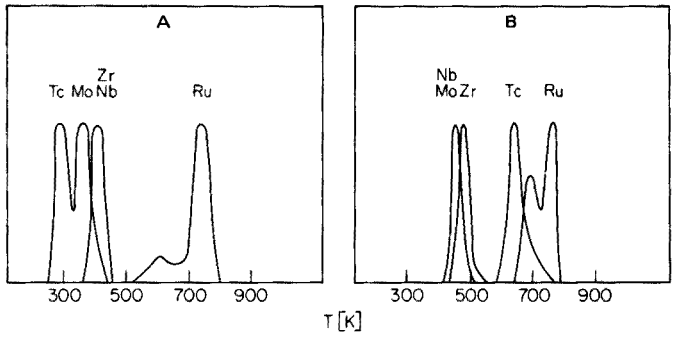


Fig. 6. Separation of 2nd transition row elements on SiO_2 (A) and on $BaCl_2$ (B).

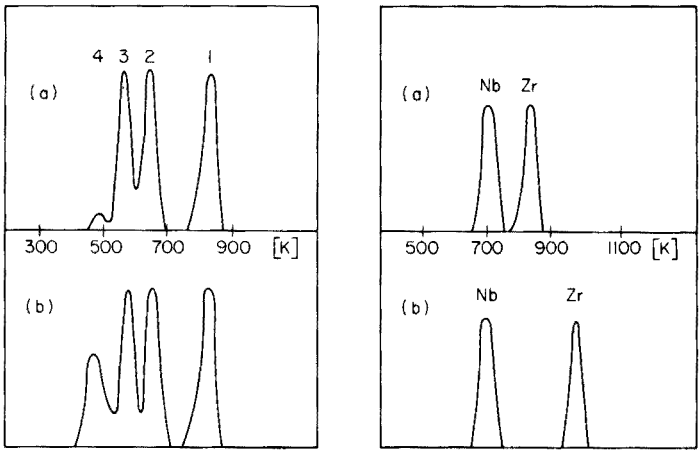


Fig. 7. (left) The distribution of the Tc-activity in the thermochromatographic column. (a) Water impurities removed from the carrier gas. (b) Water impurities not removed from the carrier gas. Peaks 1—4 are identified in the text.

Fig. 8. (right) (a) The separation of Zr and Nb on CsCl. (b) The same separation after changing the temperature gradient.

bdenum, $MoCl_5$, is so sensitive to O_2 and water impurities that even very small concentrations are enough for partial oxidation and formation of $MoOCl_4$ [10].

A comparison of the behaviour of homologous transition group elements is of interest. These results may be useful for the prediction of the behaviour and some properties of the higher homologous elements 104—108. The deposition of $ZrCl_4$ and $HfCl_4$ on KCl and CsCl did not show any measurable difference, the deposition temperature being 753 K on KCl and 873 K on CsCl (beginning of coating). The deposition of $NbCl_5$ on CsCl is very similar to $TaCl_5$ and the difference in adsorption temperature is quite small, whereas the KCl—$TaCl_5$ complex appears to be more stable than the corresponding niobium compound.

The results become more complex for the elements of the 6th and 7th

transition groups. In addition to the sensitivity towards oxygen and water already mentioned, the higher homologues tend to form chlorides of higher oxidation states. Unfortunately, the nuclear reactions used for the production of these radioactive nuclides did not produce any suitable tungsten isotope for γ-spectroscopic measurement, so that a comparison of molybdenum and tungsten is not possible. However, the behaviour of technetium and rhenium can give some information about the gas-phase chemistry of these elements. The deposition temperatures of $TcCl_4$ are much higher than those of the volatile rhenium compound. Some work on the stability of $Me^I_2TcCl_6$ and $Me^I_2ReCl_6$ [13] has indicated that the rhenium complex should be more stable than the technetium complex. This leads to the conclusion that a different rhenium chloride was transported, which is in agreement with studies that report $ReCl_5$ as the only chlorination product of rhenium [14].

Both ruthenium and osmium were deposited at the highest possible temperature on the alkali metal chloride coatings. It was assumed that the tetrachlorides were the volatile species and that the deposition was due to formation of the thermally stable complexes $Me^I_2RuCl_6$ and $Me^I_2OsCl_6$, respectively. Ruthenium, however, showed a second maximum in its distribution whether quartz or $BaCl_2$ was used. This must be due to the formation of the trichloride. The second peak disappeared when KCl or CsCl-coated tubes were used because of overlapping of the deposition ranges.

It would be of interest to develop a general concept based on thermodynamic considerations and to be able to predict the behaviour of any volatile inorganic compound in the temperature gradient. The theoretical treatment of Eichler and Zvara [4] appears to be useful for calculations of some thermodynamic quantities related to adsorption of the volatile compounds on quartz. The deposition temperatures were correlated to the adsorption enthalpies. However, this approach cannot be used when chemical reactions take place at the column walls to produce solid compounds. In this case, the free energies of the reactions must be taken into account, i.e. the reaction entropy has a strong effect. This becomes obvious from the behaviour of $ZrCl_4$ on KCl and CsCl. Although the reaction enthalpies for the formation of K_2ZrCl_6 and Cs_2ZrCl_6 are almost identical (Table 1), the deposition temperatures in the thermochromatographic column are quite different; this happens because the contribution of the reaction entropy leads to a more negative free energy for the formation of Cs_2ZrCl_6 (Table 2). Some partly extrapolated data for the chlorides of Nb, Ta, Zr and Hf indicated that higher deposition temperatures correspond to higher free energies of reaction.

The deposition at the start of a coating is not characteristic; this was shown by a simple experiment (Fig. 8). Curve (a) shows the deposition behaviour of Zr and Nb on CsCl; curve (b) was produced when the column was moved 30 min after the start, so that each position came to a final temperature 150 K higher than initially. $NbCl_5$ moved to reach its characteristic deposition temperature again, whereas $ZrCl_4$ was not affected by the change because Cs_2ZrCl_6 is stable also at higher temperatures. This is a simple procedure for increasing the resolution.

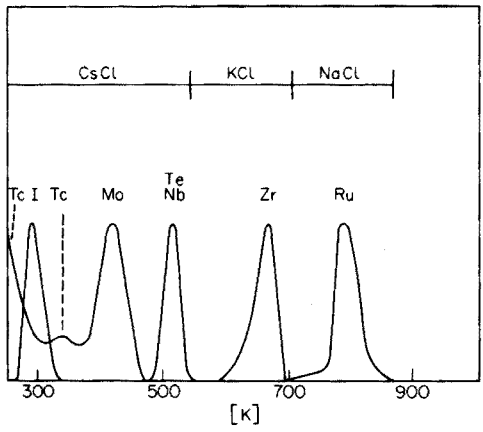


Fig. 9. A separation with several alkali metal chloride coatings in a row.

It was shown that for each element the deposition temperature was higher with increasing radius of the alkali metal cation. There is an obvious separation of two groups of compounds on CsCl. The tetrachlorides (Zr, Hf, Tc, Ru, Os) are deposited at the beginning of the coating and the pentachlorides (Nb, Ta, Mo, Re) in the temperature region 673—573 K. On KCl there is a higher resolution of the elements of each group.

An attempt was made to optimize the separation effect by using several alkali metal chloride coatings in the same column. The sequence used was NaCl, KCl, CsCl, i.e. an increase in cationic radii with decreasing temperature. The result is presented in Fig. 9. A good separation of the second transition row elements was achieved, each one being deposited at a different position.

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ELECTROCHEMICAL MICROBIOASSAY OF VITAMIN B_1

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SUMMARY

A microbioassay of vitamin B_1 is possible with a new electrode assembly consisting of a platinum anode and a silver peroxide cathode. The response time of the electrode decreases with increasing amount of the bacterial suspension (*L. fermenti*) injected. A linear relationship is obtained between the steady-state current and the concentration of vitamin B_1 in the culture broth. The microbioassay can be completed within 6 h, with relative errors of ±8%. A possible mechanism of the current generation is discussed.

As bacteria require specific nutrients for their growth, bioassay of amino acids, vitamins and antibiotics is possible with the use of special bacteria such as *Lactobacilli*, *Streptococci*, etc. Generally, microbioassays of such biologically active materials are carried out by turbidimetric or titrimetric methods. However, these methods require a lengthy incubation of bacteria, e.g., the turbidimetric method requires cultivation for more than 16 h and the titrimetric method needs cultivation for more than 72 h at 30°C [1]. Moreover, colored samples cannot be used for the microbioassay by turbidimetry.

Recently, many methods have been developed for electrochemical monitoring of enzymatic reactions [2—4]. Biochemical fuel cell systems based on immobilized enzymes and whole cells have been applied in clinical analysis and to the estimation of Biochemical Oxygen Demand (BOD) [5—7].

In the present paper, a new electrode system is described for the microbioassay of vitamin B_1. A possible mechanism of current generation is discussed.

EXPERIMENTAL

Materials

The basic assay medium (Takara Kosan Co.) had the composition shown in Table 1. Thiamine was purchased from Tokyo Kasei Co. Other reagents were analytical reagents or laboratory-grade materials. Deionized water was used in all procedures.

TABLE 1

The composition of microbioassay medium
(Double strength, 100 ml, pH 6.8)

Casamino acid	1 g	$FeSO_4 \cdot 7H_2O$	1 mg	Riboflavin	0.1 mg
L-Cysteine	40 mg	$MnSO_4 \cdot 4H_2O$	1 mg	Pyridoxin	0.1 mg
L-Tryptophan	10 mg	NaCl	1 mg	Pyridoxal	0.1 mg
Glucose	2 g	NH_4Cl	300 mg	Pantothenate	0.1 mg
CH_3COOK	2 g	Adenine	1 mg	Nicotinic acid	0.1 mg
K_2HPO_4	50 mg	Guanine	1 mg	*p*-Aminobenzoic acid	0.02 mg
KH_2PO_4	50 mg	Uracil	1 mg	Folic acid	0.001 mg
$MgSO_4 \cdot 7H_2O$	20 mg	Xanthine	1 mg	Biotin	0.001 mg

Lactobacillus fermenti ATCC 9338 was employed for the assay of vitamin B_1. It was maintained in peptone-yeast agar and transferred into fresh medium every 10—14 days.

Preparation of bacterial suspension

L. fermenti was cultured at 37°C for 24 h in 100 ml of a mixture (pH 6.8) containing 0.5 g of yeast extract, 1.0 g of peptone, 1.0 g of glucose, 1.0 g CH_3COONa, 25 mg K_2HPO_4, 25 mg KH_2PO_4, 10 mg $MgSO_4 \cdot 7H_2O$, 0.5 mg $FeSO_4 \cdot 7H_2O$, 0.5 mg $MnSO_4 \cdot 4H_2O$ and 0.5 mg NaCl. The cells were centrifuged at 5°C and 8000 g, washed twice with physiological saline and resuspended in physiological saline. This cell suspension was diluted suitably for use.

Apparatus

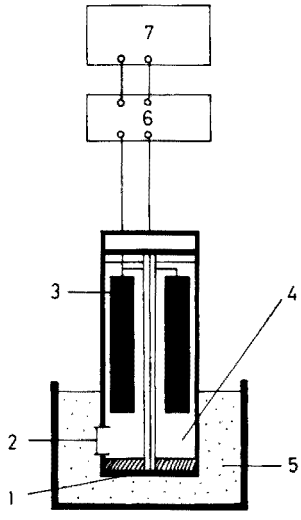
A schematic diagram of the electrochemical sensor for microbioassay is shown in Fig. 1. The cathode (1 cm × 4 cm) was silver peroxide (Ag_2O_2) and the anode (diameter, 2.2 cm) was platinum. Phosphate buffer (0.1 M, pH 7.0) was used as the catholyte. An anion-exchange membrane (Selemion Type AMV, Asahi Glass Co.) was used to separate the electrochemical cell from the culture broth. The current was measured by a milliammeter (Kikusui Electronic, Model 114) and the signal was displayed on a recorder (Riken Denshi, Model SP-J3C).

Assay procedure

A portion (5 ml) of the double-strength basic medium and 5 ml of a sample solution containing appropriate amounts of vitamin B_1 were placed in a test tube and sterilized for 15 min at 120°C. Then 50 μl of bacterial suspension was added to the medium containing vitamin B_1. After incubation for 6 h at 37°C, the electrode system (Fig. 1) was inserted in the culture broth and the current was measured.

Detection of the electroactive organic acids and gases

Volatile organic acids were detected by gas chromatography (Simadzu Seisakujo, Model GC 6 AM) on a Chromosorb 101 (60—80 mesh) column



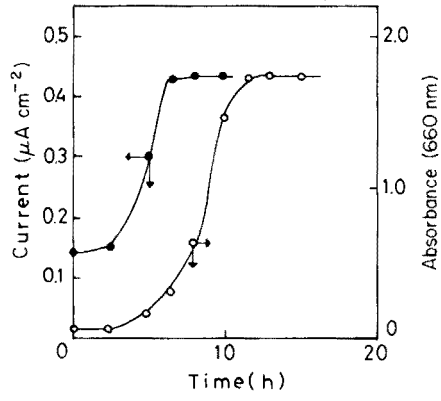


Fig. 1. The scheme of electrode for electrochemical microbioassay. 1. Anode (Pt). 2. Anion-exchange membrane. 3. Cathode (Ag_2O_2). 4. Electrolyte (0.1 M phosphate buffer). 5. Sample solution (medium, vitamin B_1, bacteria). 6. Ammeter. 7. Recorder.

Fig. 2. The time-course of bacterial growth and the current generated. The culture medium contained 50×10^{-9} g ml^{-1} of vitamin B_1. The amount of bacterial suspension injected was 5×10^{-6} g wet cells ml^{-1}.

(1 m × 3 mm i.d. glass) with argon carrier gas and flame-ionization detection (column temperature, 170°C; injection port 200°C). Hydrogen and carbon dioxide were detected by gas chromatography on a molecular sieve 5 A (60—80 mesh) and Porapak Q (60—80 mesh) column (3 m × 3 mm i.d.) with argon carrier gas and thermal conductivity detection (column temperature, 40°C). Lactate was determined by enzymatic analysis with lactate dehydrogenase [8].

RESULTS

Incubation time of bacteria

The time-course of bacterial growth and the current generated are shown in Fig. 2. *L. fermenti* grew normally in the culture medium containing 50×10^{-9} g ml^{-1} of vitamin B_1. After injection of the *L. fermenti* suspension, the electrode was inserted in the culture medium and the current was measured. The current increased with increasing incubation time. The maximum current was obtained at the middle point of the exponential bacterial growth. There was no increase in current from the medium in the absence of vitamin B_1.

The time required to obtain the maximum current decreased with increasing amount of the injected bacterial suspension. The minimum time

for microbioassay was 6 h when 5×10^{-6} g wet cells/ml was injected into the incubation medium. Further increase of the amount injected did not shorten the incubation time. An amount of 5×10^{-6} g ml^{-1} was therefore employed for further work.

Response of electrode

The culture medium for microbioassay was incubated for 6 h at 37°C before the electrode was inserted. The response time (the time required for the current to reach steady state) is shown in Fig. 3. The open-circuit anode potential was −420 mV vs SCE and the cathode potential was 220 mV vs. SCE. As the anode was polarized, a high current was obtained initially. Then the anode potential became constant. Diffusion of the electroactive substances produced became the rate-determining factor and a steady-state current was obtained. As shown in Fig. 3, the steady-state current was attained within 15 min in all cases.

Calibration

Figure 4 shows the relationship between the steady-state current and the concentration of vitamin B_1. A linear relationship was obtained between the steady-state current and the vitamin B_1 concentration below 25×10^{-9} g ml^{-1}. The steady-state current was reproducible within ±8% of the relative error when a medium containing 25×10^{-9} g ml^{-1} of vitamin B_1 was used. The standard deviation was 10^{-9} g, when various concentrations of vitamin B_1 were measured by this method.

Mechanism of current generation

Lactic acid, acetic acid, carbon dioxide and traces of formic acid were detected as metabolites. Hydrogen was not detected by gas chromatography.

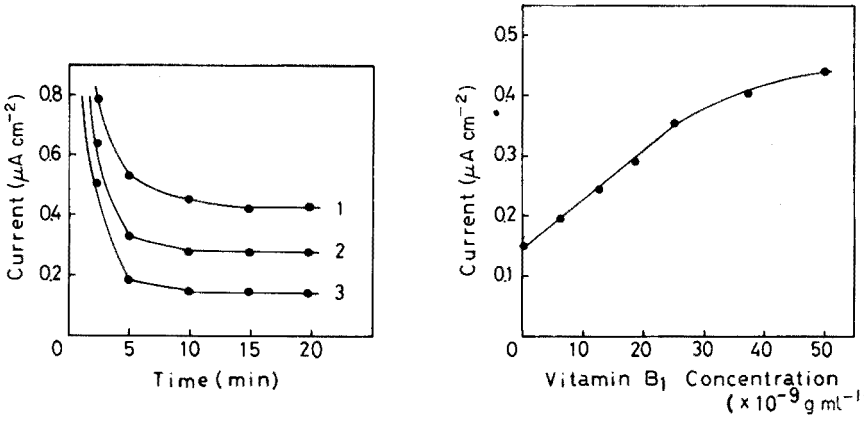


Fig. 3. Response curves. The concentration of vitamin B_1 was 1. 50×10^{-9} g ml^{-1}. 2. 12.5×10^{-9} g ml^{-1}. 3. None. The amount of bacterial suspension was 5×10^{-6} g wet cells ml^{-1}. The current was measured after incubation for 6 h at 37°C.

Fig. 4. Calibration curve. The experimental conditions were the same as described for Fig. 3.

Traces of formic acid would not contribute much to current generation. Lactic acid — a main metabolite of *L. fermenti* — acetic acid and carbon dioxide were not oxidized at the anode. Table 2 shows the steady-state currents of various culture broths. The steady-state currents obtained from the culture broth with and without vitamin B_1 were 0.42 and 0.15 $\mu A\ cm^{-2}$, respectively. When the bacteria were removed from the culture broth by centrifugation, the steady-state current obtained from the supernatant solution was 0.19 $\mu A\ cm^{-2}$. The relationship between the steady-state current and the amount of bacteria suspended in the culture medium is given by the equation

$$I = 0.21\ C + 0.19$$

where I is the current density ($\mu A\ cm^{-2}$) and C is the concentration of bacteria ($\times 10^{-3}$ g wet cells ml^{-1}). The current increased linearly with increasing amount of bacteria. However, when the active bacteria were inactivated by boiling the suspension for 15 min, the current decreased to the value which was obtained from the culture broth without bacteria. This result shows that only active bacteria contribute to current generation.

DISCUSSION

A rapid microbioassay is desirable for the determination of biologically active substances. It is possible that a large injection of bacterial suspension will shorten the time required for microbioassay, but this cannot be used for the turbidimetric method because the turbidity of the medium increases. It has been shown that microorganisms produce electroactive substances such as formate and hydrogen [9], so that electrochemical methods could be applied to microbioassay, which would be useful for the assay of colored samples.

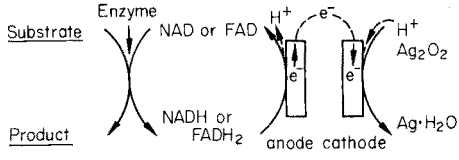
When the new electrode assembly for the bioassay of vitamin B_1 was immersed in the culture medium, a current of 0.14 $\mu A\ cm^{-2}$ was obtained initially, which indicated the presence of trace amounts of unknown electroactive substances in the basic assay medium. The current increased with increasing incubation time, maximum current being obtained at the middle

TABLE 2

The steady state current of various broths

Culture broths	Steady state current ($\mu A\ cm^{-2}$)
(a) Culture broth containing vitamin B_1	0.42
(b) Supernatant solution of the broth	0.19
(c) Culture broth containing vitamin B_1 (measured by modified electrode)	0.19
(d) Culture broth without vitamin B_1	0.15

stage of bacterial growth; but the maximum current was attained more quickly as the amount of bacterial suspension injected was increased. Therefore, it is probable that the electroactive substances were produced by active *L. fermenti.* As previously reported [5, 9], immobilized *Clostridium butyricum* produced hydrogen and formic acid as electroactive substances. However, no hydrogen and only traces of formic acid were detected in the culture broth of *L. fermenti.* The current obtained from a culture broth without bacteria was about the same as that from a fresh broth (Table 2). A main electroactive substance was not found in the culture broth. Furthermore, the current was also the same when a modified electrode (the surface of the platinum anode was covered with cellulose dialysis membrane) was inserted in the culture broth containing bacteria. These facts suggest that most of the current generated is due to the direct transfer of electrons from bacteria to the electrode. *Lactobacillus* is a Gram-positive bacterium and the cell walls contain glycerol teichoic acid and peptidoglycan [10], but these substances are not electroactive. As previously reported [7, 11], co-enzymes such as NAD(P)H, and $FADH_2$ are electroactive. Enzymes such as glucose dehydrogenase, glucose-6-phosphate dehydrogenase and glutamate dehydrogenase require NAD, and amino acid oxidase requires FAD as the co-enzyme. These reduced co-enzymes located on the bacterial cell wall may be oxidized at the platinum electrode and the co-enzymes reduced again as follows



Current could then be obtained continuously from the electrode. However, enzymes and co-enzymes located on the cell wall cannot contribute to current generation, when the bacteria are inactivated with heat as described above. However, the detailed mechanism of the current generation is far from being fully understood.

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Analytica Chimica Acta, 98 (1978) 31—37

ALCOHOL, LACTATE AND GLUTAMATE SENSORS BASED ON OXIDOREDUCTASES WITH REGENERATION OF NICOTINAMIDE ADENINE DINUCLEOTIDE

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SUMMARY

Flowthrough enzyme electrodes are reported for determinations of alcohol, lactate and glutamate. Oxidoreductases mixed with immobilized NAD^+ cofactor are held between a suitable platinum electrode and a semipermeable membrane. The coenzyme is readily regenerated either directly by electrochemical oxidation or by using phenazine methosulphate (PMS^+) as intermediate. Continuous flow conditions are used. The sensitivity obtained with the alcohol dehydrogenase electrode was 50, 620 or 810 nA mol^{-1} of ethanol, respectively, when regeneration was done electrochemically or with 0.1 or 0.5 mM PMS^+. The sensitivities for the lactate and glutamate sensors in the presence of 0.5 mM PMS^+, were 14 and 50 nA $mmol^{-1}$ for D,L-lactate and L-glutamate, respectively. The calibration curves were linear for concentrations up to 0.5, 1.5 and 100 mM of glutamate, lactate and ethanol, respectively. The sensitivity of the alcohol and lactate sensors decreased by 50—55% within 60 h and that of the glutamate sensor within 6 h.

Enzymatic methods of analysis are finding increasing use. However, the performance of large numbers of analyses requires considerable amounts of enzyme, and automation of these processes is very difficult. The application of immobilized enzymes, and the development of enzyme sensors, as well as tubular and column analytical reactors, has led to the construction of new analytical devices [1—4]. The immobilization of coenzymes offers possibilities of developing such sensors on the basis of enzymes which require cofactors to function. Among these enzymes, the oxidoreductases with NAD^+ as coenzyme are of primary importance.

The possibility of developing analytical systems with enzymatic regeneration of coenzymes was first shown by Davies and Mosbach in 1974 [5]. Electrochemical regeneration of NAD^+ in bioelectrochemical cells was reported by Suzuki et al. [6]. However, in this case, the employment of non-immobilized NAD^+ does not afford suitable preconditions for reliable regeneration of the coenzyme. Recently, an immobilized derivative of NAD^+ has been used to construct a reagentless lactate sensor with electrochemical regeneration of NAD^+ [7]. However, the high potential applied imposes some limitations to the employment of such sensors.

The present paper concerns the development of flowthrough enzyme electrodes on the basis of oxidoreductases and a high-molecular-weight, water-soluble derivative of NAD^+; the NAD^+ is regenerated electrochemically or by using an intermediate. Enzyme electrodes sensitive to ethanol, lactate and glutamate, based on alcohol, lactate and glutamate dehydrogenases are reported.

EXPERIMENTAL

Materials

The following materials were used: alcohol dehydrogenase (ADH) from horse liver (EC.1.1.1.1; 2 units/mg), lactate dehydrogenase (LDH) from pig muscle (EC.1.1.1.27; 200 units/mg), NAD^+ and NADH* (all from Reanal, Hungary), glutamate dehydrogenase (GDH) from cattle liver (EC.1.4.1.3; 120 units/mg; Ferak, Berlin), *N,N'*-dicyclohexylcarbodiimide (Chemapol; Czechoslovakia), dextran (molecular weight 40000; Polfa, Poland), phenazine methosulphate (Gee Lawson, Great Britain), 2,4,6-trichloro-s-triazine (cyanuric chloride; Yerevan Chemical Reagents, U.S.S.R.).

Preparation of a high-molecular-weight soluble derivative of NAD^+

Dextran (20 g) was dissolved in 150 ml of water, 2 g of cyanuric chloride was added and the mixture was activated at pH 11 during 30 min. Then 10 g of 1,8-diamino-octane was added; the solution was adjusted to pH 9.5, stored for 3 days at 30°C and then poured into a 10-fold volume of acetone. The precipitate of 8-amino-octyldextran was dissolved in 50 ml of water, and succinyl-NAD^+ obtained from 0.4 g of NAD^+ [8] was added. Pyridine (50 ml) containing 0.4 g of *N,N'*-dicyclohexylcarbodiimide was added; the solution was then acidified to pH 6.0, stored for 3 days at 30°C and poured into 1 l of acetone. The precipitate was dissolved in 40 ml of water and dialyzed in water at room temperature for 5 days, during which time the water was changed periodically. The course of the dialysis was checked spectrophotometrically by the absorbance at 260 mm. When dialysis was complete, the solution was poured into 400 ml of acetone, and the precipitate was collected and dried in vacuum.

The content of enzymatically active NAD^+ in this derivative was determined by measuring the change of absorbance at 340 nm in the alcohol dehydrogenase-catalyzed reaction with ethanol [9] and was found to be 17% of the bonded NAD^+.

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*NAD^+ and NADH are the oxidized and reduced forms of nicotinamide adenine dinucleotide, respectively. PMS^+ and PMSH are oxidized and reduced phenazine methosulphate (*N*-methylphenazonium methosulphate).

Polarographic measurements and construction of enzyme electrode

A polarograph (PPT-1) with a recorder (KSP-4) was used for the measurements. The sensitivity of the polarograph was 0.4 nA mm^{-1} (internal resistance 13 kohm). All the solutions used contained 0.1 M KCl and 0.01 M phosphate buffer pH 8.0. The measurements were carried out at 30°C.

The voltammetric characteristics of NADH oxidation were determined at a platinum electrode (P-101; surface area 56 mm^2; Radiometer, Denmark). A positive potential was applied to the electrode with a sweep rate of 2 mV s^{-1}. The reference electrode was a standard silver/silver chloride electrode. During the measurements the concentration of NADH was held at 10^{-4}—10^{-5} M.

The construction of the enzyme sensor is shown in Fig. 1. A platinum disk electrode of 2.5-mm diameter (1) is used with a silver/silver chloride reference electrode in the form of a chloridized silver wire spiral (2) immersed in a solution of 0.1 M KCl—0.01 M phosphate buffer pH 8.0 (3), which was isolated from the microcell by a conductive gel layer. A solution (0.05 ml) containing 8.9 mg of immobilized NAD^+ and enzyme was deposited on the surface of the platinum electrode. The concentration of the coenzymatically active form of NAD^+ was 0.89 mM. The concentration of the enzyme was 8.8, 4.4 and 4.6 mg ml^{-1} for alcohol, lactate and glutamate dehydrogenase, respectively. The surface of the electrode was covered with a dialysis membrane (6; 55-μm thick) which was secured with a rubber ring (7). The test solution was thermostated in the heat exchanger (5) before being pumped through the microcell, which had a volume of 0.3 cm^3 (4). A micropump (Model MC 706.1, Czechoslovakia) was used at a constant speed of 0.53 ml min^{-1}. In order to standardize all experiments, the test solution was pumped through the sensor for 3 min, and then the buffer solution without sample was passed until the current fell to the original value. The potential applied in the direct oxidation at the platinum electrode was +0.75 V. When PMS was used, the potential was not applied; in this case, 1 ml of test solution was

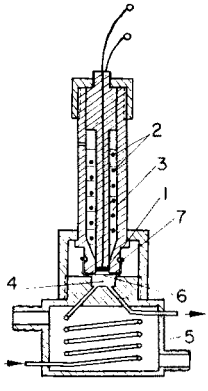

Fig. 1. Construction of enzyme electrode. (1) Platinum disk; (2) reference electrode; (3) solution of 0.1 M KCl + 0.01 M phosphate buffer pH 8.0; (4) microcell; (5) heat exchanger; (6) semipermeable membrane; (7) rubber ring. The arrows show the direction of pumping of the solution through the microcell.

mixed with 1 ml of PMS^+ solution of appropriate strength just before addition to the buffer solution for pumping.

In order to measure the stability of response of the sensors with time, the sensors were stored at 4°C, and 3—4 measurements were carried out at 30°C during 15—20 min, at intervals over a period of time.

RESULTS

When the solution is pumped through the microcell, the enzyme substrate diffuses across the semipermeable membrane to a region near the surface of the electrode. The enzymatic reaction with reduction of coenzyme then occurs. Direct oxidation of NADH at the platinum electrode, or indirect oxidation with PMS^+, causes regeneration of the coenzyme. The value of the current then obtained in the external circuit depends on the concentration of substrate. The dependence of the current of the ethanol sensor on the concentration of ethanol is given in Fig. 2. The regeneration of NAD^+ in this sensor was carried out at 0.75 V, i.e. at the maximum of the wave for the NADH oxidation. Figure 2 shows that the steady-state current depends linearly on the substrate concentration up to 100 mM; above this concentration, the slope decreases. The data presented are linear in inverse coordinates (Fig. 3).

A current is also attained in the alcohol sensor when ethanol and PMS^+ are added to a buffer solution and an external potential is not applied. The steady-state current of the sensor then depends linearly on the ethanol concentration up to 40 or 80 mM when 0.1 or 0.5 mM PMS^+, respectively, is added. With 40 mM ethanol, the electrode current was 25 or 35 nA for these respective concentrations of PMS^+.

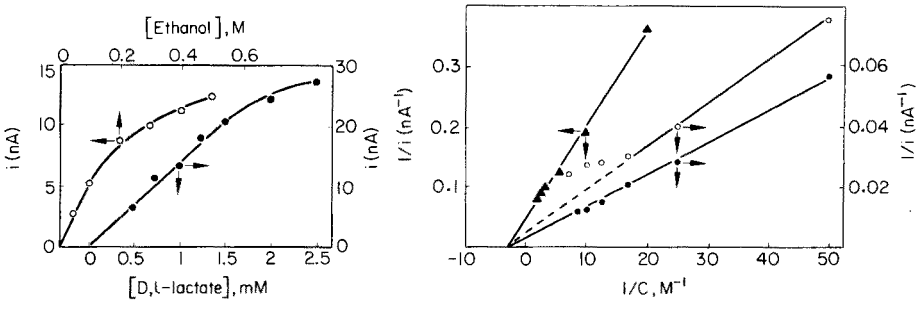

Fig. 2. Dependence of current intensity on ethanol concentration in the case of electrochemical regeneration of NAD^+ at a potential of +0.75 V (○), and dependence of current intensity on D,L-lactate concentration in the case of regeneration of NAD^+ with 0.5 mM PMS^+ (●). pH 8.0, 30°C.

Fig. 3. Dependence of current intensity on ethanol concentration in inverse coordinates with regeneration of NAD^+ electrochemically (▲), and with 0.1 mM PMS^+ (○) or 0.5 mM PMS^+ (●). pH 8.0, 30°C.

The dependence of the current on the lactate concentration for the LDH electrode, based on chemical regeneration of the coenzyme with 0.5 mM PMS^+, is shown in Fig. 2. The calibration curve is linear up to 1.5 mM lactate. A limiting value of the electrode current, calculated by extrapolation of this plot, was found to be 70 nA.

A linear dependence of the current of the GDH electrode based on chemical regeneration of coenzyme with 0.5 mM PMS was observed up to 0.5 mM glutamate.

The response times of the sensors did not exceed 3 min when PMS^+ was used. The zero current level was also reached after 3 min, when basic buffer was pumped through the microcell. In the case of direct electrochemical regeneration of coenzymes, the response time increases to 5—6 min, and the zero level is re-established only after the buffer solution has been pumped for 20—30 min.

A significant parameter for the application of these enzyme sensors is their stability with respect to the responses obtained with suitable concentration of substrate over a period of time. It was shown that the current increases initially and then decreases when a substrate of definite concentration is pumped uninterruptedly through the microcell. The changes with time in the readings obtained with lactate and glutamate sensors which were stored at 4°C and tested periodically are shown in Fig. 4. The sensitivity of the lactate sensor, as well as that of the alcohol sensor, falls by 50—55% within 60 h. For the glutamate sensor, the current increases almost 3-fold within the first 1.5 h, but the sensitivity then decreases rapidly, the rate constant being 0.16 h^{-1}.

DISCUSSION

The oxidation of substrates (AH_2) by the action of dehydrogenase and the regeneration of the coenzyme occurs in the layer held against the working electrode; this layer contains both the enzyme and the immobilized coenzyme. In these reactions, the coenzyme and substrate are consumed equimolecularly. To achieve continuous oxidation of the substrate the oxidized form of the coenzyme must be regenerated.

Direct oxidation of NADH at the platinum electrode is of interest, for this oxidation proceeds without any addition of chemicals. However, this

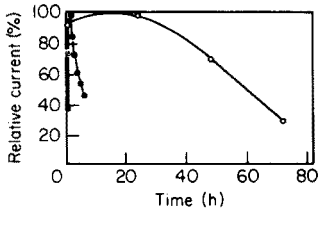

Fig. 4. Change of readings of lactate (○) and glutamate (●) electrodes with time. The concentration of D,L-lactate was 1 mM, and that of L-glutamate was 0.4 mM.

process has some limitations. First, oxidation of NADH occurs only at high potentials, which considerably decreases the selectivity of this process in real solutions which contain other electrochemically active compounds. Secondly the rate of NADH oxidation is rather slow. Consequently, the electro-oxidation of NADH can limit the entire conjugated process. To eliminate these problems, the electrochemical regeneration of coenzyme may be carried out via intermediates [6, 10]. In the present work, PMS^+ was used as such. As is known, this compound oxidizes NADH quickly and its reduced form may be readily oxidized electrochemically [11]. The sequence of conjugated reactions occurring at the anode may be illustrated as follows:

$$AH_2 + NAD^+ \overset{\text{ADH, LDH, GDH}}{\rightleftharpoons} A + NADH + H^+$$

$$NADH + PMS^+ \rightleftharpoons NAD^+ + PMSH$$

$$PMSH \overset{Pt}{\rightleftharpoons} PMS^+ + H^+ + 2e^-$$

The equilibrium concentration of the oxidized and reduced forms of the intermediate was established when the buffer solution with PMS^+ was pumped through the microcell. This ratio was found to be 8.5×10^7 at the standard potential of PMS^+ (0.08 V [11]) with a reference electrode potential of 0.314 V. When the substrates are injected into the solution they are enzymatically oxidized to produce NADH. The reaction of NADH with PMS^+ leads to the formation of PMSH which is discharged at the platinum electrode. In this case, the electrodes behave as a bioelectrochemical cell which is discharged through a high internal resistance partly to prevent destruction of the reference electrode.

If the action of the sensors depends on enzyme kinetics and mass transfer across a semipermeable membrane, the dependence of current intensity on the concentration of substrate must be linear in inverse coordinates [12]. Then the intersection with the x-axis is equal to the reverse negative constant of K_m(app). As can be seen from Fig. 3, K_m(app) is 0.3 M for the ethanol sensor, both for direct oxidation of NADH and for oxidation with 0.5 mM PMS^+. When the concentration of PMS^+ is lower (0.1 mM) the K_m(app) value is close to 0.3 M within the range of lower concentrations of substrate. However, when the concentration of ethanol exceeds 0.16 M, the linear dependence disappears. This indicates that a subsequent process — probably the chemical oxidation of NADH — becomes a limiting stage. Consequently, the enzymatic reaction approaches equilibrium and the current reaches a maximum.

The K_m(app) values obtained under conditions of fast regeneration of coenzyme are 2.3 times higher than the constant for a native enzyme. Consequently, under these conditions the rate of mass transfer of substrate is comparable to that of enzyme kinetics [12].

The limiting current of the ethanol sensor was found to be 23 nA under the conditions for direct oxidation of NADH, and 200 or 300 nA in the

presence of 0.1 or 0.5 mM PMS^+, respectively. Accordingly, the intermediate must react more quickly at the electrode than NADH. Furthermore, in the absence of the intermediate, the zero-concentration level was established rather slowly. Probably, this is due to slow desorption of the coenzyme from the electrode at the high positive potential used in electrochemical regeneration.

The changes with time in the measurements require comment. The increase in sensitivity in the initial stage of a continuous measurement is due to an increase in the rate of mass transfer across the membrane and to the change of viscosity in the solution next to the platinum electrode. This was indicated by tests with glutamate dehydrogenase when a solution of the enzyme in 50% glycerol was used. A rise in sensitivity with time has been observed for other amperometric electrodes [13]. The decrease in sensitivity of the sensors over a long period of time depends on the nature of the enzyme, and a long-term change of sensitivity can be attributed mainly to deactivation of the enzyme. Of the three enzymes used here, GDH is least stable. The stability of the LDH electrode is close to that of the ADH electrode.

Electrodes based on regeneration of NAD^+ should offer significant advantages, especially in the construction of automatic analyzers working under continuous flow conditions.

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Analytica Chimica Acta, 98 (1978) 39—46

THE DETERMINATION OF TRACE METALS IN MINERAL WATERS
Part I. Atomic Absorption Spectrometric Determination of Cd, Co, Cr, Cu, Ni and Pb by Electrothermal Atomization After Concentration by Co-precipitation

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SUMMARY

Procedures based on flameless atomic absorption spectrometry are described for the determination of Cd, Co, Cr, Cu, Ni and Pb in mineral waters. Because of matrix effects and the inadequate detection limits for direct determinations, the metals are separated from the macrocomponents by precipitation of their tetramethylenedithiocarbamates with iron(III) as collector, or by co-precipitation on $Fe(OH)_3$. The detection limits are 0.005, 0.3, 0.08, 0.10, 2 and 0.10 $\mu g\ l^{-1}$ for Cd, Co, Cr, Cu, Ni and Pb, respectively, and are satisfactory for the determination of these elements in mineral waters. The precision is 2—7%.

The increasing exploitation of mineral waters, not only as drinking waters but for their medicinal properties, requires as complete an evaluation of all the more important sources as possible. Not only main constituents but also trace components should be determined. While the chemical occurrence and importance of individual macrocomponents are already fairly well known, present knowledge about trace elements is still limited and methodical studies are required. Unlike drinking waters, the analysis of mineral waters meets with problems because of the higher concentrations of individual components and their widely varying ratios (Table 1).

The occurrence of many metals can be low in mineral waters. This requires the use of sensitive methods of analysis and the elaboration of procedures with very low detection limits. Atomic absorption spectrometry (a.a.s.) with graphite tube atomization is suitable for the direct determination of some metals in mineral waters; but for the majority of metals, separation from the macrocomponents is necessary before atomization because of matrix effects and insufficient sensitivity.

The literature contains few data on the determination of microelements in mineral waters by flameless a.a.s. However, some procedures have been described for the extraction of metals from drinking waters and especially sea waters and their determination by a.a.s. [1—8]. Traces of metals in waters have often been concentrated by co-precipitation techniques [9—14].

TABLE 1

Normal concentration ranges of some components in mineral waters

Component	mg kg^{-1}	Component	mg kg^{-1}	Component	mg kg^{-1}
Li^+	0.35—1.8	$Fe^{2+,3+}$	0.02—10	HPO_4^{2-}	0.0—0.5
K^+	2—220	F^-	0.1—1.7	HCO_3^-	480—9000
Na^+	8—5200	Cl^-	1—1700	HBO_2	1.6—40
Ca^{2+}	3—540	Br^-	0.25—0.75	H_2SiO_3	25—100
Mg^{2+}	2—1300	I^-	0.07—1.3	CO_2 (g)[a]	40—3800
Sr^{2+}	0.5—13.1	SO_4^{2-}	1—2200		

[a] CO_2 free.

The present paper reports the determination of traces of metals (Cd, Co, Cr, Cu, Ni, Pb) in mineral waters by flameless a.a.s. after their separation by (co)precipitation.

EXPERIMENTAL

Apparatus and chemicals

Measurements were made with a Perkin-Elmer graphite-tube furnace, HGA70, in conjunction with a Perkin-Elmer 300S atomic absorption spectrometer. Westinghouse (Cr, Cu, Pb) and Varian-Techtron (Co, Mn, Ni) hollow-cathode lamps, as well as a Perkin-Elmer high-frequency electrodeless lamp (Cd) were used.

All chemicals were of A.R. quality. Redistilled water from a Heraeus quartz apparatus was used.

Determination after co-precipitation with iron(III)

Iron(III) standard solution (1 ml of a 1 mg ml^{-1} solution) is added to 200 ml of the water sample, carbon dioxide is boiled out, and $Fe(OH)_3$ is precipitated with ammonia solution (1 + 3). The precipitate is collected on a filter, washed with dilute ammonia solution (1 + 10) and dissolved in 10 ml of nitric acid (1 + 3). The solution is evaporated to dryness and the residue is dissolved in 10 ml of 0.1 M H_2SO_4. An aliquot (10 μl) of the solution is injected into the graphite furnace, and the absorption of the element under test is measured; the conditions are summarized in Table 2.

Determination after precipitation with ammonium tetramethylenedithiocarbamate

The water sample (200—250 ml) is poured into a beaker, 0.5 ml of Fe^{3+} standard solution (1 mg ml^{-1}) is added, the pH is adjusted to 2—3, and CO_2 is boiled out. The ammonium tetramethylenedithiocarbamate solution (2.5 ml of 2% w/v) is added. The precipitate is collected on a filter and dissolved with 3 ml of concentrated nitric acid, the filter being washed with

TABLE 2

Conditions for measurement
(In all cases, the drying temperature was 100°C for 30 s. The ashing and atomization times were 30 and 20 s, respectively.)

Element	Wavelength (nm)	Ashing temp. (°C)	Atomization temp. (°C)
Cd	228.8	300	1600
Co	240.7	1100	2450
Cr	357.9	1100	2500
Cu	324.7	750	2450
Mn	279.5	1100	2450
Ni	232.0	1100	2450
Pb	283.3	500	2450

10 ml of 5% (v/v) HNO_3. The solution is then evaporated to dryness and the residue is dissolved in 5 ml of 0.1 M H_2SO_4. An aliquot (10 μl) of the solution is then injected into the graphite furnace for the absorption measurement (Table 2).

Calibration

Appropriate amounts of standard solutions of the test elements are poured into 25-ml measuring flasks, 2.5 ml of Fe^{3+} standard solution (1 mg ml^{-1}) is added, and the mixture diluted to the mark with 0.1 M H_2SO_4. These solutions are then taken through the recommended procedures, including co-precipitation.

RESULTS AND DISCUSSION

Mineral waters contain high concentrations of alkali metals, alkaline-earth metals, sulphate, chloride, and hydrogencarbonate ions. These components can affect the determinations when their compounds cause co-volatilization of the element under consideration during evaporation in the ashing step. They can also evaporate in the atomization step, creating unspecific absorption signals.

Figures 1 and 2 show the effects of calcium, sodium and some anions on the absorptions of cadmium, cobalt, chromium, copper, manganese, nickel and lead. The elements which form relatively involatile compounds (cobalt, chromium, copper, manganese and nickel) can be determined directly at sodium or calcium ion concentrations up to 500 mg l^{-1}. In larger amounts these ions cause unspecific absorption effects which cannot be eliminated with deuterium background compensation. Volatile elements (cadmium, lead) cannot be determined directly in mineral waters because of matrix interferences. Sulphuric acid has no really significant effect on the determination of the elements mentioned above; unspecific absorption occurs only at higher concentrations of acid. Higher concentrations of hydrochloric and

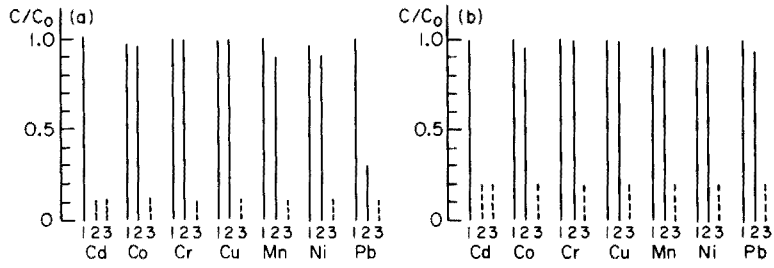

Fig. 1. The influence of (a) sodium and (b) calcium on the absorption of cadmium, cobalt, chromium, copper, manganese, nickel and lead. 50 μl, t_1 = 90 s, t_2 = 60 s; other conditions in Table 2. (1) 5 mg l^{-1} Na^+ or Ca^{2+}, (2) 500 mg l^{-1} Na^+ or Ca^{2+}, (3) 5000 mg l^{-1} Na^+ or Ca^{2+}. ----- Nonspecific absorption. C/C_0 is the ratio of apparent and real concentrations.

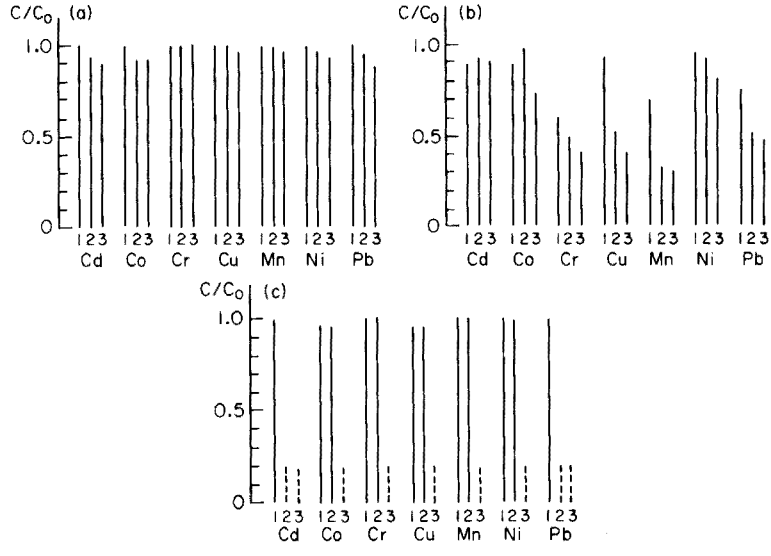

Fig. 2. The influence of (a) hydrochloric, (b) nitric and (c) sulphuric acids on the absorption of cadmium, cobalt, chromium, copper, manganese, nickel and lead. 50 μl, t_1 = 90 s, t_2 = 60 s; other conditions in Table 2. (1) 0.2 M; (2) 1 M; (3) 2 M acid. For (-----) and C/C_0, see Fig. 1.

especially nitric acid tend to decrease the absorption. To avoid these difficulties and sources of errors, prior separation of the metals from the matrix by co-precipitation seemed advisable. Such techniques, in comparison with solvent extraction, should have the advantage of making it possible to determine the total content of a metal, regardless of the species in which it occurs in mineral water. Moreover, co-precipitation should allow adequate preconcentration of the metal (50—100 ×).

Since most mineral waters contain iron in comparatively large quantities, the suitability of iron(III) hydroxide and of iron(III) tetramethylenedithiocarbamate (TMDTC) for the co-precipitation of Cd, Co, Cr, Cu, Ni and Pb was examined.

Iron(III) hydroxide as a collector gave promising results for pure aqueous solutions, but the precipitation with ammonia from mineral water samples produced colloidal precipitates which could not be filtered. Voluminous and readily filterable precipitates were obtained only after the addition of iron(III) to the sample; even then, co-precipitation of the metal ions was not complete. Table 3 shows the results obtained for aqueous solutions and for mineral water samples. The precipitation at controlled pH of the solution within the range 3—9 also did not give quantitative results. The effect of electrolytes in larger amounts was not investigated because these would increase the blank. Table 3 shows that only chromium can be separated quantitatively from mineral waters, the statistically determined detection limit being 0.08 $\mu g\ l^{-1}$ with a standard deviation of 15%.

Table 3 also shows clearly that in the presence of Fe—TMDTC, cadmium, cobalt, copper, nickel and lead are precipitated quantitatively. The yield for chromium was below 10% because under the precipitation conditions used, chromium did not produce an insoluble chelate. The standard deviations were satisfactory (2—7%).

High blanks were observed for copper and lead. Analysis of the reagents showed that the iron(III) solution and the acids used contributed to the high blank values. Blanks obtained from ten parallel determinations with relative standard deviations and statistically calculated detection limits are given for some elements in Table 4. For some elements, matters could be improved by purification of chemicals or by precipitation of the metals from larger volumes of sample (500 ml or more of mineral water).

The solutions in which cadmium, cobalt, chromium, copper, nickel and lead were determined contained large amounts of iron(III); therefore their effect on the absorption of the test elements was studied. Figure 3 shows the influence of iron (0.1 and 0.2 mg $Fe^{3+}\ ml^{-1}$) on the absorption of the elements examined. It can be seen that iron increased only the absorption of cobalt; the effect was slight.

TABLE 3

Co-precipitation of some metals from twice-distilled and mineral waters. (a) $Fe(OH)_3$. (b) Fe—TMDTC

Element	Added (ng/10 μl)	Found (ng/10 μl)			
		Distilled water		Mineral water	
		a	b	a	b
Cd	0.10[a] 0.20[b]	0.12	0.20	<0.05	0.20
Co	1.0	1.1	1.05	0.5	1.0
Cr	1.0	1.05	<0.1	1.0	<0.1
Cu	1.0	1.0	0.95	0.5	0.95
Ni	2.0	2.0	2.1	<0.5	2.0
Pb	1.0	0.95	0.95	0.3	1.0

TABLE 4

The blanks and detection limits for some elements after the precipitation with Fe—TMDTC

Element	Blank[a]		Limit of detection[b]
	$\bar{x}_i$	s_r	
Cd	0.008	12	0.005
Co	<0.05	—	0.3
Cu	0.49	13	0.10
	0.2[c]		
Ni	<0.3	—	2
Pb	0.15	12	0.10

[a]In ng/10 μl. [b]In μg l^{-1} (200 ml of water sample; final volume 5 ml). [c]After purification of iron with dithizone.

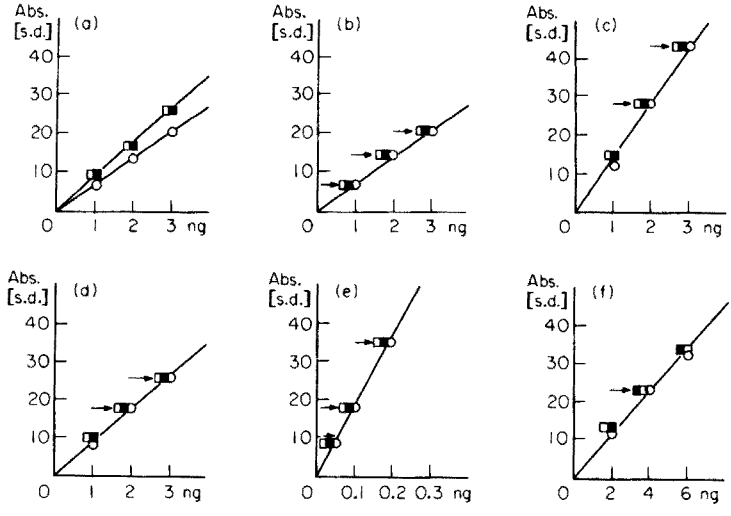

Fig. 3. Influence of Fe(III) on the absorption of (a) cobalt, (b) lead, (c) chromium, (d) copper, (e) cadmium and (f) nickel. ○ 0.1 M H_2SO_4; □ 0.1 M H_2SO_4 + 0.1 mg Fe^{3+} ml^{-1}; ■ 0.1 M H_2SO_4 + 0.2 mg Fe^{3+} ml^{-1}. Volume 10 μl; other conditions in Table 2.

When the trace elements are co-precipitated, the precipitate also adsorbs ions of the main constituents, i.e. alkali and alkaline-earth metals, aluminium and other ions. As expected, the adsorption of these elements on the precipitate of Fe—TMDTC is much lower than on iron(III) hydroxide (Table 5). Therefore, interferences are not expected when cadmium, cobalt, copper, lead and nickel are determined, and this was confirmed by the method of standard additions. The determination of chromium is also free from interferences under the conditions applied. Hence all the elements can be determined by means of calibration curves.

The accuracy of the procedure was established by independent methods. Thus, copper, lead and cadmium were determined by anodic stripping

TABLE 5

Analysis of Fe—TMDTC and $Fe(OH)_3$ precipitates (in $\mu g\ ml^{-1}$)

Element	Fe—TMDTC	$Fe(OH)_3$
Al	<0.5	<1
Ca	30	100
K	0.4	1
Mg	30	110
Mn	<0.02	1
Na	20	80
Si	<0.5	1

TABLE 6

Comparison of results obtained by flameless a.a.s. and anodic stripping voltammetry (a.s.v.) or emission spectrometry (e.s.)
(All results are given in $\mu g\ l^{-1}$)

Sample	Cd		Cu		Pb	
	A.a.s.	A.s.v.[a]	A.a.s.	A.s.v.	A.a.s.	A.s.v.
1	0.06	<0.2	4.0	4.3	0.20	0.20
2	0.07	<0.2	5.3	5.1	4.5	4.7
3	0.04	<0.2	3.2	3.2	0.45	0.43
4	0.05	<0.2	15	13.4	0.60	0.50
	Ni		Co		Cu	
	A.a.s.	E.s.[b]	A.a.s.	E.s.	A.a.s.	E.s.
5	10	12	6.5	6.8	4.9	5.0

[a]A.s.v. at a mercury hanging drop electrode, 0.5 M HCl, electrolysis time 10 min at —0.8 V vs. SCE.
[b]E.s. at a d.c. arc (10 A) after preconcentration of the TMDTC chelates on $Al(OH)_3$.

voltammetry, whereas cobalt, copper and nickel were determined by emission spectrometry. Results for five samples of mineral waters are given in Table 6.

The proposed method was used to analyse some mineral waters of Slovenia. The results obtained are given in Table 7. The samples were acidified with HCl to pH 2 immediately after sampling. These solutions were stable enough for completion of the analysis. From the results it can be concluded that the procedure is suitable for the determination of these trace metals in mineral waters and could be applied to some other metals (e.g. Ag, As, Bi, V).

TABLE 7

Analysis of some mineral waters of Slovenia (in $\mu g\ l^{-1}$)

Spring	Cd	Co	Cr	Cu	Ni	Pb	Zn[a]	Mn[a]
Radenci-1	0.04	0.8	1.2	15	5.3	0.50	20	250
Radenci-2	0.05	<0.3	0.2	8.1	0.7	0.60	30	220
Očeslavci	0.04	0.3	0.8	1.5	1.8	0.75	20	350
Nuskova	0.06	<0.3	7.5	1.3	2.8	0.80	20	350
Kotlje	<0.005	2.7	0.2	0.35	4.2	0.70	5	360
Rogaška Slatina-1	0.04	6.3	7.8	17	5.5	1.1	100	150
Rogaška Slatina-2	<0.005	0.5	7.2	1.9	<1	<0.1	55	90

[a]Direct flame a.a.s. determination.

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Analytica Chimica Acta, 98 (1978) 47—57

AN IMPROVED METAL EXTRACTION PROCEDURE FOR THE DETERMINATION OF TRACE METALS IN SEA WATER BY ATOMIC ABSORPTION SPECTROMETRY WITH ELECTROTHERMAL ATOMIZATION

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SUMMARY

A rapid carbamate extraction method with pyrrolidinedithiocarbamate and diethyldithiocarbamate is described for the simultaneous determination of Cd, Co, Cu, Fe, Ni, Pb and Zn in sea water by atomic absorption spectrometry with a graphite atomizer. The metal—carbamate complexes are extracted from 500 ml of sea water into Freon TF and back-extracted into 10 ml of 0.3 M nitric acid. The method has considerable advantages over previously recommended extraction procedures. The metals are transferred to a solution in which their concentrations do not change with time, and which can be easily stored for transportation. The sensitivity is high enough for analysis of open ocean waters.

The determination of trace elements, particularly the heavy metals, has received increasing attention in pollution studies. To assess accurately the environmental hazards, methods capable of analyzing trace metals at their background levels in unpolluted waters are needed. In addition, there is considerable interest in the natural cycles of these elements both in open sea and estuarine areas.

The analytical methods published up to the beginning of 1974 have been reviewed by Riley [1]. Recently, several attempts to analyze sea water directly with electrothermal atomizers and a.a.s. have been undertaken. In most cases, however, the sensitivity was too low for unpolluted waters, as found by Campbell and Ottaway [2]. The only direct method with sufficient sensitivity was found to be a.s.v. [3]. Most separation methods in use are based on the formation of metal—carbamate complexes. Kremling and Petersen [4] used APDC—MIBK (ammonium pyrrolidine dithiocarbamate—methyl isobutyl ketone) extraction to separate trace metals from 1 cm^3 of sample, while Boyle and Edmond [5] used coprecipitation with cobalt pyrrolidine dithiocarbamate. The metal—carbamate extraction system has been treated in some detail by Kinrade and Van Loon [6].

The aim of this work was to develop a method for the determination of trace metals in sea water by utilizing the very high sensitivity possible with the graphite furnace. Many of the published methods were tested; the modification developed has the advantages of ease of operation and of stabilization of the extracted metals. The procedure eliminates the drawbacks of the poor stability of dithiocarbamates in the hexone (MIBK) solvent. The extraction procedure described is suitable for the determination of Cd, Co(II), Cu(II), Fe(III), Ni, Pb and Zn traces in sea water.

EXPERIMENTAL

Reagents

Stock standard solutions of the different metals were prepared from Titrisol ampoules which were diluted to 1000 ppm in a final nitric acid concentration of 0.14 M. From the stock solutions mixed standards were prepared with metal concentrations in proportion to those normally present in sea water.

Water used for preparing reagents and diluting the standards was deionized water from a laboratory supply followed by either (1) double distillation in a quartz still, or (2) a Milli-Q water purification system. No difference could be found between these two kinds of water.

The organic solvent Freon TF (1,1,2-trichloro-1,2,2-trifluoroethane, density 1.565 g cm^{-3} (25°C), b.p. 47.6; Du Pont, 1211-Geneva-24, Switzerland) was used as purchased after checking the contamination level.

A solution of ammonium pyrrolidinedithiocarbamate (APDC) and diethylammonium diethyldithiocarbamate (DDTC), 1% (w/v) of each, was used as the extraction reagent. The solution was prepared as follows: 0.5 g of APDC and 0.5 g of DDTC were weighed into a measuring cylinder, dissolved in 50 ml of water and purified by shaking with 50 ml of Freon TF. An insoluble substance present in some batches of carbamate as well as most metal contamination was removed by this treatment.

The buffer was a 0.5 M diammonium hydrogencitrate solution, which was purified by repeated carbamate extractions until negligible concentrations of metals were found in the extract.

The nitric acid used was of Suprapur grade.

Analytical-grade chemicals were used for preparing synthetic sea water. The synthetic sea water contained 234.7 g of NaCl, 106.4 g of $MgCl_2 \cdot 6H_2O$, 39.2 g of Na_2SO_4, 14.7 g of $CaCl_2 \cdot 2H_2O$ and 1.92 g of $NaHCO_3$ per 10 kg. The approximate metal concentrations in synthetic sea water are given in Table 1.

Apparatus

A Perkin-Elmer 403 atomic absorption spectrometer equipped with a standard single-slot burner head was used for the determination of zinc. The light source was a Perkin-Elmer Intensitron hollow-cathode lamp.

TABLE 1

Typical metal concentrations in synthetic sea water

Metal	Cd	Co	Cu	Fe	Ni	Pb	Zn
Concentration ($\mu g\ l^{-1}$)	0.1	<0.01	2	5	4	0.5	5—25

For the other elements a Perkin-Elmer 370 spectrometer supplied with a heated graphite atomizer (HGA 2100) was used. The light sources used were Intensitron hollow-cathode lamps for Cu, Ni and Fe, a Cathodeon hollow-cathode lamp for cobalt and Perkin-Elmer electrodeless discharge lamps for cadmium and lead.

Borosilicate glass separatory funnels with Teflon stopcocks were used. All glassware was washed with 7 M nitric acid (analytical grade); new glassware was allowed to stand for several hours in acid. After three rinses with deionized water and three rinses with further purified water, the glassware was stored under dust-free conditions.

Finnpipette adjustable micropipettes were employed for pipetting small volumes on to the HGA and for spiking samples with standard solution.

Preliminary studies

Different separation methods in the literature were tested for their adaptability to the HGA. Coprecipitation techniques were unsatisfactory because the reagent gave rise to severe matrix effects in the HGA and electrochemical techniques could be used only for a few elements. Although extraction methods have been widely employed and are fairly well documented, these methods have some severe drawbacks when used with the HGA. For example, the final solution is an organic solvent which has wetting properties that make it difficult to use in the graphite furnace. The solvent most often used, MIBK, has a fairly high solubility in water and phase separation is slow.

To avoid these problems, a heavy organic solvent was selected so that it could be easily transferred to test tubes after extraction. The organic solvent was added in two portions, 20 + 10 ml. The last drops of Freon were always left in the funnel, to avoid introducing sea water into the extract, and the second portion was used to collect the metal complexes in these drops to facilitate complete transfer. A back-extraction into an acidified aqueous solution was then performed. This gives a solution well suited for analysis with the HGA.

Choice of organic solvent. Two heavy organic solvents are widely used in extractions, chloroform and carbon tetrachloride; both are known to be possible carcinogens. The less toxic Freon TF is considered almost harmless when used in industrial applications as a degreasing agent and has many virtues in extractions. It is almost insoluble in water (0.017% w/w at 21.1°C) and separates quickly from the aqueous phase after shaking. The solvent has a weak, innocuous odour and is inexpensive. The technical quality can be

used without purification since it has a sufficiently low metal concentration level. The only drawback is the low solubility of the complexes in Freon TF as compared to chloroform, although for trace metal analysis the solubilities and distribution constants are sufficiently high.

Choice of complexing agent. A.a.s. is a sensitive and selective technique for the final trace metal analysis. Therefore emphasis was placed on the use of non-selective complexing agents, the most popular of which are the carbamates. The main disadvantage is the poor stability of the metal carbamates in the organic solution, which limits the time available for analysis after extraction and permits only one or two elements to be determined in the same extract. This problem is avoided if the metals are transferred to an aqueous solution by back-extraction.

Kinrade and Van Loon [6] found an enhancement in the stability of metal carbamates in MIBK when a mixture of equal amounts of APDC and DDTC was used. Since use of this mixture might also broaden the pH range, it was adopted as the extraction reagent in the present procedure.

Recommended procedure

Transfer 500 ml of the sample to a separatory funnel. Adjust the pH to about 5 with 3 ml of purified buffer. Add 3 ml of the purified mixed extraction reagent followed without delay by 20 ml of Freon; shake the funnel vigorously for 150 s. Allow the phases to separate and drain the lower organic layer into a stoppered test tube. Add another 10 ml of Freon and shake the funnel for 30 s. Combine the two extracts and add 0.2 ml of concentrated nitric acid. Shake the tube for 20 s and let stand for at least 5 min. Add 10 ml of water and shake for another 20 s. The extracts are now ready for determination with a.a.s. Operating parameters for the HGA follow the manufacturer's recommendations and the sample volume used has to be chosen according to the concentration level present.

Standardization

Extraction efficiencies measured for different types of water samples show only slight variations, making it possible to use one standard addition curve for the evaluation of many samples extracted on the same occasion.

The following procedure was used. Transfer 500 ml of the sample to each of four separatory funnels. After pH adjustment, add small amounts of a mixed standard. Select the concentrations in the mixed standard so that the first addition raises the metal content by approximately 50%, the second by a further 50% and the third by a further 100%. After extraction by the recommended procedure, use the slope of the standard addition curve obtained to evaluate the samples. The total blank (see below) was subtracted from the sample concentration.

RESULTS AND DISCUSSION

Effect of pH on extraction efficiency

A large batch of synthetic sea water was prepared and stored in a glass bottle under continuous stirring. The same batch was used for all experiments concerning pH variation. It was considered important to measure the pH variation as close to the natural conditions as possible and thus the synthetic sea water was spiked only with cobalt. This was because the cobalt content of the salts used was too low to yield a readily determinable concentration. The pH was adjusted with Na_3PO_4 and HCl and monitored with a pH meter. Phosphate was used as the buffer instead of citrate, so that one buffer system could be used over the whole pH range studied. The results (Fig. 1) prove that pH has no influence on extraction efficiency for these metals in the range 3—7. The decline in efficiency above pH 7 is probably due to precipitation of $Fe(OH)_3$, $Mg_3(PO_4)_2$ or both. On the acid side (pH <3), the decline is due to decomposition of complexing agents or extraction of their protonated forms.

Effect on extraction efficiency of amount and relative composition of extraction reagent

A batch of synthetic sea water was prepared and extractions were performed at pH 3.5—4 with different mixtures of APDC and DDTC (the total

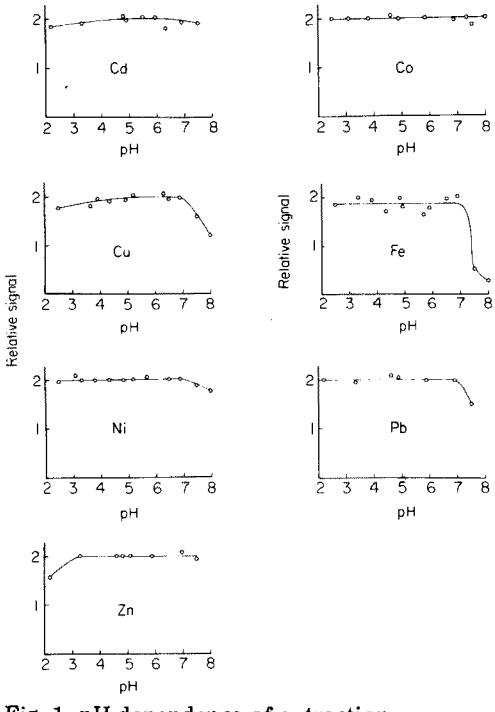

Fig. 1. pH dependence of extraction.

volume of mixture was kept constant at 3 ml). The relative proportions were varied from 100% APDC to 100% DDTC. Although the solubilities of the complexes in Freon were found to vary with the relative amounts of complexing agents, no variation in extraction efficiency was found.

The influence of the total amount of extraction reagent was also studied. Between 1 and 10 ml were added to 500 ml of synthetic sea water, but no trend could be seen, and 3 ml were used in subsequent experiments.

Formation of complexes at the nanogram and microgram levels

At the very low concentrations found in natural waters, it was suspected that fairly long shaking times would be necessary. A study of the effect of varying the shaking time from 5 to 120 s, both in natural and synthetic sea water, showed that extraction is extremely fast (Fig. 2, Table 2). For iron, both extraction efficiency and precision are adversely affected when the shaking time is less than 30 s. This delay is probably due to the transformation of iron from an unreactive, e.g. colloidal, to a reactive form.

TABLE 2

Necessary shaking time

Metal	Synthetic sea water (s)	Sea water (s)	Metal concentration in sea water ($\mu g\ l^{-1}$)
Cd	15	15	0.03
Co	5	—	<0.01
Cu	5	5	0.9
Fe	30	30	2
Ni	5	30	0.6
Pb	5	5	0.13
Zn	5	5	3

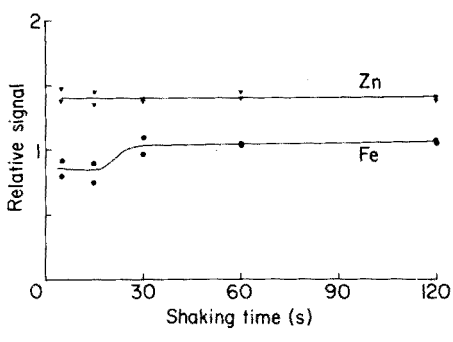

Fig. 2. Extraction efficiency as a function of shaking time for Zn and Fe in synthetic sea water: (•) Fe, (▾) Zn.

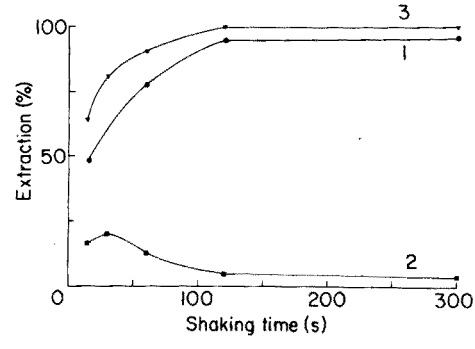

Fig. 3. Percentage extraction of copper (20 $\mu g\ l^{-1}$) as a function of shaking time for spiked sea water. (1) First portion of Freon, (2) second portion, (3) total extraction.

The speed of extraction was also investigated for waters with higher concentrations of metals. These waters were spiked to concentrations typical of the highest standard addition in slightly polluted waters. In this case, the time-dependence of the extraction efficiency was quite different, as shown in Fig. 3 for copper. At these concentrations it was necessary to shake for about 150 s to achieve acceptable extraction. The reason for this behaviour is probably related to precipitate formation in the aqueous phase, when the extraction reagent mixture is added. The subsequent dissolution of this precipitate introduces a time lag so that longer shaking times are needed. For the final procedure, 150-s shaking times were used to ensure that small variations in time would not cause errors.

Shaking times of 30 s and 150 s were used in studies of the useful concentration ranges of the method. A mixed standard was added in varying amounts to portions (500 ml) of sea water and these samples were extracted at pH 3.8. The metal concentrations in the extracts were determined and compared to standards with the same acid strength. Some typical results are shown in Figs. 4 and 5 and summarized in Table 3. Clearly, the ranges are wide enough to encompass most natural waters when a shaking time of 150 s is used. The highest concentration of total metal added was 245 $\mu g\ l^{-1}$. The extraction efficiencies were excellent for all metals except cadmium, which was only 80%. The reason for this anomalous behaviour is being investigated.

Stability of metal complexes in the organic phase

One of the shortcomings of the common APDC—MIBK extraction system is the instability of the complexes in the organic phase. To test this stability in the present system an extraction with a large volume of organic solvent was performed. The aqueous phase was spiked with a mixed standard so that the final concentration in the organic phase would approximate that obtained in a typical sea-water extraction. After extraction, 15 g of the extract were transferred to a stoppered test tube and back-extracted. The rest of the

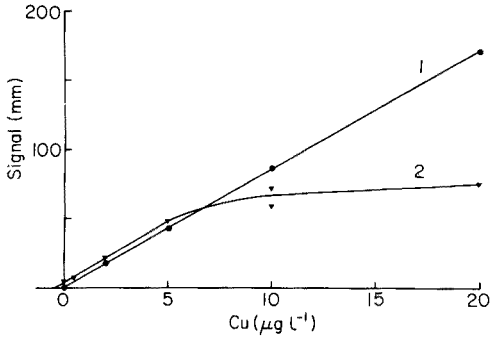

Fig. 4. Concentration range for copper with 30-s shaking time. (1) standards; (2) extracted samples.

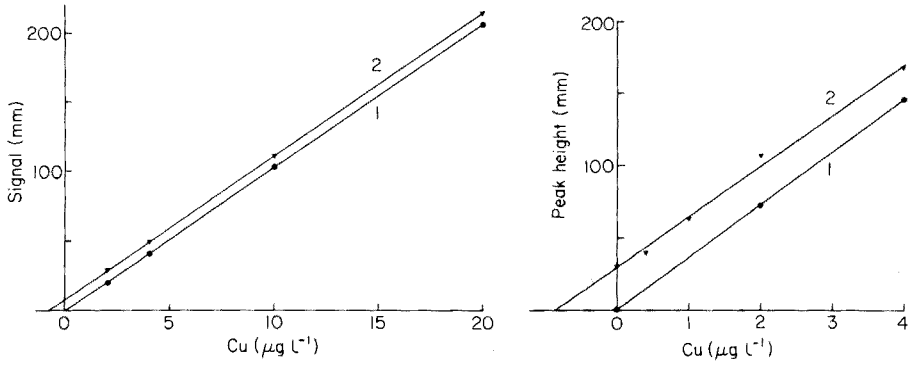

Fig. 5. Concentration range for copper with 150-s shaking time. (a) Higher concentration range (flame a.a.s.). (b) Lower concentration range (graphite-furnace a.a.s). (1) Standards, (2) extracted samples.

TABLE 3

Concentration ranges (in $\mu g\ l^{-1}$) with constant extraction yield at two different shaking times

Metal	Shaking time	
	30 s	150 s
Cd	0—0.5	0—1[a]
Co	0—2	0—2[a]
Cu	0—5	0—20[a]
Fe	0—10	0—100[a]
Ni	0—20	0—20[a]
Pb	0—0.5	0—1[a]
Zn	0—30	0—100[a]

[a]Maximum concentration tested.

organic phase was left in contact with the water in the separatory funnel. At intervals new aliquots of organic phase were withdrawn and immediately back-extracted. The last extract was collected after 192 h. The results are shown in Table 4. Zinc showed least stability, but the extract could be stored for at least 19 h at room temperature without decomposition. However, experience has shown that the stability is subject to large variations; it is therefore recommended to not store the extract longer than necessary.

Back-extraction

The back-extraction was necessary to transfer the metals to an acidified aqueous solution. Nitric acid is the most suitable acid for the HGA and was consequently used. The amount for complete back-extraction was investi-

TABLE 4

Percentage of metal left in the organic phase after storage

Metal	Time (h)					
	6	19	48	72	144	192
Cd	100	100	100	100	<1	<1
Co	100	100	100	100	100	100
Cu	100	100	100	100	100	100
Fe	100	100	100	100	89	71
Ni	100	100	100	100	90	85
Pb	100	100	100	100	87	41
Zn	100	100	61	18	2	<1

gated. A large batch of extract was collected and poured in 30-ml portions into test tubes. Different amounts (50—1000 μl) of nitric acid were added and the tubes were shaken for 10 s. After 1 h, 10 ml of water were added and the tubes were reshaken. The quantity of acid used had no influence on the back-extraction efficiency; 200 μl was chosen to ensure complete decomposition of the metal complexes. The final nitric acid concentration resulted in only a slight reduction of the analytical sensitivity.

The reaction time between the addition of acid and the addition of water was also examined. Reaction times below 10 s gave low values for Cd, Co, Cu and Ni, but after 5 min all metals were completely back-extracted. The carbamates in the organic phase are decomposed by the nitric acid, which can be seen from the brown colour of the nitrogen oxides formed.

Stability of the aqueous solution

Determinations on the same extracts were made 1, 2, 4, 15, 30 and 70 days after back-extraction. Losses of metals in 0.3 M nitric acid by adsorption onto vessel walls was hardly expected, but a slow leaching of metals from the test tubes was thought possible. However, no significant change in the concentration of metals was found during the storage period studied. This is an important advantage over most other extraction methods for trace metal analysis.

Interferences

Salts from small amounts of sea water sometimes accompanying the organic phase will end up in the final solution and decrease the signal in the determinations of cadmium and lead. This problem can be minimized by careful control of ashing and atomizing temperatures, but it is also advizable to avoid introducing sea water into the test tubes. This can be done by careful handling of the separatory funnels. The slight overpressure formed during shaking should be released through the stopper, so that sea water will not be blown into the stopcock and be removed later with the Freon.

The acid concentration used in the final solution affects the signal slightly, and the standards must be of the same acidity as the samples.

Precision and accuracy

The precision of the method was evaluated with synthetic sea water (Table 5). In view of the low contents measured, the figures are acceptable. Table 6 shows the results from four separate determinations made on the same sea-water sample. The sample was taken with a 5-l plastic sampler (Hydrobios) from a 3224-m depth in the Gulf of Aden during the cruise with RV Academic Kurchatov 1976. Cobalt was below the limit of detection (10 ng l^{-1}) and lead was not determined. The results indicate that this sample has been contaminated with zinc, probably from the sampler. Even though the other results are fairly low, the precision is quite good. The overall precision of any trace metal analysis will be limited by contamination during the sampling and handling stages.

The accuracy of trace metal analysis in natural waters cannot be thoroughly evaluated, since, for example, no standard samples are available. Spikes added to sea water are not always relevant measures because the metal added might be in a different form from the metal originally present.

The recovery of spikes with this method is excellent, being between 90 and 100% for all metals tested. Furthermore, addition of EDTA in amounts much larger than the amount of strong complexing agent present in sea water did not affect the extraction yield. However, extraction of iron from filtered sea water without prior acidification to dissolve colloidal iron yields low results. It can be concluded that the method measures metals originally present in the form of hydrated ions and other complexes. Metals in kinetically robust complexes and colloids must be solubilized before extraction.

TABLE 5

Precision of the method based on 19 separate determinations with synthetic sea water

Metal	Cd	Cu	Fe	Ni	Pb	Zn
R.s.d. (%)	6	3	12	3	9	3

TABLE 6

Metal content found in four separate determinations on Indian Ocean deep water from the same sample

Sample	Cd (ng l^{-1})	Cu (μg l^{-1})	Fe (μg l^{-1})	Ni (μg l^{-1})	Zn (μg l^{-1})
1	50	0.50	0.58	0.65	10.8
2	46	0.52	0.60	0.67	11.1
3	50	0.54	0.49	0.65	10.8
4	48	0.49	0.57	0.70	10.2

TABLE 7

Blanks (in ng l^{-1}) following the recommended procedure

Metal	Reagent blank	Total blank
Cd	<3	1
Co	<3	<10
Cu	32	24—43
Fe	<80	60—120
Ni	<30	60—86
Pb	<4	24—36
Zn	66	60—80

Blank

The blank in the extraction procedure consists of metals in the reagents used as well as metals introduced during sampling and extraction. The metal content in the purified buffer and in the purified extraction reagent was determined by standard addition. The metal content in Freon was determined by extraction with nitric acid. In most cases, the metal content in the reagents was close to or below the detection limit for the HGA. The maximum reagent blanks in a 500-ml sample are given in Table 7.

To determine a total extraction blank, 3 ml of extraction reagent and 3 ml of buffer were poured into a 500-ml separatory funnel. Freon was added and the recommended extraction procedure was thereafter followed. The results are shown in Table 7. Extractions were done at an ordinary laboratory bench and the somewhat high lead value in the total blank is probably due to contamination from the laboratory air.

Conclusions

The main advantage of this method is the transfer of the metals to a solution where their concentrations do not change with time. In contrast to most other methods, one extraction is sufficient for the determination of Cd, Co, Cu, Fe, Ni, Pb and Zn in a sea-water sample. The final solution is well suited for analysis with the HGA and the sensitivity is good enough for analysis of open ocean waters. Furthermore, samples can be extracted immediately after sampling and the extracts stored, thus greatly alleviating storage problems.

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SPECIES DETERMINATION OF SELENIUM IN NATURAL WATERS

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SUMMARY

A method is described for the determination in natural waters of selenite, selenate, dimethyl selenide, and dimethyl diselenide. The detection limits are in the parts per trillion range. The volatile methyl species are removed from the sample with a stripping gas. The inorganic forms are selectively reduced to the hydride, and also stripped from the sample. A liquid nitrogen trap is used to collect both the selenides and the generated hydrides. Separation of the methyl species is accomplished by gas chromatography. All the species are detected by an atomic absorption spectrometer equipped with a quartz tube furnace.

In the study of selenium speciation in natural waters, the major obstacle to be overcome is one of sensitivity. Typically, the values are in the parts per trillion range. To handle this problem, previous techniques, such as the piazselenol method [1], require extensive sample treatment. Others have resorted to concentrating the sample from a large volume of water [2]. Increased sample treatment tends to make the method less than quantitative and often introduces large blank values. Another vital and often overlooked factor to be considered is sample handling and storage. This is of particular importance when dealing with such low concentrations. Sample loss by adsorption, reaction, or even volatilization must be kept to an absolute minimum.

The method described herein is capable of determining Se(IV) and Se(VI) in water samples without concentration steps or sample preparation. The procedure involves the selective volatilization of Se(IV) + (VI) similar to the technique of McDaniel et al. [3]. The method can be used in conjunction with that of Chau et al. [4] for the determination of dimethyl selenide and dimethyl diselenide. Detection of the selenium species is by atomic absorption spectrometry.

EXPERIMENTAL

Apparatus

Figure 1 shows the gas stripper and supporting apparatus which is used to remove the hydride from the solution. The stripper is made from Pyrex glass,

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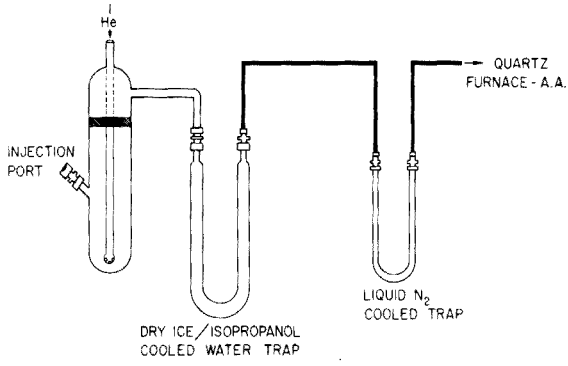

Fig. 1. Apparatus for the stripping and trapping of volatilized inorganic selenium species.

all tubing in the system is Teflon, and all Swagelok connectors used are either made of Teflon or nylon. The stripper itself can hold ca. 170 ml. The 34/45 ground-glass joint is fitted with a Teflon sleeve. The injection port consists of a Teflon Swagelok connector housing a Teflon-backed silicone rubber septum. The drying column is a Pyrex U-tube (36 cm long, 8 mm i.d.) immersed in a dry ice—isopropanol bath. The sample trap consists simply of a Pyrex U-tube (18 cm long, 6 mm o.d.) packed with silanized glass wool.

The detector, a Varian AA6 atomic absorption spectrometer, utilizes a hollow-cathode selenium lamp. The unit is fitted with a quartz tube furnace (9 mm o.d. and 6.5 cm long) which burns an air—hydrogen mixture. It is modified after a burner design used by Chau et al. [4], and is physically the same burner used by Andreae for arsenic determinations [5]. A strip chart recorder equipped with an electronic integrator records the signal. The operating parameters are: He stripping gas, 70 cm^3 min^{-1}; burner gases, 200 cm^3 min^{-1} air and 330 cm^3 min^{-1} H_2; lamp current, 8—10 mA; wavelength, 196.0 nm; slitwidth, 1.0 nm.

Reagents and standards

Standard solutions (1000 ppm) of sodium selenite and sodium selenate were made, and diluted working solutions were prepared daily. (Note: they were remade if older than 4—5 h.) The 4% sodium borohydride solution employed was made slightly alkaline (2 ml of 2 M NaOH per 50 ml of borohydride solution) to stabilize it.

The Baker Analyzed Reagent hydrochloric acid used appeared to be a major contributor to the small blank values seen as Se(IV). Typical blank values were $1—2 \times 10^{-11}$ g ml^{-1} of acid, varying from batch to batch. Doubling the borohydride concentration did not noticeably affect these values. When Baker Ultrex hydrochloric acid was used, the blank value was reduced by 69%.

Procedures

Selenite. A 100-ml sample is added to the reaction vessel of the stripper and brought to slightly above 4 M with 52 ml of HCl. The two sections of the stripper are connected and the helium stripping gas is introduced. After a sufficient time to remove air from the system (ca. 4 min), the sample trap is placed in liquid N_2. Borohydride solution (6 ml) is added through the septum with a syringe at a rate of ca. 1.5 ml min^{-1}. With too fast an addition, the vigorous generation of hydrogen can cause the furnace flame to be extinguished. The solution is stripped for exactly 10 min, including the time to add the borohydride. At this time the sample trap is removed from the liquid N_2 and allowed to warm. At the given helium flow rate (70 cm^3 min^{-1}) the signal appears in 17—20 s.

Selenate (as total inorganic selenium). A 100-ml sample is added to a beaker, brought to ca. 4 M with 52 ml of HCl, and covered with a watch glass. The solution is boiled vigorously for 4—5 min, depending on the nature of the sample (see Discussion). The solution is added to the lower portion of the stripper and quickly cooled to room temperature. Then the selenite procedure is followed, giving a Se(IV) + (VI) value.

The conversion and employment of the inorganic species apparatus for the determination of methyl selenides is described later in the text.

DISCUSSION

Hydride generation and collection

The generation of the hydride is most significantly affected by acid concentration. Using radiotracers, McDaniel et al. [3] found that a concentration of 4 M is optimal for selenium in the tetravalent state. At this concentration no detectable amounts of Se(VI) are converted to the hydride. Thus, Se(IV) can be selectively determined, and Se(VI) can be determined from a total Se(IV) + (VI) value.

The borohydride concentration needs simply to be in excess. Adding the borohydride to the stripping vessel as a solution affords easier control over the reaction than adding pellets. Moreover, the profuse hydrogen bubbles assist the helium carrier gas in stripping the hydride from the solution. The narrow diameter of the stripping vessel itself is designed to provide maximum contact between the solution and the stripping gas.

A liquid N_2 sample trap is employed to concentrate the small amount of hydride from a sample into a single, sharp pulse. However, this trap necessitates the use of a drier system since water will clog the trap. Calcium chloride is effective as the desiccant, but also traps a portion of the generated hydride. A U-tube immersed in a dry ice—isopropanol bath solved this retention problem; the results obtained were then 38% higher than those with the calcium chloride desiccant. The dry ice trap could be used all day without needing to be regenerated or repacked. Past experience with liquid N_2 traps has shown that silanized glass wool is one of the best general packing materials; the results remain quantitative and reproducible.

Interference studies [3, 6] have shown no appreciable effect on hydride generation from various ions. The ion concentrations tested were far above those found in environmental samples. The only major interference was found when attempts were made to analyze drinking water which had been chlorinated. It appears that either the chlorine interferes with the reduction or any chlorine contamination in the system oxidizes the highly reduced selenium hydride; complete loss of signal has been observed. Thus, this method cannot be used for the analysis of selenium in chlorinated water.

Reduction of selenium(VI) to selenium(IV)

In order to determine the concentration of Se(VI) in a sample, it must be quantitatively converted to Se(IV) so that a total selenium value can be obtained. A common procedure involves heating the sample with an acidic mixture of tin(II) chloride and potassium iodide [7]. Unfortunately, when sodium borohydride and tin(II) chloride are used at an acid concentration of 4 M, stannane is copiously produced from Sn(II). The tin hydride signal completely masks the selenium signal, even when background correction is used. In the standard method [8], the Se(VI)-containing solution is heated with ammonium chloride in 4 M hydrochloric acid. It was found that the ammonium chloride interferes with the production of the hydride from the selenite form. According to Walker et al. [9], the acidic tin(II) chloride—potassium iodide solution is not necessary, and a sulfuric acid—hydrochloric acid mixture can be used in its place for both Se(IV) and Se(VI). Once again this procedure cannot be employed in a method based on sodium borohydride as the reductant. Two of the above methods use a zinc or aluminum slurry to liberate the hydride [7, 9]. However, with sodium borohydride, the catalysts or acid mixtures produce an undesirable side-product (stannane), or interfere with hydride evolution (ammonium chloride), or involve a reaction too vigorous to be contained in the stripper apparatus safely (sulfuric—hydrochloric acids).

A method of quantitative reduction was found in simply boiling an acidified solution containing selenate. It is important to note that if the mixture is boiled for too long, reduction to the metallic state results. To check this, spiked selenite samples were analyzed by the selenate procedure and a boiling time curve was constructed. Any significant decrease in the selenite value was assumed to be a result of selenite reduction prior to volatilization. This is presented along with the boiling time curve for selenate in Fig. 2. As shown, boiling the 100-ml sample for longer than 5 min results in reduction of selenite. Since a total inorganic selenium value is sought, the boiling time must be watched carefully. The actual time of boiling at 4 M HCl depends on the sample volume and on the nature of the sample. Seawater samples require a boiling time of ca. 1 min less than fresh waters, and the curves are shifted appropriately. At the optimum boiling time, the conversion is 98% efficient, within the experimental error of the method. Analysis of duplicate samples shows the relative standard deviation for the reduction to be 4.4%.

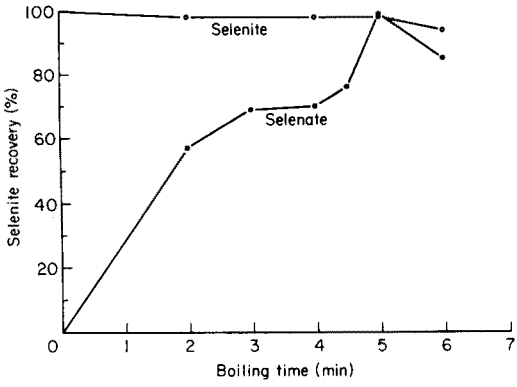

Fig. 2. Boiling time curves for 20 ng of selenite and 20 ng of selenate in distilled water. Sample volume, 100 ml; HCl concentration, 4 M. ○ Selenite; • selenate.

Detection

The flow rates of hydrogen and air used in the furnace, although optimal, are not extremely critical; variations of less than 20% can be tolerated. The recommended selenium lamp current is given as a range, since it is lamp-dependent; a current versus relative response curve must be constructed for replicate samples, to determine the optimum point for a given lamp. The variation in sensitivity over a small change in lamp current (1—2 mA) is not very large; thus, operating the lamp at a lower current has the obvious advantage of prolonging the life of the lamp without significantly altering the detection limits. For quantities under 50 ng, the responses for peak area and peak height are linear, falling off slightly for values to 200 ng (Fig. 3). Nevertheless, the reproducibility for peak area is far better than that for peak height. Analysis of a series of 5-ng Se(IV) samples gave a relative standard deviation of 1.6% with peak area, but of 7.6% with peak height. Under the conditions recommended, with peak area, the detection limit for

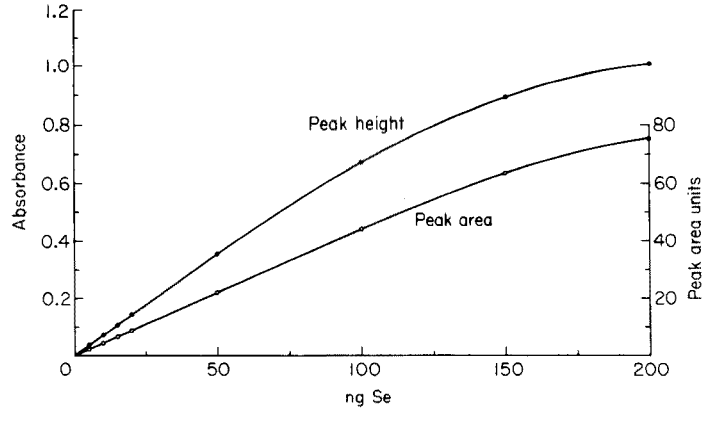

Fig. 3. Calibration curve for Se(IV) and Se(VI). Sample volume, 100 ml; HCl concentration, 4 M; lamp current, 8 mA; wavelength, 196.0 nm. • Peak height; ○ peak area.

the hydride is 0.5 ng. In a 100-ml sample this gives a relative detection limit of 5.0 parts per trillion.

Conversion of the apparatus for methyl selenides

With some minor modifications and the placement of a gas chromatograph between the stripping—trapping apparatus and the a.a.s. detector, methyl selenides can be detected in natural waters. The detection of dimethyl selenide and dimethyl diselenide with a g.c.-coupled a.a.s. apparatus has been previously described by Chau et al. [4]. These compounds are sufficiently volatile to be stripped from an aqueous sample with only helium as the stripping gas.

The impinger tip of the stripper must be replaced with a fritted glass cylinder. Since oxygen-free conditions are necessary, the stripper remains closed prior to sample addition and flushed with helium. The sample can be introduced through the septum of the injection port. The dry ice—isopropanol water trap must be replaced with a calcium chloride drying tube. The sample trap system, g.c. column, and related parameters are the same as those described by Andreae [5]. The a.a.s. parameters recommended under Experimental remain the same, and the 10-min stripping time is also used. The stripping and trapping efficiency of this system was found to be 93%, based on a comparison of a direct injection onto the column versus an addition to the stripper filled with distilled water. Sensitivity and precision are equivalent to those reported by Chau et al. [4]. Samples as large as 150 ml can be analyzed.

Sample storage

The rate of loss of selenite by adsorption on to various containers has been studied by Shendrikar and West [10]. In their work, Pyrex glass showed the lowest losses for both acidified and non-acidified samples. For selenium studies in this laboratory, various containers and caps were explored. For selenite, Pyrex bottles with aluminum foil-lined caps are used. Acidification is unnecessary for periods up to 2 weeks. Adsorption of selenate was found to be a significant problem. In polyethylene bottles, 59% loss of the sample was noted in 5 days. An unacidified sample in a Pyrex bottle exhibited a 50% loss in 7 days. Freezing the samples was also tried. Freezing spiked samples in polyethylene bottles with dry ice and subsequent storage for 3 days in a freezer resulted in a 12% loss of selenite, and a 48% loss of selenate. However, quick-freezing the samples in polyethylene bottles with liquid nitrogen gave satisfactory results; there was an initial 2% loss for selenite and 3.2% loss for selenate, with no detectable loss thereafter, when the samples were stored in a freezer.

Acidification can also be employed to preserve the samples, but it must not be excessive because the speciation can be changed. A selenium(VI)-spiked sample stored at 4 M hydrochloric acid for 7 days showed a 60% conversion to Se(IV). Sample storage at 1.0 M HCl preserves the Se(VI)

TABLE 1

Inorganic selenium species in natural waters (as ng Se l^{-1})

Location and sample type	Se(IV)	Se(VI)[a]
Penasquitos Marsh, Del Mar, Calif.	50.85	7.10
Lake Arrowhead, Calif.	17.70	<5.0
Sea water, Scripps Pier, La Jolla, Calif.		
Sept. 25, 1977	<5.0	80.20
Oct. 2, 1977	<5.0	58.40
Sea water, Santa Catalina Basin		
Surface	<5.0	NA
120 m below surface	20.63	NA
250 m below surface	33.09	NA
960 m below surface	50.57	NA
1310 m below surface (bottom)	70.22	NA
Rain, La Jolla, Calif.		
Oct. 6, 1977	52.4	<5.0

[a]NA = Not analyzed.

as well as the Se(IV). Pyrex bottles are used, but it is necessary to use caps with Teflon liners, because of etching of the aluminum foil liners by the acid. Although acidification can be used, quick freezing the samples is preferable. The introduction of variables such as blank values is avoided. In addition, the frozen samples can also be used for the analyses of other elements and compounds. Storage of the methyl species in air-tight containers is only good for one day before a complete loss of sample occurs.

Conclusion

The method described provides a simple yet sensitive analysis of inorganic and certain organic selenium species in the parts-per-trillion range. Analysis time for Se(IV) is ca. 15 min/sample, and the time for Se(VI) and the methyl species is 25—30 min/sample. The procedures have been applied in the analysis of a variety of samples. Some of the results are given in Table 1. To date, no free methyl selenides have been detected in these samples, possibly because they have disappeared during sample storage.

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Analytica Chimica Acta, 98 (1978) 67—75

DETERMINATION OF DISSOLVED BORON IN FRESH, ESTUARINE, AND GEOTHERMAL WATERS BY D.C. ARGON-PLASMA EMISSION SPECTROMETRY

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SUMMARY

A d.c. argon-plasma emission spectrometer is used to determine dissolved boron in natural (fresh and estuarine) water samples. Concentrations ranged from 0.02 to 250 mg l^{-1}. The emission—concentration function is linear from 0.02 to 1000 mg l^{-1}. Achievement of a relative standard deviation of $\leq 3\%$ requires frequent restandardization to offset sensitivity changes. Dilution may be necessary to overcome high and variable electron density caused by differences in alkali-metal content and to avoid quenching of the plasma by high solute concentrations of sodium and other easily ionized elements. The proposed method was tested against a reference method and found to be more sensitive, equally or more precise and accurate, less subject to interferences, with a wider linear analytical range than the carmine method. Analyses of standard reference samples yielded results in all cases within one standard deviation of the means.

Knowledge of the concentration of boron in geothermal and estuarine waters is valuable because boron frequently acts conservatively and is, therefore, sometimes a useful test, along with chloride, for the presence of geothermal waters and for the occurrence of mixing or evaporative phenomena in them. In addition, boron forms several complexes with fluoride and hydroxide, which can be of significance in the chemical modeling of waters and hydrological systems. Boron is of ecological significance in that concentrations of 1—2 mg l^{-1} in irrigation waters often adversely affect citrus and other plants [1].

Commonly-used methods for boron determination are the curcumin, carmine, and potentiometric acid—base titration methods [1], and the dianthrimide method [2—5]. A hollow-cathode boron lamp exists for atomic absorption spectrometry (a.a.s.), but the detection limit is so high (ca. 5 mg l^{-1} [6]) that the a.a.s. boron technique is useless for all but the most concentrated natural waters. The above methods all suffer from at least one of the following limitations: (1) poor sensitivity; (2) interference by commonly-occurring solutes, through their introducing uncontrollable errors or necessitating lengthy separations; (3) requirement for very rigid control of experimental conditions such as temperature, container shape, fumes in the air, necessity to use concentrated acids; or (4) high cost of analyst time per sample.

A recently-developed d.c. argon-plasma emission spectrometer (Spectrametrics, Inc., Andover, MA) has been described by Cox [7]. The use of a high-resolution Echelle grating (maximum resolution of 0.0015 nm at 200.0 nm and entrance and exit slit widths of 25 μm) combined with a very high-energy excitation source in the d.c. argon plasma makes this instrument a powerful analytical tool, which is reported to be virtually interference-free [7]. This type of instrument has been used in the determination of 34 elements, including boron [7]. Other investigators have reported a similar boron analysis technique, but used an inductively coupled plasma as the emission source [8, 9].

There appears to be no detailed documentation in the literature reporting specific d.c. argon-plasma analytical methodology for boron. The proposed method is demonstrated to be equal or superior to the cited methods in terms of accuracy and precision, simplicity, speed of analysis, and freedom from interferences.

EXPERIMENTAL

Apparatus and reagents

The instrument used is a Spectraspan III plasma-emission spectrometer operated in the direct-reading, single-element mode. The signal is monitored on a 10-V potentiometric recorder with a strip chart (chart speed 1 cm min^{-1}).

Instrumental settings are as follows (scfh = std. cu. ft. h^{-1}): plasma current, 7.0 A; plasma argon flow, 3.8 scfh (1.8 l min^{-1}); nebulizer argon flow, 5.5 scfh (2.6 l min^{-1}); analysis mode, direct-1; entrance slit, 25 × 300 μm; exit slit, 50 × 300 μm; wavelength, 249.773 nm; amplifier gain setting, 10—63 as required for adequate signal; photomultiplier voltage setting, 2—9 as required for adequate signal.

Sample introduction is by a 2-channel peristaltic pump fitted with tubing and fittings designed to mix automatically a 2.5% (w/v) lithium buffer solution with all samples and standards at very precise 1:10 ratio (0.148: 1.48 ml min^{-1}) at the time of analysis [10]. Waste fluid drains by gravity from the nebulizer to a collection bottle through an 8-in. coiled tubing trap.

The only reagent required is the buffer solution containing 2.5% lithium to promote and equalize sample excitation. It is prepared by dissolving the appropriate amount of ultrapure (99.999%) lithium carbonate in dilute nitric acid. Owing to its purity, this solution should be usable in the determination of other elements as well, except lithium. Standards are prepared in distilled water for samples containing less than 1000 mg l^{-1} of alkali metals. For the best accuracy in sea-water samples, 1/5 dilutions of samples or standards prepared in the appropriate matrix are used. No other sample preparation is required.

Procedure

Leave the photomultiplier on continuously except when starting the plasma. Start and warm up the instrument according to the manufacturer's instructions, continuously aspirating lithium buffer through the small-bore tubing. Optimize the wavelength and plasma positioning while aspirating a 10—20-mg l^{-1} standard of boron in distilled water through the large-bore tubing. Analyze the samples by pumping sequentially into the plasma.

Sensitivity is periodically re-optimized by re-adjustment of the plasma position and the wavelength setting. Although emission is a linear function of boron concentration throughout the 0.02—1000-mg l^{-1} range, for maximum accuracy analyze standards of several concentrations and use their emission values to construct a standard curve from which the sample concentrations can be read.

RESULTS AND DISCUSSION

Interference studies

Very few interferences were noted when the proposed technique was tested. There is a minor interference from the differential enhancement of tungsten relative to boron in solutions containing high concentrations of alkali metals, such as sea water [11]. The effect of this is to increase the background when sea water is being analyzed, and it can be mitigated by using synthetic sea water as a blank, by dilution, or by analysis by the method of standard additions.

A table of spectral-line intensities [12] was consulted in order to determine potential spectral interferences. A brief list of the major lines found appears in Table 1. Iron, tested analytically, was found to interfere significantly at concentrations exceeding 100 mg l^{-1}, if present in solutions containing 1 mg l^{-1} of boron or less (Table 2). Above the 1 mg B l^{-1} level,

TABLE 1

Potential interferent lines near the boron analytical lines (Wavelength data from Meggers et al. [12] except as noted.)

Wavelength (nm)	Element	Relative intensity	Wavelength (nm)	Element	Relative intensity
249.473	Be	1000	249.699[a]	Fe	—
249.526	W	630	249.748	W	95
249.570	Sn	1100	249.773	B	4800
249.631	Cr	170	249.796	Ge	900
249.653	Fe	140	249.828	Mo	85
249.664	W	230	249.889	Fe	100
249.671	Co	380	249.943	W	95
249.678	B	2400	249.969	W	140
249.697	W	50			

[a]Suggested by M. Bankston (Wood Hole Oceanographic Institution, Woods Hole, MA).

TABLE 2

The effect of varying iron—boron ratios at different boron concentrations on the error in the boron value
(The precision (relative standard deviation) of the boron determination is indicated at each concentration; percentage changes in apparent boron concentration are those due to the presence of interferent.)

Boron		% changes at different Fe : B ratios					
Conc.	s_r	0	100	250	500	750	1000
0.1	10	−5.0	−4.0	−2.0	+6.0	+5.0	+10.0
1.0	3	+2.5	+2.0	+4.6	+7.5	+10.6	+15.6
10	3	0.0	+1.6	+4.6	+10.4	+14.6	+15.3

TABLE 3

Effect of various concentrations of several interfering substances on apparent boron concentrations in distilled water in the two boron methodologies
(The boron concentration is 5 mg l^{-1} except for the NaCl series, where it is 2.5 mg l^{-1}.)

Ca		Mn		Al		SiO_2		NaCl	
(mg l^{-1})	(%)[a]	(mg l^{-1})	(%)	(mg l^{-1})	(%)	(mg l^{-1})	(%)	(mg l^{-1})	(%)
A. Plasma emission method (detection limit, 0.02 mg l^{-1})									
50	−1.3	100	−0.04	100	+1.0	100	+3.5	100	−0.8
100	−0.1	200	+0.24	200	−1.1	200	+2.0	1000	−1.6
200	−1.6	500	+4.3	500	+0.3	500	−0.2	5000	+2.7
500	+0.2	2000	+2.9	5000	−3.5	5000	−7.6	10000	+0.2
						10000	+0.6	50000	−22.5[b]
								100000	−55.4[b]
B. Carmine method (detection limit, 0.1 mg l^{-1})									
50	+4.0	200	+1.8	100	+1.0	500	−4.0	100	−1.6
100	+3.0	500	+5.0	200	+1.0	5000	−42[c]	1000	−1.6
200	+1.0	2000	0	500	+4.3	10000	−68[c]	5000	+4.0
500	+1.0			5000	+2.6			10000	−1.2
								50000	+2.8
								100000	−1.6

[a]Percent change in apparent boron concentration caused by interferent.
[b]Low recovery caused by quenching of plasma by excess Na^+.
[c]Low recovery caused by coprecipitation of boron with silicon dioxide during acidification step.

iron interferes significantly at values of the Fe/B ratio greater than approximately 175. The following potential interferents were found not to interfere at or below the stated level: 500 mg l^{-1} of Ca; 1000 mg l^{-1} of Mg, K, PO_4^{3-}, NO_3^-, HCO_3^-, or organic carbon as sucrose; 2000 mg l^{-1} of Mn; 10000 mg l^{-1} of Na or SiO_2. Further details of interference investigations are in Table 3. The absence of interference by silica contrasts sharply with the carmine

technique, in which the silicon dioxide precipitate scavenges boron during preparation of samples containing much silicic acid, resulting in a large absorbance depression.

Sensitivity and precision

The analytical sensitivity of the instrument tends to decrease with time because of slight displacement of the region of the plasma where maximum emission intensity occurs. The displacement is caused by continuous sputtering of the anode, which is much faster when the anode protrudes more than about 4 mm from its sleeve [13] and by slight but significant fluctuations of the optimum grating positioning, which is probably due to vibration [14]. Because of this, a standard must be read every 2 or 3 samples for high-precision analyses (i.e. relative standard deviation $\leqslant 3\%$). Insulating the instrument against vibration may correct the wavelength-drift problem; however, the slow tungsten-burn contribution to instability of sensitivity remains a difficult challenge, in that the resultant movement of the region of maximum emission intensity of the plasma is impossible to control except by frequent re-adjustment.

Sensitivity changes which frequently occur as abrupt shifts are believed to be caused by the release of particles of tungsten from within the electrode sleeve enclosing the anode. The buildup of tungsten on the inner surface of the anode sleeve is initially relatively rapid, and it alters the argon flow pattern as it accumulates. The release of a large tungsten particle in turn causes sudden changes in the flow rate and pattern and therefore in the sensitivity. This problem may be partly alleviated by optimizing the arc current, which affects the tungsten deposition rate, and by altering the flow of argon to the plasma. Reducing the flow enlarges the region of maximum excitation but increases the tungsten deposition rate. Increasing gas flow slows the rate of tungsten deposition but also reduces the size of the region of maximum excitation, making plasma positioning more critical.

Baseline drift is less of a problem than the sensitivity shifts. It is, however, highly significant at boron concentrations below 0.3 mg l^{-1}. The strip-chart recorder and the direct-mode readout of the microprocessor can be used to monitor baseline drift continuously for correction.

Some initial instrumental problems were encountered. Rapid deterioration of the ceramic electrode sleeves resulting in deterioration of sensitivity and stability was corrected by replacement of a defective plasma jet. Stability was noticeably improved by mounting the spray chamber alongside the plasma head and connecting the two with a U-tube and drain. This down-horizontal-up flow pattern (compared to the previous up-only pattern) generates a more uniform cloud of fine spray particles delivered to the plasma by removing larger droplets. This configuration is standard on currently manufactured instruments. Another source of sensitivity drift may be instability of the peristaltic pump, which regulates sample flow. Replacement with a highly precise pump would be indicated in this event.

No significant memory effects have been observed from high-level standards or samples; however, samples very high in boron should not be interspersed with background-level samples, to avoid cross-contamination by the sample tubing. Viscosity effects are minimized by using the peristaltic pump for sample introduction.

The simplicity and rapidity of the present technique imply relative freedom from contamination from reagents or from exposure to the laboratory environment because only acid preservative is added to the sample and total preparation/analysis time is less than 5 min.

The linear emission-concentration range extends from 0.02 to 1000 mg l^{-1} of boron, but precision and accuracy were evaluated only in the concentration range of the samples analyzed. Within-run precision was investigated by analyzing two sets of similar water samples withdrawn over a 36-h period from an essentially invariant source (Octopus Spring, Yellowstone Park, WY [15]). The results (Table 4) indicate a relative standard deviation (s_r) of less than 3% for both sets of samples.

Accuracy was examined by comparing the analytical results with a reference method, by determining the recovery of known amounts of boron added to various samples and by analyzing interlaboratory reference samples by both methods. Results of these three studies clearly demonstrate the excellent comparability of the present method to the classical carmine method for estimating the boron concentrations of a very broad range of water types.

Comparison with the carmine method

The carmine method was chosen for comparison because it is in routine use in U.S. Geological Survey laboratories and is a relatively simple, rapid technique with a reported sensitivity similar to that of the proposed method (detection limit, 0.1 mg l^{-1}). Hatcher and Wilcox [16] adapted the qualitative boron technique of Zorkin [17] for quantitative work, using 0.05% carmine (an anthraquinone dye derived from cochineal) in concentrated sulfuric acid. The color of carmine changes from bright red to blue to reddish-blue in the presence of boron. This color develops in about 45 min and is stable for 4 h; absorbance of the solution is read on a spectrophotometer from 585 to 600 nm. High concentrations of silicon interfere by coprecipitation of boron with gelatinous silicon dioxide, which is formed by the addition of strong acid, as noted previously. This interference can only be reduced by diluting

TABLE 4

Precision data for 9 replicate plasma emission boron analyses

Sample	$\bar{x}$ (mg l^{-1})	s (mg l^{-1})	s_r (%)
Hot spring side pool	3.07	0.07	2.3
Drainage 30 m below pool	3.21	0.09	2.9

the sample. Nitrate and nitrite interferences are eliminated by adding hydrochloric acid. Fluoride and phosphate interfere, but to a lesser extent.

Figure 1 illustrates the comparability of the values obtained by the two methods. The deviant values at the upper end of the scale are believed to be the result of interferences from silicon encountered in the carmine technique. The low values correspond to portions of samples 75WA198 and 75WA199 (Table 5) with silicon added to them and are quite obviously in error. Table 5 illustrates known standard addition recovery by the two techniques. The greater sensitivity of the plasma emission technique is undoubtedly the reason for the superior recovery of boron at this standard addition level; however, it can also be seen that the range of recoveries for the carmine technique at this standard addition level is enormous, suggesting that the technique is quite unreliable at five times the claimed detection limit.

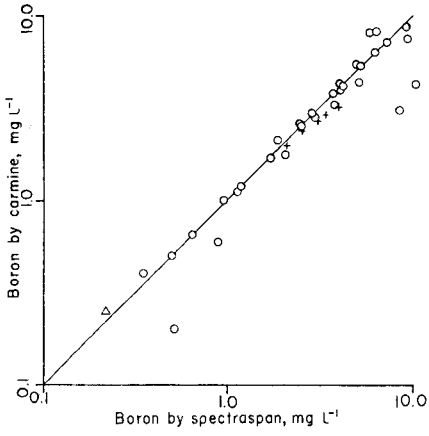

Fig. 1. Comparison of analytical methodologies for boron by the carmine vs. the plasma emission technique. ○ Geothermal water. + Estuarine water. △ River water.

TABLE 5

Comparison of recoveries of known additions obtained by the two methods (single determinations)

Sample	Plasma emission				Carmine			
	Original (mg l^{-1})	B added (mg l^{-1})	Recovery (mg l^{-1})	(%)	Original (mg l^{-1})	B added (mg l^{-1})	Recovery (mg l^{-1})	(%)
74WA117	3.25	0.5	3.73	96	2.9	0.5	3.3	80
75WA183	5.98	0.5	6.45	94	8.2	0.5	8.2	0
75WA184	0.53	0.3	0.88	117	0.5	0.3	0.6	33
75WA196	2.01	0.5	2.47	92	2.0	0.5	2.4	80
75WA198	9.39	0.5	9.83	88	8.9	0.5	4.3	−49
75WA199	8.30	0.5	8.88	116	7.3	0.5	3.1	−60
				$\bar{x}$ = 101%				$\bar{x}$ = 14%
				s_r = 12.7%				s_r = 61%

TABLE 6

Analysis of interlaboratory reference samples
(All results are given as mg l^{-1}.)

Sample no.	Reported value		Plasma emission[a]		Carmine[b]
	Av., $\bar{x}$	$s_{\bar{x}}$	Av., $\bar{x}$	$s_{\bar{x}}$	
40[c]	0.051	0.057	0.047	0.01	0.2
50[c]	0.23	0.094	0.21[b]	—	not determined
51[c]	0.046	0.032	<0.02	—	<0.1
IAGC 4[d]	24.7	2.4	25.9	0.5	24
IAGC 6[d]	28.3	2.1	28.4	0.4	32

[a]Duplicate determinations. [b]Single determination. [c]U.S. Geological Survey, Water Resources Division, Quality Assurance Laboratory, Denver, CO. [d]International Association of Geochemistry and Cosmochemistry.

Results of boron determinations of five interlaboratory reference samples (Table 6) illustrate the admirable performance of both methods over this fairly broad range of sample matrices and boron concentrations. Data obtained by the proposed method without exception fall within the standard deviation listed for the reference samples.

A single operator can routinely process more than 150 samples per day. Total automation should also be straightforward, given control over sensitivity fluctuations. However, it has not so far been possible to control sensitivity fluctuations sufficiently to permit operation in the automatic mode where a relative standard deviation of 3% or less is required.

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SPUTTERING OF ALUMINIUM ALLOYS IN THE GRIMM GLOW LAMP FOR EMISSION SPECTROCHEMICAL ANALYSIS

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SUMMARY

The effects of surface roughness, grain size and target thickness on the cathodic sputtering and emission intensities of spectral lines in the Grimm glow lamp have been investigated for samples of aluminium alloys. The intensities of the alloying elements changed with discharge time because the θ phase (Al_2Cu) and β phase (Si) are sputtered selectively. The selective sputtering of the θ phase in an Al—8% Cu alloy can be decreased and the intensities can be made constant during discharge by polishing the target surface with coarse sand paper before discharge. When a fine-grained Al—4.9% Cu—7.2% Si alloy sample was used as a target, the intensities remained nearly constant during discharge. The sample preparation for obtaining fine-grained samples involves casting the melt in a metal mould. The thickness of disk samples influences not only the sample temperature but the current, sputtering and intensities. Special attention should be paid to the thickness for the determination of copper in aluminium alloys.

Various types of light sources have been investigated to improve precision and accuracy in emission spectrochemical analysis. A glow discharge lamp was exploited by Grimm in 1967 [1] as a source for the direct analysis of metals. This lamp, called the Grimm glow lamp, operates in the region of abnormal glow discharge. Material is sputtered from the surface of a metal sample by the positive ions generated from the carrier gas and are emitted at the negative glow. This light source has several useful characteristics, such as high stability of emission, relative freedom from inter-element effects and a negligible degree of self-absorption [2—4]; it has been successfully applied to the determination of fineness of gold [5] and the in-depth analysis of the surface of alloys [6, 7].

However, some inter-element interferences which may cause a lowering of the accuracy have also been reported. Jäger indicated that the presence

of copper in a gold sample reduces the sputtering rate [8, 9], and the lead-rich inclusions contained in a brass sample are more resistant to sputtering than the matrix [10]. Selective sputtering has also been observed at the grain-boundaries of a pure copper sample whose surface is polished with a fine sand paper [11]. It is useful therefore to compile fundamental information on the sputtering as an aid for finding optimum conditions in routine analysis.

This study is primarily concerned with the effects of surface roughness, grain size and thickness of aluminium alloy samples on the sputtering and the emission intensities of spectral lines.

EXPERIMENTAL

A Grimm glow lamp with a demountable anode tube [11] was employed. The anode tube (4.5 mm i.d.) can be exchanged for another having a different inner diameter. The anode and cathode (sample) distance was adjusted to 0.12 mm. The lamp was operated at a constant argon pressure of 5 torr, with an almost constant voltage of 870 V and a variable current, unless otherwise stated.

The schematic diagram of the crater produced by sputtering on a sample surface was recorded with the Surface-Roughness and Waveness Measurement Instrument (Kosaka Laboratory Ltd., Model TR100X). The sputtering rate ($\mu g\ s^{-1}$) was calculated from the schematic diagram of the sample discharged for 120 s. The emission intensities of spectral lines were measured with a Jarrell-Ash DR-2 vacuum spectrometer.

The metals used were pure aluminium and copper, Al—8%Cu alloy and Al—4.9%Cu—7.2%Si alloy. Cubic samples (35 × 35 × 35 mm) were prepared from ingots (50 mm in diameter and 200 mm high) of aluminium alloys cast in a metal mould. To study the effects of sample thickness, metal disks 3—30 mm thick (40 mm diameter) were also used as samples. For all experiments except the study of surface roughness, the surfaces of samples to be sputtered were polished with No. 190 sand paper. An EPMA (Japan Laboratory Co. Ltd., Model JXA-5A) was used to observe the metallurgical structures of aluminium alloys.

RESULTS AND DISCUSSION

Effect of roughness of sample surface

The roughness of a sample surface was equated with the particle size of the sand paper with which the sample was polished. Samples were polished with No. 100 (coarse) and No. 400 (fine) papers.

Al—8%Cu alloy was used to investigate the effect of the surface roughness on the sputtering and the intensities of spectral lines. This alloy consists of an α phase which is an aluminium matrix containing a small amount of copper and a θ phase which is an intermetallic compound of Al_2Cu, as

illustrated in Fig. 1. The intensity—time curves of the Al I (308.2 nm) and Cu I (327.4 nm) lines were recorded for two kinds of surface roughness. The results are shown in Fig. 2.

For the rough surface, the intensities changed with the discharge time; the intensity of the Al I line increased with the discharge time, whereas the Cu I lines showed maximum intensity immediately after burn-off and gradually decreased. The change in the intensities can be best explained in terms of selective sputtering, or more accurately selective sputtering of the copper-rich (θ) phase in the initial period of discharge. A microscopic examination revealed that when the surface is smooth, the θ phase is selectively sputtering in the early period of discharge and the hole thus created becomes larger with discharge time; for the rough surface, selective sputtering of the θ phase decreases in comparison with the smooth surface.

The surface roughness influences the working curves for alloying elements contained in aluminium alloys. An example of how the slopes may change is shown in Fig. 3. These working curves were obtained at a constant pre-burn

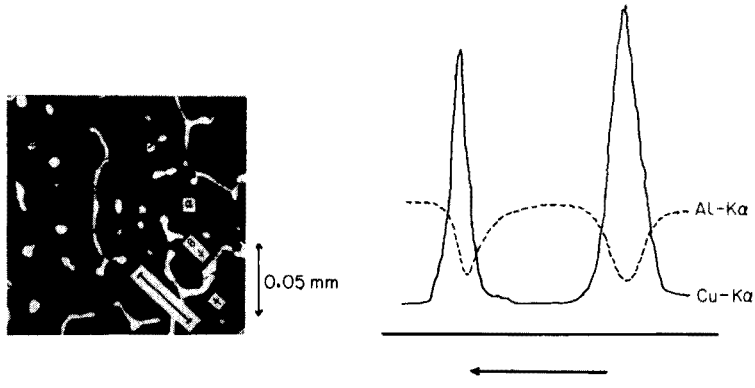

Fig. 1. EPMA study of Al—8%Cu alloy sample.

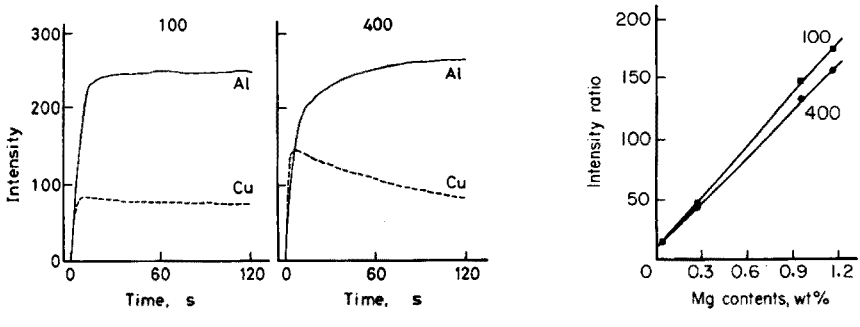

Fig. 2. Intensity—time curves of Al I (308.2 nm) and Cu I (327.4 nm) lines for Al—8%Cu alloy samples with rough (#100) and smooth (#400) surfaces.

Fig. 3. Working curves for magnesium in aluminium alloy samples with rough (No. 100) and smooth (No. 400) surfaces. Intensity ratio: Mg I (383.2 nm)/Al I (256.8 nm).

time (30 s) and exposure time (10 s). The use of a longer pre-burn time (60 s) did not eliminate the effect of surface roughness on the intensity ratios Mg I (383.2 nm)/Al I (256.8 nm), Cu I (327.4 nm)/Al I, Si I (251.6 nm)/Al I and Ni I (321.6 nm)/Al I. The selective sputtering caused by the existence of metallurgically different phases might be found for other aluminium alloys, copper alloys, steels, etc. To decrease the effect of surface roughness and improve accuracy in routine analysis, the sample surfaces should be polished with a coarser sand paper.

Effect of grain size

The photographs in Fig. 4 show an EPMA study of Al—4.9%Cu—7.2%Si alloy samples which have two different structures, gross and fine grains. The gross-grain sample was prepared by cooling the melt slowly in a graphite crucible and the fine-grain sample by casting it into a metal mould. This alloy consisted of α, θ and β phases; the β phase is silicon containing a small amount of aluminium and copper. The concentration of elements in the respective phases were much the same as those in the fine one. The intensity–time curves of the Al I (256.8 nm), Cu I (327.4 nm) and Si I (251.6 nm) lines for these two samples are shown in Fig. 5.

For the gross-grain sample, the Cu I intensity showed a maximum immediately after burn-off and then a gradual decrease, while the Si I intensity reached a maximum after ca. 1 min. To investigate the effect of increasing target area on the intensities, the 4.5-mm i.d. anode tube was replaced by a 7.0-mm i.d. one; these curves (Fig. 5) show greater changes than were obtained with the 4.5-mm i.d. tube. The surfaces of the fine- and gross-grain

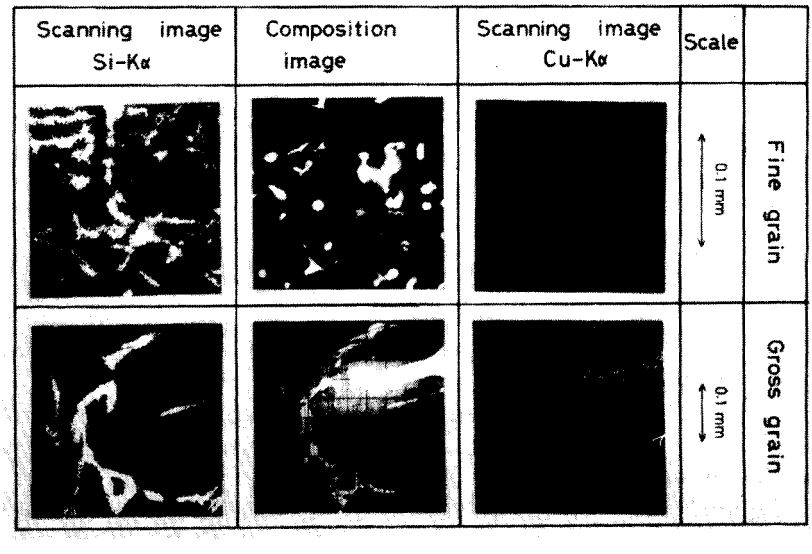

Fig. 4. EPMA study for Al—4.9%Cu—7.2%Si alloy samples with different grain sizes.

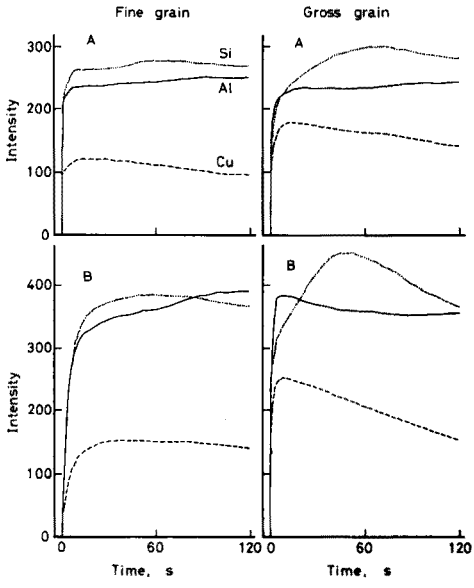

Fig. 5. Intensity—time curves of Al I (256.8 nm), Cu I (327.4 nm) and Si I (251.6 nm) for Al—4.9%Cu—7.2%Si alloy samples with different grain sizes. Anode tube: A, 4.5 mm i.d.; B, 7.0 mm i.d.

samples were examined with a microscope before and after sputtering for 120 s, but clearer information was obtained with a scanning electron microscope, Fig. 6 shows micrographs of the sputtered surfaces of the gross-grain samples for 30- and 60-s discharges. Etch pits produced by selective sputtering of the θ phase are easily seen on the sputtered surface for a 30-s discharge but those of the β phase are almost invisible; for a 60-s discharge, the etch pits of both β and θ phases are clearly visible. Thus the θ and β phases were selectively sputtered at different periods of the discharge time. The intensities were closely related to the sputtering and the intensity—time curves showed the change in the selective sputtering during the discharge. The selective sputtering of one phase among the various phases of aluminium alloys may be due to an increase in ion-bombardment of that phase because of distortion of the electrical field [10, 12]; such distortion will change with changes in the sample conditions caused by the discharge. Further research will be needed to explain this point more precisely.

For the fine-grain sample, when the α phase at the surface is sputtered immediately after the θ and β phases at the surface have been selectively sputtered within a short time, new θ and β phases appear at the surface. Accordingly, selective sputtering was again observed but the intensities were nearly constant during the discharge.

In order to reduce the effect of the grain size on the sputtering and on the intensities of spectral lines, samples should be prepared by casting the melt

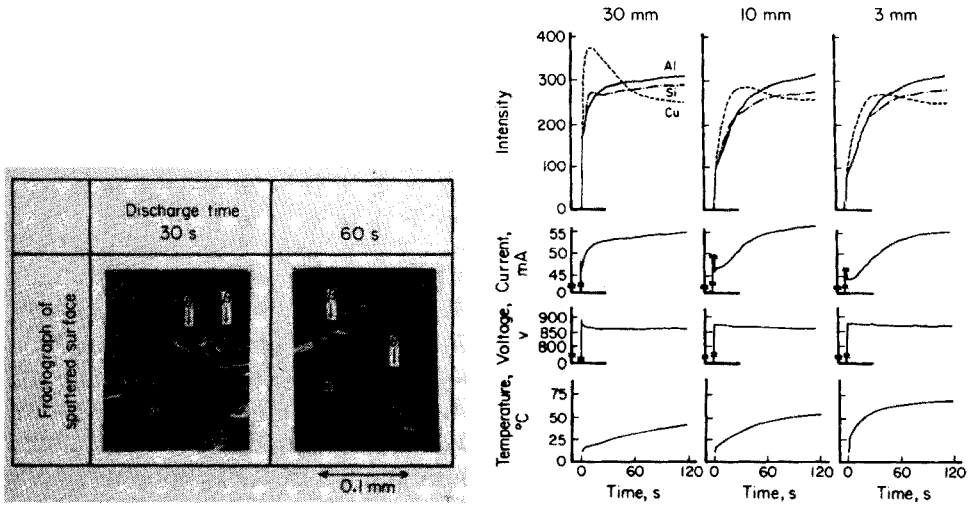

Fig. 6. Scanning electron micrographs of sputtered surface of the gross-grain sample.

Fig. 7. Temperature of sample, current, voltage, and intensities of the Al I (256.8 nm), Cu I (327.4 nm) and Si I (251.6 nm) lines as a function of time for Al—4.9%Cu—7.2%Si alloy samples of different thicknesses.

into a metal mould and should be analysed with a Grimm glow lamp which has an anode tube of a small inner diameter.

Effect of thickness of sample

A knowledge of the sample temperature would be useful in discussing the effect of sample size on selective sputtering. To measure the sample temperature generated by the discharge, a hole of 1.1-mm diameter was bored in each disk sample (3—30 mm thick, 40-mm diameter) along the central axis from the bottom, i.e. the reverse side from the target surface, to a position 1 mm short of the target surface, and a thermocouple (CA) was inserted into the hole. The temperature of the sample before burn-off was 15°C in all cases.

The temperature of the sample, the voltage, the current and the intensities of the spectral lines Al I (256.8 nm), Cu I (327.4 nm) and Si I (251.6 nm), were recorded simultaneously. The results obtained are shown in Fig. 7. During the initial period of discharge, the thicker the sample, the lower the temperature, and the larger the current. The selective sputtering of the θ phase was most remarkable for the 30-mm thick sample. This is more clearly illustrated in Fig. 8. The thicker the sample, the larger the value of the intensity ratio for Cu I/Al I, whereas the Si I/Al I ratio remained constant with change of thickness. Thus, special attention should be paid to the sample size in the determination of copper in aluminium alloys.

Additional experiments on the effect of sample thickness were carried out with pure copper and aluminium disk samples. A clear effect of thickness on

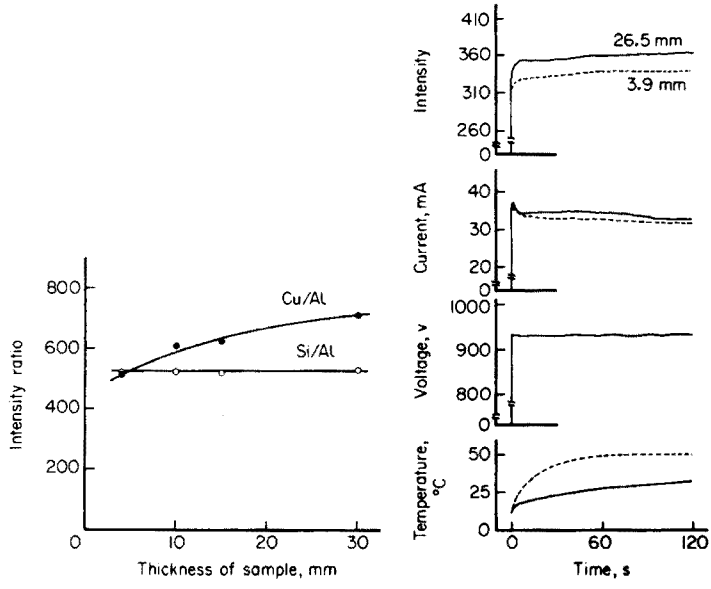

Fig. 8. Intensity ratios of Cu I (327.4 nm)/Al I (256.8 nm) and Si I (251.6 nm)/Al I plotted against thickness of samples. Pre-burn time, 30 s; exposure time, 10 s.

Fig. 9. Temperature of sample, current, voltage, and intensities of the Cu I (327.4 nm) line as a function of time for pure copper samples of different thicknesses.

sputtering and intensity was observed for pure copper but not for pure aluminium. The sample temperature, voltage, current and intensity obtained for two different thicknesses of pure copper as a function of time are shown in Fig. 9. For the thin sample, the temperature of sample was higher, the current was smaller and the intensity was weaker than those for the thick one. This is in agreement with the results for the aluminium alloy samples (Fig. 7).

It is known that the temperature of the sample surface (cathode) influences the γ function (γ is the number of electrons released from the surface by one argon ion) and the space charge, and so the thickness of the sample will influence the sputtering and the intensity. Figure 10 shows that the sputtered surface of the thin sample is less rough than that of the thick sample. This may be caused by a change in the distortion of the electrical field, because of the difference in temperatures of the samples. The temperature of this sample surface was higher than that shown in Figs. 7 and 9.

Measurements of the sputtering rate ($\mu g\ s^{-1}$), the intensity and the current were made with various thicknesses of pure copper disk samples (Fig. 11). The current and intensity represented are the average values for the period 60—120 s after burn-off. Linear relationships were obtained between the intensity and the current, and between the intensity and the sputtering rate. Similar relations will probably hold for copper contained

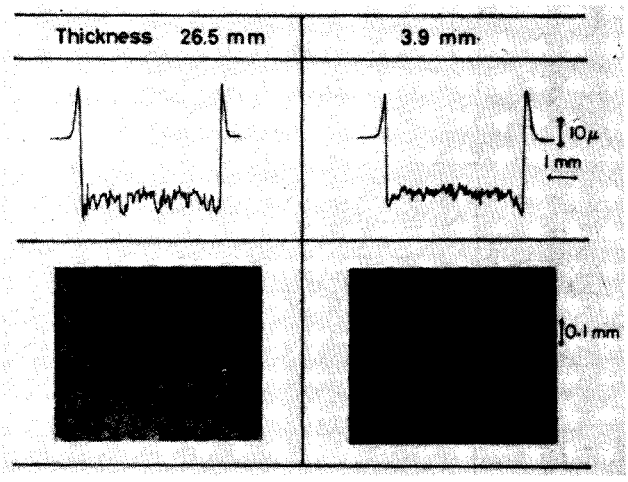

Fig. 10. Schematic diagrams of craters and microscope photographs of sputtered surfaces for pure copper disk samples of different thicknesses.

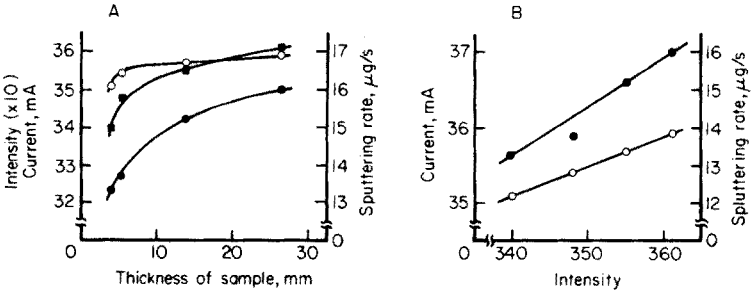

Fig. 11. (A) Intensity of the Cu I (327.4 nm) line, current and sputtering rate plotted against the thickness of pure copper disk samples. (B) Current and sputtering rate plotted against intensity of the Cu I line. Voltage, 930 V. (▪) intensity; (○) current; (●) sputtering rate.

in aluminium alloys. This can be presumed from the behavior of the Cu I intensity shown in Fig. 7.

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Analytica Chimica Acta, 98 (1978) 85—91

PERIODATE OXIDATION ANALYSIS OF CARBOHYDRATES
Part IX. Evaluation of the use of Pyridine as Reaction Solvent for Oxidation of Water-insoluble Carbohydrate Materials

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SUMMARY

Oxidation of methyl glycopyranosides with periodic acid in pyridine was studied by analysis of the reaction products by the dithioacetal method. Both C_2—C_3 and C_3—C_4 bonds were cleaved yielding three types of dialdehydes; secondary attack of these dialdehydes by periodic acid did not occur. Oxidation in aqueous pyridine proceeded in normal Malapradian fashion, but more slowly than for oxidation in water. Molar proportions of aldehydes were theoretical, when methyl glycosides were oxidized with periodic acid in a 1:1 (v/v) mixture of pyridine and water. The structures of carbohydrate moieties in a few saponins and glycolipids were confirmed by analysis of the oxidation products obtained under similar conditions.

Periodate oxidation of free carbohydrates is usually performed in water, but a considerable number of carbohydrate materials are either insoluble or difficultly soluble in water. Although mixtures of water with methanol [1], ethanol [2], and dioxane [3] may be used to effect solubilization, oxidation reactions slow down and interference with medium-induced, non-specific oxidation becomes a serious problem through prolongation of the reaction time. Therefore, determination of the structures of carbohydrate materials from periodate reduction is liable to be misleading, without careful measurement of blank values.

Recently a simple gas chromatographic method for simultaneous determination of the conjugated aldehydes in dialdehyde compounds by their direct conversion to dithioacetals, followed by trimethylsilylation was developed [4]. Versatile analysis of the products of oxidation of carbohydrates by this method offers more reliable information on the structures of carbohydrates, and the mode of oxidation of carbohydrates in pyridine, a universal solvent for this group of compounds, has been examined. This paper gives details of the oxidation of methyl glycosides with periodic acid in this solvent, and demonstrates the applicability of oxidation in aqueous pyridine to the analysis of interglycosidic linkages in water-insoluble carbohydrate materials, such as saponins and glycolipids.

EXPERIMENTAL

Materials

Pyridine was refluxed with barium oxide and distilled before use. A solution of periodic acid in this dehydrated pyridine was prepared as follows. A slight excess of reagent-grade periodic acid dihydrate (Wako Pure Chemical Industries, Ltd., Doshomachi, Osaka) was dissolved in pyridine, a large excess of Drierite was added, and the mixture was stored in darkness for a few days. Then the mixture was centrifuged, the content of periodic acid in the supernatant solution was assayed by iodimetry [5], and the concentration was adjusted to 0.10 M by the addition of dehydrated pyridine. Sodium metaperiodate and all other reagents were of the highest grade commercially available. Methyl α-D-glucopyranoside (Wako Pure Chemical Industries, Ltd.) and other methyl glycosides (Sigma Chemical Co., St. Louis, Missouri) were used. Cholesterol glycosides were prepared [6] by a modified Königs–Knorr condensation between the corresponding acetobromo sugars and cholesterol in benzene in the presence of silver carbonate. The samples of diacylglycerides were isolated from spinach leaves [7] and purified on a column of silica gel. D-Glucopyranosyl ceramide was purchased from the Sigma Chemical Co. All samples of these carbohydrate materials gave single spots on t.l.c. (Merck t.l.c. Plate Silica Gel 60; 60 : 35 : 8 (v/v) chloroform–methanol–water; spray, concentrated sulfuric acid).

Oxidation of methyl glycosides with periodic acid in pyridine

Equal volumes of solutions of a methyl glycoside in dehydrated pyridine (0.01 M) and periodic acid in pyridine (0.1 M) were mixed; the resultant solution was kept at 25°C in darkness. Aliquots (2 ml) were removed at intervals, and treated with a 1% solution of ethylene glycol in pyridine (1 ml); the mixture was then allowed to stand for 15 min in darkness. The reaction mixture was evaporated to dryness, and the residue was dissolved in a small volume of water. The solution was passed through a column of Amberlite CG-120 (H^+ form, 100 mesh, 0.5 ml) and Amberlite CG-400 (OAc^- form, 100 mesh, 0.5 ml), and the column was washed with water (20 ml). The eluate and the washing fluid were combined and evaporated to dryness. The residual syrup was dissolved in aqueous acetone, and the volume was adjusted to 2.00 ml. A 200-μl portion was evaporated in a small sample tube (0.5 cm i.d., 5 cm long), and the residue was subjected to component analysis.

Oxidation of methyl glycosides with periodic acid in a 1:1 (v/v) mixture of pyridine and water

Equal volumes of an aqueous solution of a methyl glycoside (0.01 M) and of periodic acid in pyridine (0.1 M) were mixed; the resultant solution was kept at 25°C in darkness. The reaction mixture was worked up as for oxidation in pyridine, and the deionized product was subjected to component analysis.

The molar ratios of D-glyceraldehyde to glyoxal, obtained by component analysis of methyl α- and β-D-glucopyranosides, were 1.02 and 1.00, respectively. The molar ratio of glycolaldehyde to glyoxal for methyl β-D-xylopyranoside was 1.03.

Oxidation of methyl β-D-glucopyranoside with sodium metaperiodate

Equal volumes of aqueous solutions of methyl β-D-glucopyranoside (0.01 M) and sodium metaperiodate (0.1 M) were mixed; the resultant solution was kept at 25°C in darkness. The reaction mixture was worked up as for oxidation in pyridine, except that the addition of ethylene glycol and subsequent evaporation of the reaction mixture were omitted, and the deionized product was subjected to component analysis.

Simultaneous determination of the conjugated aldehydes in products of oxidation

The procedure is essentially the same as described previously [4]. A syrupy product in a sample tube, obtained by deionization or extraction of the reaction mixture, was dissolved in a freshly prepared mixture (10:1, v/v, 20 μl) of ethanethiol and trifluoroacetic acid, and the solution was kept for 30 min at 25°C. A solution (50 μl) of D-xylitol (internal standard, 1 μmol) in pyridine was added to the reaction mixture, followed by hexamethyldisilazane (100 μl) and trimethylchlorosilane (50 μl). The mixture was kept for 30 min at 50°C with occasional shaking and then centrifuged. The supernatant (1 μl) was analyzed by gas chromatography (Shimadzu 4BMPF chromatograph with a hydrogen flame ionization detector). Gas chromatography conditions: column, 3% OV-1 on Chromosorb W (2 m, glass); column temperature, 180°C; detector temperature, 240°C; carrier (N_2), 50 ml min^{-1}. Peaks were integrated with a Shimadzu ITG-2A integrator.

Glycolaldehyde, D-glyceraldehyde, and D-erythrose were converted quantitatively to the trimethylsilyl derivatives of their diethyl dithioacetals, and detected at retention times (r.r.t.) relative to the trimethylsilyl derivative of D-xylitol, of 0.36, 0.70, and 1.74 with relative molar response factors (r.m.r.f.), 0.464, 0.641, and 0.772, respectively. Glyoxal was converted to the bis-dithioacetal which appeared at r.r.t. 1.53 with r.m.r.f. 0.521. Hydroxymalonaldehyde was converted to the trimethylsilyl derivative of the bis-dithioacetal (r.r.t. 4.14, r.m.r.f. 0.753). The r.m.r.f. values for the methyl pentosides (r.r.t. 0.5—0.9) and hexosides (r.r.t. 1.2—2.0) were 0.643 and 0.869, respectively.

Analysis of the glycosidic linkages in model saponins and glycolipids

A carbohydrate material was dissolved in the solution of periodic acid in pyridine (0.1 M, 100 μl), and to this solution was added water (100 μl). In the case of the ceramide, a minimum volume of chloroform was also added to give complete dissolution of the material. The resultant solution was kept for 24 h at 25°C. A solution of ethylene glycol in pyridine (1%,

100 μl) was added, and the mixture was allowed to stand for 15 min in darkness and evaporated to dryness. The residue was dried in vacuo, and extracted with chloroform (20 ml). The extract was concentrated to a small volume, transferred to a small tube, and evaporated to dryness. The residue was subjected to component analysis as described above. The results are given in Table 1.

RESULTS AND DISCUSSION

Figure 1(a) shows a typical example of oxidation of glycosides with periodic acid in pyridine. Formation of each aldehyde from methyl β-D-glucopyranoside increased gradually to reach a plateau in a few hours. This

TABLE 1

Molar proportions of aldehydes for model saponins and glycolipids, oxidized with periodic acid in aqueous pyridine[a]

Saponin, glycolipid	Amount of sample (μg)	Molar ratio, D-Glyceraldehyde[b] / Glyoxal
β-D-Glucopyranosyl cholesterol	551	0.99
β-Gentiobiosyl cholesterol	603	0.95
β-D-Galactopyranosyl diacylglyceride	1,032	0.93
α-D-Galactopyranosyl-(1→6)-β-D-Galactopyranosyl diacylglyceride	855	0.96
β-D-Galactopyranosyl ceramide	626	1.00

[a]Reaction time, 24 h. [b]Theoretical value is 1 for all saponins and glycolipids.

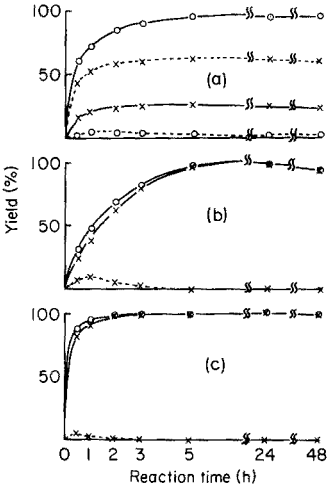


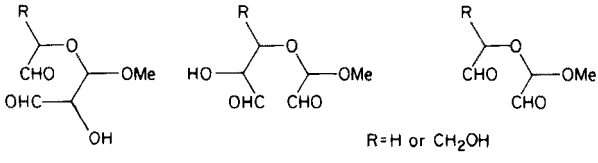
Fig. 1. Oxidation of methyl β-D-glucopyranoside with (a) periodic acid in pyridine, (b) periodic acid in a 1:1 mixture of pyridine and water, and (c) sodium metaperiodate in water. o—o D-glyceraldehyde, o----o D-erythrose, x—x glyoxal, x---- x hydroxymalonaldehyd

is comparable with oxidation with sodium metaperiodate in water (Fig. 1(c)), where the yield of glyoxal increased and that of hydroxymalonaldehyde decreased after it reached a maximum value, but the total value was equal to the yield of D-glyceraldehyde throughout the course of oxidation [8]. It is also noticeable that, unlike oxidation in water, a considerable amount of D-erythrose was found, and the total yield of the hydroxyaldehyde components (D-glyceraldehyde and D-erythrose) was maintained equal to that of the dicarbonyl components (glyoxal and hydroxymalonaldehyde). This relationship was also observed for other hexopyranosides, but relative yields of aldehydes differed with the configurations of the glycosides. For pentopyranosides, glyceraldehyde should be replaced by glycolaldehyde and tetrose by glyceraldehyde in this relationship.

Periodate cleaves either the C_3—C_4 or C_2—C_3 bond, or both, in methyl glycosides, yielding the type *a*, *b* or *c* dialdehyde (see Table 2), respectively. The amount of the type *b* and *a* dialdehydes can be estimated from the yields of tetrose (for hexosides) or glyceraldehyde(for pentosides) and hydroxymalonaldehyde (for both), respectively. The amount of the type *c* dialdehyde is calculated as the difference of the yields between glyceraldehyde (for hexosides) or glycolaldehyde (for pentosides) and hydroxymalonaldehyde (for both). Molar proportions of these three types of dialdehyde, obtained for oxidation of each glycoside, were almost constant without regard to reaction time within 24 h. Table 2 gives their average values. After 24 h, the proportions of the type *a* and *b* dialdehydes decreased, and

TABLE 2

Molar proportions[a] of three types of dialdehydes formed on oxidation of methyl glycopyranosides in pyridine



Type *a* Type *b* Type *c*

Glycoside	Molar proportion of dialdehydes		
	Type *a*	Type *b*	Type *c*
Methyl α-D-galactopyranoside	66	1	33
Methyl β-D-galactopyranoside	82	0	19
Methyl α-D-glucopyranoside	69	13	17
Methyl β-D-glucopyranoside	74	5	21
Methyl α-D-mannopyranoside	79	9	11
Methyl β-L-arabinopyranoside	81	16	1
Methyl β-D-xylopyranoside	50	48	4

[a]Average values for the oxidation for 0.5, 1, 2, 3, 5, and 24 h.

accordingly that of the type *c* dialdehyde increased very slowly. Table 2 shows that the most abundant type of dialdehyde is *a*, for all glycosides. However, the selectivity in C_3—C_4-bond scission is not as high as in oxidation in water. It is striking that the degree of cleavage of the C_2—C_3 bond in methyl β-D-xylopyranoside was approximately equal to that of the C_3—C_4 bond. The presence of considerable amounts of the type *c* dialdehydes for all glycosides is also remarkable. Since the type *a* and *b* dialdehydes in pyridine are considered to exist in cyclic forms in which the secondary hydroxyl groups are blocked by hemiacetal bonds, α-hydroxyaldehyde groups are not available for further oxidation. Equilibria between the cyclic and dissociated forms arising from traces of water in the solvent may be considered, but such large amounts of the type *c* dialdehydes are unlikely to be formed by secondary attack by periodate. If secondary oxidation of the dissociated forms does occur, the molar proportions of dialdehydes would not be maintained constant, but proportions of the type *c* dialdehydes would continue to increase fairly quickly after the initial stage of oxidation. The mechanism of formation of the type *c* dialdehydes is uncertain, but contributions of tridentate complexes [9, 10] as intermediates may be postulated. Periodate reduction measured by iodimetry [5] was slightly higher than expected from the amounts of aldehydes. For instance, 1 mol of methyl α-D-glucopyranoside reduced 1.49 mol of periodate after 24 h. The expected value was 1.33 mol. Over-estimation was probably a result of interaction with pyridine.

The results obtained above differ greatly from those reported by Yu and Bishop [11] for the oxidation of methyl glycosides with periodic acid in dimethylsulfoxide. Their work, done by qualitative analysis of the hydrolysates of the borohydride-reduced oxidation products, suggested selectivities in C_2—C_3- and C_3—C_4-bond scission for methyl β-D-xylopyranoside and both methyl β-L-arabino- and α-D-galacto-pyranosides, respectively. Methyl α-D-gluco- and α-D-manno-pyranosides were reported to be oxidized non-selectively. Since only the hydroxyaldehyde components were detected, i.e., the dicarbonyl components were missed, the type *a* and *c* dialdehydes could not be differentiated in their experiments. Therefore, formation of the type *c* dialdehydes by oxidation in dimethylsulfoxide may also be possible. The results in Table 2 indicate that, for oxidation in pyridine, none of these glycosides have selective sites of oxidation in the strict sense, though the degree of cleavage of each bond differs for the different structures of the glycosides.

Oxidation in aqueous pyridine took a course intermediate between that of oxidation in pyridine and that in water. Thus, the oxidation of methyl β-D-glucopyranoside in a mixture (1:1, v/v) of pyridine and water yielded D-glyceraldehyde and glyoxal in moderate speeds as shown in Fig. 1(b). Hydroxymalonaldehyde was first formed, but disappeared in a few hours. The molar ratio of D-glyceraldehyde to glyoxal was exactly unity after oxidation for 24 h. For other glycosides similar results were obtained.

Periodate reduction in this solvent system was abnormal; the amount of periodate reduced by 1 mol of methyl α-D-glucopyranoside after 24 h reached 6.34 mol, apparently because of non-specific reduction by the reaction medium.

A few model saponins and glycolipids were oxidized under similar conditions and the products were analyzed. The ceramide necessitated the addition of a minimum volume of chloroform for complete dissolution. Table 1 gives results that agree well with the theoretical values.

The data presented in this work indicate that the use of aqueous pyridine as a solvent for periodate oxidation is useful for the elucidation of glycosidic linkages in carbohydrate materials that are insoluble or difficultly soluble in water, if aldehydic products are analyzed by the dithioacetal method. Measurement of periodate reduction, however, is of no value for linkage analysis, because of interaction with the reaction medium.

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Analytica Chimica Acta, 98 (1978) 93—99

THE POLAROGRAPHIC BEHAVIOUR OF THE ANTIDEPRESSANT DRUG CHLORIMIPRAMINE

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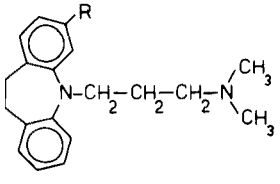
SUMMARY

The polarographic activity of chlorimipramine is due to surface catalytic hydrogen evolution; there is no reduction of the chlorimipramine itself at the d.m.e. The occurrence of the catalytic process is confirmed by the effects of the supporting electrolyte and of the buffer pH and composition.

Tricyclic antidepressants such as the dibenzazepines have been determined by many sophisticated analytical techniques, e.g. gas chromatography with mass spectrometry [1] or with a specific nitrogen detector [2], h.p.l.c. [3—5] and t.l.c. of ion pairs [6, 7]. Surprisingly, no electrochemical methods have been used until recently. Some electrochemical detectors for h.p.l.c. have been developed which make it possible to detect antidepressants such as imipramine and its derivatives by oxidizing them at a graphite electrode [8—12].



R = H imipramine
R = Cl chlorimipramine

Polarographic determinations of tertiary amines have been described. Hoffmann [13] reported the polarographic determination of different amines after oxidation to the corresponding amine oxides. This method of converting the amino group to a reducible group has been followed up by other investigators [14, 15].

Rojo et al. [16] developed a suitable polarographic analysis for some cyclic psychopharmaceutical drugs which involved pretreatment with nitric—sulphuric acid mixture before the polarographic measurements. Unfortunately this method is not applicable to analysis for the imipramine derivatives.

The present paper concerns the electrochemical behaviour of 1,4-dibenz-[bf]azepine derivatives. Imipramine and chlorimipramine are known [17] to show some polarographic activity and can be determined in pharmaceutical preparations such as Tofranil and Anafranil. The influence of the medium on the polarographic behaviour of chlorimipramine is reported here and an explanation of the phenomena observed is given.

EXPERIMENTAL

Apparatus and chemicals

A Princeton Applied Research model 174 A polarograph equipped with a PAR model 174/70 drop timer was used with an Omniscribe recorder. The three-electrode assembly consisted of a dropping mercury electrode (PAR capillary model 9309), a saturated calomel reference electrode (Metrohm EA 410) and a platinum wire auxiliary electrode. A Radiometer PHM 62 standard pH meter was used.

All chemicals were of analytical grade (p.a., Merck) and were used without further purification. Nitrogen was purified by passage through vanadium(III) chloride solution. The antidepressant drug was kindly supplied by Ciba-Geigy.

Procedures

D.c. polarography or differential pulse polarography (modulation amplitude 25 mV) was used at a scan rate of 5 mV s^{-1} (drop time, 1 s; time constant of the low pass filter, 0.3 s; mercury column height, 66 cm).

The solutions were deaerated by passing through nitrogen for 10 min. Surfactants were not used. The pH of the buffered solutions was adjusted with 2 M NaOH solution. The buffer solutions used were Britton-Robinson buffers, phosphate buffers and borate buffers of various concentrations. The exact conditions are given in the Figure legends.

RESULTS AND DISCUSSION

The chlorimipramine wave corresponds to catalytic evolution of hydrogen. This was indicated by the shape of the wave (Fig. 1, Curves A, C, D), the lack of dependence of the wave height on the height of the mercury column, and the non-linear dependence on the concentration of chlorimipramine. The wave is considerably higher than the 2e diffusion-controlled wave of benzophenone (Fig. 1, Curve B), particularly at higher concentrations (Fig. 2), and is also dependent on the buffer concentration and composition (Fig. 1). The best polarograms are obtained in buffered sodium chloride solutions. Britton–Robinson, phosphate and borate buffers (Fig. 1, Curves C and D) at various concentrations and pH have been used. The variation of the instantaneous current with the time during the drop life indicates that the catalytic process is accompanied by adsorption phenomena (Fig. 3).

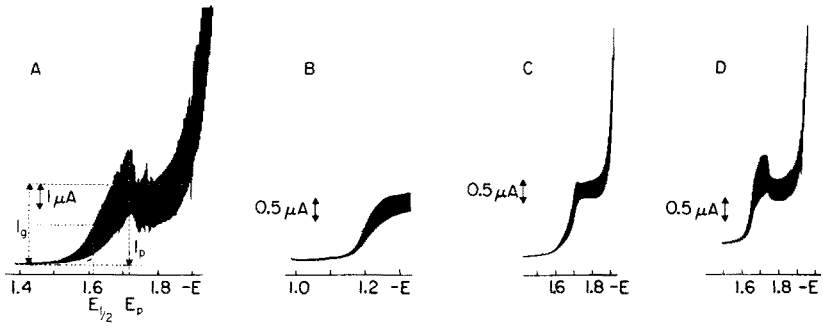


Fig. 1. Polarographic waves of (A) 1.6×10^{-4} M chlorimipramine and (B) 1.6×10^{-4} M benzophenone, in a supporting electrolyte containing 10% acetone, 0.5 M NaCl and 0.008 M phosphate at pH 7.4. (i_g = wave height, $E_{1/2}$ = half-wave potential, i_p = peak height, E_p = peak potential.) Curves C and D were obtained for 10^{-4} M chlorimipramine in supporting electrolytes of 0.5 M NaCl—0.003 M phosphate at pH 8.0, and 0.5 M NaCl—0.003 M borate at pH 8.3, respectively.

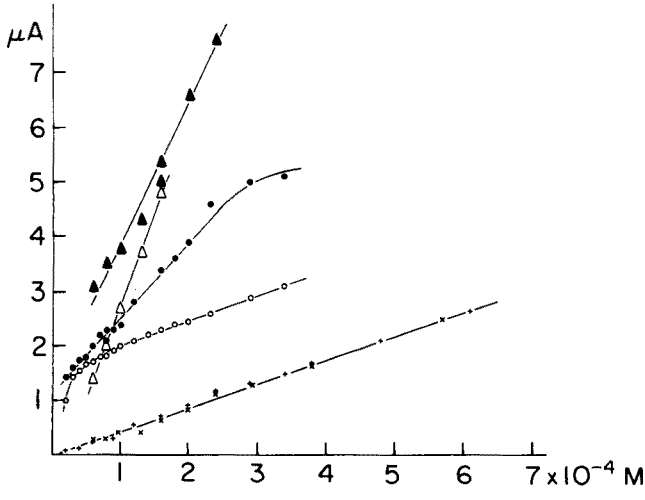


Fig. 2. Concentration of depolarizer (chlorimipramine or benzophenone) vs. the wave height or peak height at two different phosphate concentrations. Supporting electrolyte, 0.5 M NaCl—10% acetone. (+) Wave height for benzophenone at 0.004 M phosphate, pH 7.8. (×) Wave height for benzophenone at 0.008 M phosphate, pH 8.0. (○) Wave height for chlorimipramine at 0.004 M phosphate, pH 7.2. (●) Peak height for chlorimipramine at 0.004 M phosphate, pH 7.2 (△) Wave height for chlorimipramine at 0.008 M phosphate, pH 7.4 (▲) Peak height for chlorimipramine at 0.008 M phosphate, pH 7.4.

This and the shape of the wave show that the wave is a surface catalytic wave rather than a volume catalytic wave [18—21].

The shape and the height of the polarographic waves are highly dependent on the buffer concentration in the supporting electrolyte solution (Fig. 4). A linear relationship exists between the phosphate buffer concentration and

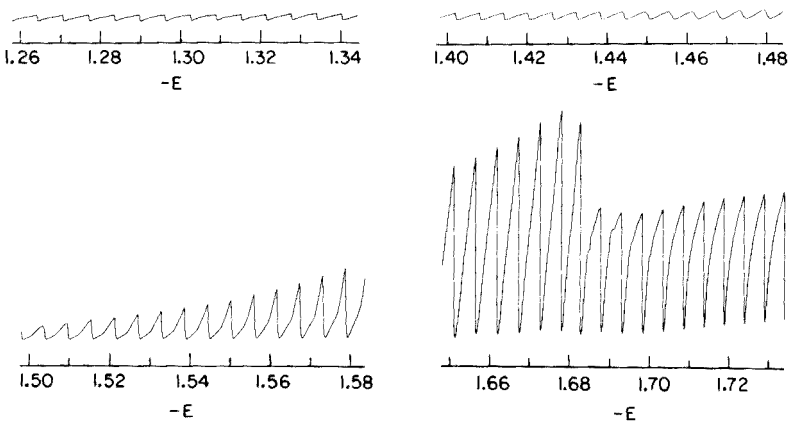


Fig. 3. Variation of the instantaneous current during the life of a single mercury drop. —1.26 V to —1.34 V: Diffusion current of the supporting electrolyte. —1.40 V to —1.48: The chlorimipramine starts to be adsorbed at the d.m.e.; change-over from diffusion to adsorption current. —1.50 to —1.58 V: Change-over from adsorption to catalytic process. —1.65 to —1.73 V: Reorientation and partial desorption of the chlorimipramine at the d.m.e. causing a peak in the polarogram.

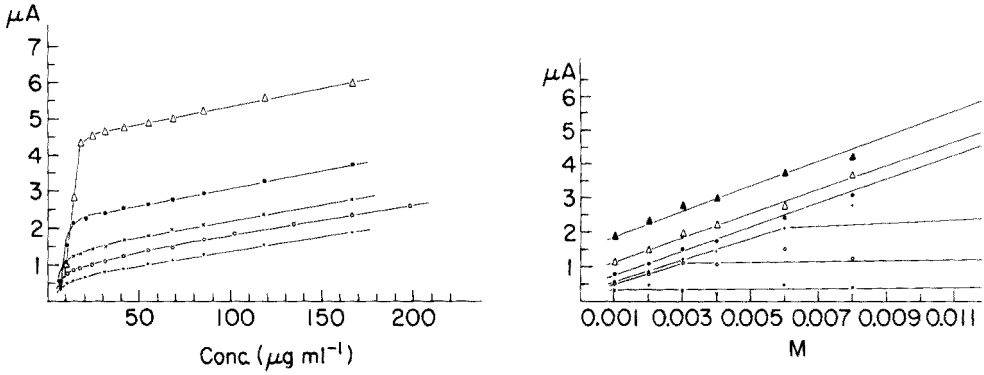


Fig. 4. Wave height vs. concentration of chlorimipramine hydrochloride at several phosphat concentrations. Supporting electrolyte, 0.5 M NaCl at pH 7.2. (+) 0.001 M phosphate, (○) 0.002 M phosphate, (×) 0.003 M phosphate, (●) 0.006 M phosphate, (△) 0.012 M phosphate.

Fig. 5. Wave height for chlorimipramine vs. concentration of phosphate at different chlorimipramine hydrochloride concentrations. Supporting electrolyte, 0.5 M NaCl at pH 7.2. Concentrations of depolarizer: (×) 7.0, (○) 10.5, (+) 13.9, (●) 31.2, (△) 68.5, (▲) 166.5 μg ml^{-1}.

the wave height at constant chlorimipramine concentration and constant pH (Fig. 5). The slope of this plot and the linear relationship between wave height and concentration of the proton donor ($H_2PO_4^-$) indicate a 1e diffusion process, although phosphate itself is not polarographically active.

The influence of the pH on the polarographic waves for chlorimipramine at constant phosphate concentration is shown in Fig. 6.

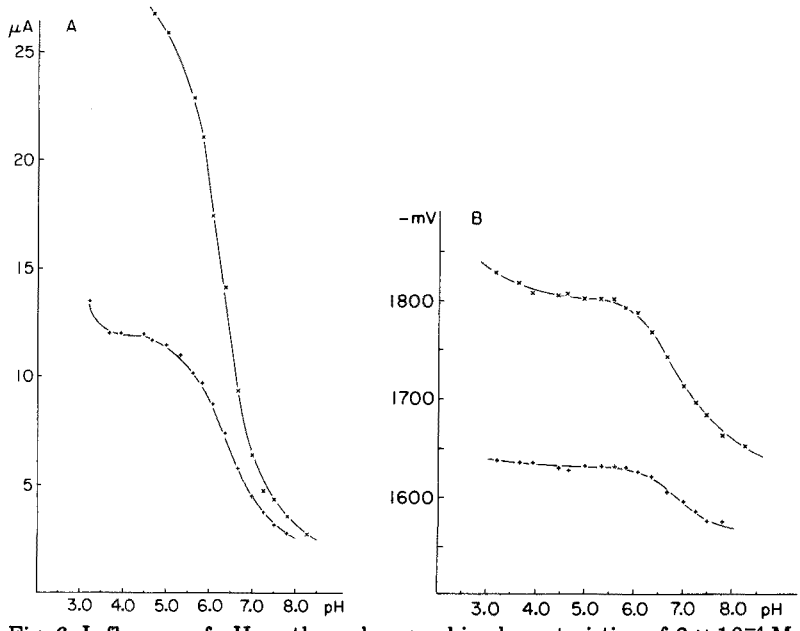


Fig. 6. Influence of pH on the polarographic characteristics of 2×10^{-4} M chlorimipramine. Supporting electrolyte, 0.5 M NaCl—0.006 M phosphate. (A) Effect on wave height (+) and peak height (×). (B) Effect on half-wave potential (+) and peak potential (×).

The course of the polarographic curves of chlorimipramine may be explained as follows. The changes in the instantaneous current of the drop life indicate strong adsorption of chlorimipramine at the d.m.e. Reorientation of the chlorimipramine molecules at the d.m.e. followed by a partial desorption produces a polarographic maximum. The chlorimipramine serves as a catalyst for the reduction of protons. The protons of the protonated chlorimipramine molecules which are adsorbed at the d.m.e. will be reduced first. Then proton donor ($H_2PO_4^-$) diffuses from the bulk solution to the d.m.e. and delivers new protons to the deprotonated chlorimipramine in order to allow additional catalytic proton reduction. This explains the linear relationship in Fig. 5. The factors limiting the wave height are the quantity of adsorbed chlorimipramine at the d.m.e. and the concentration of proton donor in the solution. When very little or no proton donor is present in the bulk solution, only the protons of the adsorbed chlorimipramine itself can be reduced. In this case, the limiting current is not a catalytic but an adsorption current (Fig. 4). The relationship between wave height and concentration of chlorimipramine resembles very well the adsorption isotherm $i = aC^{1/n}$, where i is the wave height (μA), C is the concentration of chlorimipramine (μg ml^{-1}), a is a constant (μA ml μg^{-1}) and n is a constant.

Calculation of the adsorption isotherm of Fig. 4 for a phosphate concentration of 0.001 M ($H_2PO_4^-$ concentration is much less) by the method of the least squares, gives a value of 2.0 for n and a value of 0.49 for a (corre-

lation coefficient, 0.995). When the proton donor concentration is increased, the adsorption character vanishes and the catalytic character becomes apparent (Fig. 4). Of course, the pK_a value of the proton donor must be less than the pK_a value of chlorimipramine, otherwise no proton transfer can occur; pK_a (chlorimipramine) = 9.4; pK_a ($H_2PO_4^-$) = 7.21; pK_a (H_3BO_3) = 9.23 [22, 23].

Changes in pH cause changes in the concentration of proton donor and therefore affect strongly the polarographic curves of chlorimipramine. The dissociation curve of the proton donor is sigmoidal when the pH is changed from 3 to 9 at constant chlorimipramine concentration (Fig. 6). Above pH 9, there is no polarographic activity because only the protonated form of chlorimipramine is active (the unprotonated form is insoluble).

During controlled-potential electrolysis of chlorimipramine at the d.m.e., the polarographic curve did not change. When a hanging mercury drop electrode was used, gas bubbles developed and interfered with the electrolysis. The electrolysis potential was selected at the point before the polarographic peak at −1700 mV (vs. SCE). The pH of the electrolysed solution changed from 7.09 to 7.20, in agreement with an estimate based on calculations of consumption of hydrogen ions. After the controlled potential electrolysis, a small decrease in the concentration of chlorimipramine was found by means of high-pressure liquid chromatographic analysis; possibly, some chlorimipramine was adsorbed on the mercury at the bottom of the electrolysis cell. It is not likely that the chlorimipramine had disintegrated because no other peaks could be detected in the chromatograms.

Conclusions

The effects of the composition of the supporting electrolyte, the composition and concentration of the buffer solution, and of the pH make it clear that the catalytic process involved in the electrode process has a surface character. The electrolysis experiments with the hanging mercury drop electrode and with the dropping mercury electrode prove that chlorimipramine acts as a catalyst for the reduction of protons.

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DETERMINATION OF CICLAZINDOL IN BIOLOGICAL FLUIDS BY DIFFERENTIAL PULSE POLAROGRAPHY

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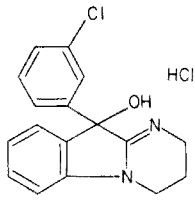
SUMMARY

A differential pulse polarographic method has been developed for determination of the antidepressant, 10-(*m*-chlorophenyl)-2,3,4,10-tetrahydropyrimido[1,2a]indol-10-ol hydrochloride in plasma and urine. The method involves solvent extraction of the drug from the plasma or urine, evaporation to dryness and dissolution of the residue in 10% methanolic 0.01 M tetraethylammonium chloride solution followed by differential pulse polarography. The mean recovery of the drug from plasma containing 0.5—5.0 $\mu g\ ml^{-1}$ is 80%; the coefficient of variation is 5.5% at the 1.0-$\mu g\ ml^{-1}$ (2.98×10^{-6} M) level on 2-ml samples. The method is not subject to interference from the chemical degradation products and metabolites. The techniques described have been applied to the analysis of human plasma; the polarographic and gas chromatographic results showed good agreement.

Ciclazindol, 10-(*m*-chlorophenyl)-2,3,4,10-tetrahydropyrimido[1,2a]-indol-10-ol hydrochloride (I) is a drug of novel structure which has been shown to possess pharmacological properties in animals that suggest it may have antidepressant activity in man [1]. The pharmacokinetics and metabolism of the drug have been studied by liquid scintillation counting of urine and plasma samples after oral administration of [2-^{14}C]-ciclazindol to human volunteers [2, 3]. Studies by Swaisland et al. [2] on the metabolism of (I) in man showed that ciclazindol was extensively metabolized and rapidly excreted to give at least three metabolites. Possible chemical degradation products and metabolites include the hydrolysis product (II), the oxidation derivative (III) and the amide (IV).

A wide range of analytical techniques is available for the analysis of drugs and metabolites in biological fluids, e.g. spectrophotometry [4], fluorescence [5], gas chromatography [6], polarography [7] and, more recently, radio-immunoassay [8], high-pressure liquid chromatography [9, 10] and mass fragmentography [11]. Each technique has its merits and the criteria for selection of methods have been considered [12].



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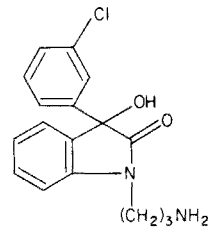

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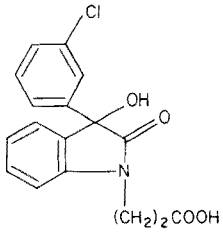

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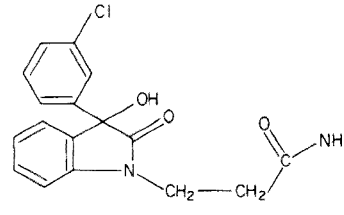

IV

Recently, a gas—liquid chromatographic method [13] has been devised for the determination of (I) in plasma after solvent extraction, but this involves derivatization of the drug to form a more volatile silylated compound.

In view of the difficulty in developing a simple, selective and sensitive method, it was decided to investigate other methods of analysis for this drug. The applicability of polarographic techniques to the analysis of pharmaceutical formulations is well recognised [7]. A stability-indicating assay based on d.c. polarography has been reported [14] for the determination of (I) in pharmaceutical preparations at concentration levels of 10^{-4} M with tetrabutylammonium chloride as the supporting electrolyte. Measurement of concentrations below 10^{-5} M, however, is greatly hindered by the increasing residual current of the supporting electrolyte. Since its introduction by Parry and Osteryoung [15], differential pulse polarography (d.p.p.) at the dropping mercury electrode (DME) has been widely employed for trace analysis of metals and drug substances at the 10^{-7} M, and in some cases at the 10^{-8} M level. The use of d.p.p. to measure drugs in biological fluids has been reported [16].

This paper describes the development of a d.p.p. method for the determination of (I) in biological fluids, after extraction into toluene of the compounds from plasma or urine. The technique is most suitable for determining intact drug at levels of 0.5—5.0 μg ml^{-1}, in 2-ml plasma or urine samples. Precautions and optimization of experimental conditions of the technique are also considered.

EXPERIMENTAL

Apparatus

All experiments were done with a Princeton Applied Research Corporation (PAR) Model 174A Polarographic Analyser equipped with a PAR Model 172A Drop Timer and electrode assembly. Polarograms were recorded on an Advance X-Y recorder (Model LR 100). The three-electrode system used contained a dropping mercury electrode (DME) as the working electrode, a saturated calomel reference electrode (SCE) and a platinum wire counter electrode. The polarographic cell made of borosilicate glass (Princeton Applied Research, Accessory 9328), had a working volume of 2—50 ml. The differential pulse mode was used with a 50-mV pulse and a 1-s drop time. The flow rate was 2.70 mg s^{-1} ($m^{2/3}$ $t^{1/6}$ = 2.332). Scans were made from —0.750 to —1.650 V vs. SCE at a scan rate of 5 mV s^{-1} with a full scan range of 3.0 V. The current sensitivity required ranged from 0.2 to 1 μA full scale deflection.

Accurate additions of standards to plasma or urine blanks were made from 100-μl syringe micropipettes (Alpha Laboratories, Greenford, Middlesex). Finnpipettes (1—5 ml; Jencons, Hemel Hempstead) were used for pipetting and delivering organic solvents.

Reagents

Compounds (I)—(IV) were prepared in the Wyeth Laboratories [17]; their purities were confirmed by thin layer chromatography: (I) 10-(*m*-chlorophenyl-2,3,4,10-tetrahydropyrimido[1,2a]indol-10-ol hydrochloride, (II) 1-(3-aminopropyl)-3-(*m*-chlorophenyl-3-hydroxy-2-indolinone, (III) 3-(3-chlorophenyl)-2,3-dihydro-3-hydroxy-2-oxo-1*H*-indole-1-propanoic acid, and (IV) 3-(3-chlorophenyl)-2,3-dihydro-3-hydroxy-2-oxo-1*H*-indole-1-propanamide.

All other reagents (analytical-reagent grade) were used without further purification except as indicated otherwise. An aqueous 0.01 M solution of tetraethylammonium chloride (TEACl, Eastman Kodak) was prepared. All aqueous reagents were prepared in glass-distilled water. Triply-distilled mercury was used in the DME.

Biological samples used were horse plasma and human urine.

Preparation of standard solutions

Dissolve 10.0 mg of (I) ($C_{17}H_{16}N_2OCl_2$; m.w. 335.228; m.p. 282°C) in 100 ml of distilled water. Dilute 10.0 ml and 25.0 ml of the resulting solutiom to 100 ml in separate flasks with distilled water to give standard solutions containing 10 and 25 μg ml^{-1}, respectively.

Extraction procedure

To a 20-ml glass-stoppered centrifuge tube, add 2.0 ml of plasma or urine and 0.1 ml of distilled water. Adjust the sample to pH 12.3 with

0.2 ml of 5 M sodium hydroxide solution, extract with 6.0 ml of toluene by shaking gently for 2 min, and centrifuge for 3 min at 3,500 rpm to separate the layers. Repeat the procedure with 2.0 ml of plasma or urine blank. Prepare standards by adding 100 μl of the 10- and 25-μg ml^{-1} solutions of (I) to 2.0-ml portions of plasma or urine blank and extract as described above. Retain the standard solutions for the determination of percent recovery.

Transfer 5.0 ml of the supernatant toluene to a 10-ml pear-shaped flask with a Finnpipette, and evaporate under a stream of nitrogen on a water bath at 80°C. Dissolve the residue in 0.2 ml of methanol by swirling for 1 min. Add 1.8 ml of aqueous 0.01 M TEACl and shake vigorously for 1 min. Transfer the contents to the polarographic cell, remove oxygen from the solution by passing purified nitrogen for 10 min, and analyse by differential pulse polarography, using the operational parameters described above. Run a reagent blank by adding 0.2 ml of methanol and 1.8 ml of aqueous 0.01 M TEACl directly to the polarographic cell.

Sample concentrations were calculated in the usual manner based on comparison with the standards in plasma or urine. The percent recovery was obtained by direct comparison of the peak currents of these standards, after correction for the aliquot taken, with those of pure standard solutions.

Decontamination of glassware

Differential pulse polarograms of reducible substances are greatly affected by the presence of surfactants [18]. In this case, successful analysis depends on careful cleaning of the glassware as follows. The bulk of the proteinaceous material adhering to the glassware in the extraction procedures is first removed by immersing overnight in a dilute solution of Decon 90. The apparatus is then washed in hot tap water to remove the excess of surfactant. The most effective way of removing traces of this surfactant is to fill the apparatus with chromic acid, and leave it for several hours. The acid is then poured off, and the apparatus is thoroughly rinsed with distilled water and allowed to drain until dry. Under the conditions described, as little as 0.5 μg ml^{-1} of the drug could be reliably measured in 2.0-ml samples of plasma and urine.

RESULTS AND DISCUSSION

Supporting electrolytes

The proposed method was developed by screening a large number of supporting electrolytes to determine their effect on peak height for ciclazindol reduction. The polarographic peak at −1.44 V vs. SCE for (I) is due to the reduction of the carbon—nitrogen double bond in the cyclic amidine ring [19]. This potential range is most easily utilized with tetraalkylammonium ions as the supporting electrolyte up to −2.5 V [20]. Tetrabutylammonium chloride was used as the supporting electrolyte for

determinations of ciclazindol in tablet formulations [14]; the residual current was negligible when the concentration was between 0.005 and 0.025 M for d.p.p. at low sensitivity (10 μA), but at high sensitivity (0.5 μA) the residual current at —1.44 V (vs. SCE) was too great to allow quantitative analysis. A more dilute electrolyte solution (0.001 M) gave a large peak at about —1.1 V; although this seemed to reflect the presence of an electroactive impurity, it was unaffected by attempts at purification.

Other tetraalkylammonium halides and phosphonium compounds were then examined to determine their residual current curves at high instrumental sensitivity. An aqueous solution of tetraethylammonium chloride (0.01 M) gave a polarographic curve at high sensitivity (0.2 μA full scale) with a baseline free from impurity peaks over the potential range —0.75 to —1.65 V vs. SCE. Subsequently, dilute solutions of the drug in this supporting electrolyte were analysed successsfully.

A linear relationship between peak height and concentration was obtained from 1.7×10^{-7} to 3.4×10^{-5} M. At higher concentrations, there is a levelling effect, and the calibration graph is no longer rectilinear.

Extraction of unchanged ciclazindol

Direct determination of (I) in plasma was not possible because the wave was greatly distorted by an adsorbed film of surface-active proteins [21] on the electrode surface. Consequently, the drug had to be separated from the proteins before polarography. A shortened version of the solvent extraction procedure of Swaisland et al. [3] was used to extract the drug from horse plasma, providing a final solution sufficiently clean to give a reproducible d.p.p. baseline. No interferences were present in the region of —1.44 V, the position of the drug activity.

In a series of experiments, various amounts of ciclazindol hydrochloride were added to 2-ml plasma samples, and extracted as described above. The drug in the clear toluene phase was easily determined by evaporating an aliquot under a stream of nitrogen on a water bath, dissolving the residue in methanolic TEACl solution, and analysing by d.p.p. from —0.75 to —1.65 V (vs. SCE). The use of a little alcohol facilitates complete transfer of the drug and minimizes possible adsorption effects. Figure 1 shows d.p.p. scans of ciclazindol after extraction from horse plasma at concentrations of $1.49—14.9 \times 10^{-6}$ M. The excellent linearity of the concentration—peak current plot would allow accurate analysis based on standard ratios.

The mean recovery of the drug from horse plasma containing 0.5—5.0 μg ml^{-1} was 80%, based on comparisons of the peak currents of the samples and pure standard solutions at —1.44 V vs. SCE.

It was shown by t.l.c. that compound (I) was unchanged on extraction. The residues obtained from the extractions by evaporating the organic solvent to dryness were re-dissolved in methanol (0.5 ml). Portions of these solutions (100 μl) together with a standard solution of the drug were spotted on silica gel plates and developed in (a) toluene/ethanol/ammonia (79:20:1)

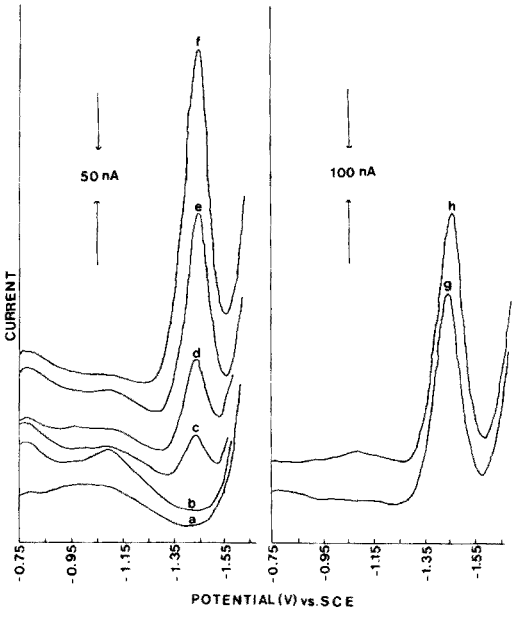

Fig. 1. Differential pulse polarograms of ciclazindol after extraction of the drug from plasma with toluene, evaporation to dryness and dissolution of the residue in 2 ml of 10% methanolic 0.01 M TEACl solution. (a) Reagent blank, (b) plasma blank, (c) 0.5 μg of (I) per ml (1.49×10^{-6} M), (d) 1.0 μg of (I) per ml, (e) 2.0 μg of (I) per ml, (f) 3.0 μg of (I) per ml, (g) 4.0 μg of (I) per ml, and (h) 5.0 μg of (I) per ml.

and (b) glacial acetic acid/methanol (3:7). Only one main spot was detected in each chromatogram (by u.v. absorption at 254 nm) for sample extracts and standard solutions, with R_F values of 0.40 and 0.52, respectively, for systems (a) and (b).

Precision of the method

The reproducibility of the polarographic method was tested for ten horse plasma samples containing a known amount of the drug (2.98×10^{-6} M). The coefficient of variation was 5.5%, which is an adequate precision for biological analysis.

Interference by metabolites and chemical degradation products

Swaisland et al. [3] showed that ciclazindol was extensively metabolized and rapidly excreted to give at least three metabolites. Possible interfering species present in urine include the hydrolysis product (II), the oxidation derivative (III) and the amide (IV), which may be co-extracted in the clean-up steps prior to polarography. The d.p.p. behaviour of 10^{-4} M solutions of each was tested in 10% methanolic tetraethylammonium chloride solution (0.01 M). Table 1 shows that all compounds exhibit one d.p.p. peak.

TABLE 1

Polarographic data of some metabolites and chemical degradation products of ciclazindol in man. Solutions 10^{-4} M in 10% methanolic tetraethylammonium chloride solution, 0.01 M

Compound	Half-wave potential (V)[a]	Limiting current (μA)
I	−1.44	5.0
II	−1.84	4.6
III	−1.58	2.5
IV	−1.48	1.2

[a] vs. SCE.

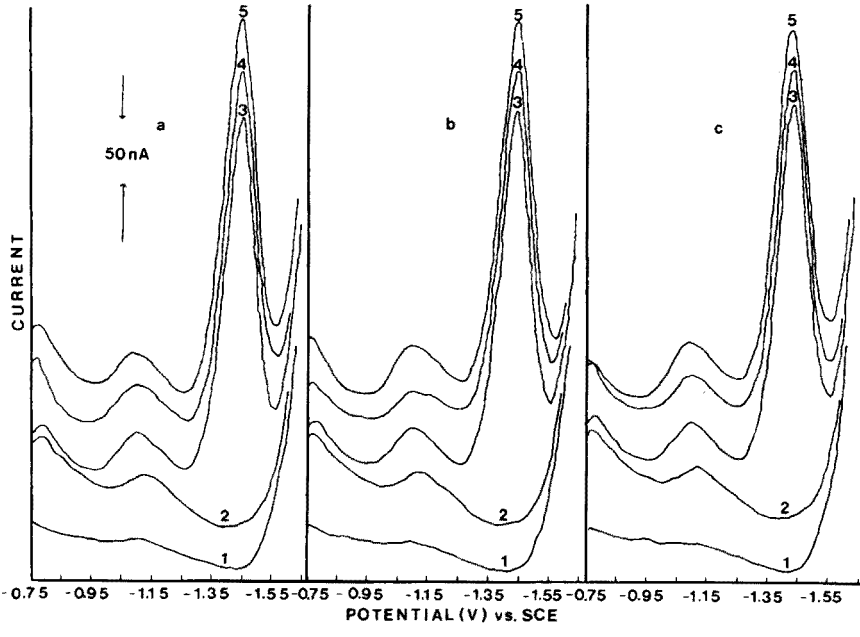

Fig. 2. Effect of interference by metabolites and chemical degradation products on differential pulse polarograms of 7.5×10^{-6} M solutions of compound (I) after extraction from urine with toluene, evaporation to dryness and dissolution of the residue in 2 ml of 10% methanolic 0.01 M TEACl solution. (a) Additions of (3) 0.0, (4) 2.5 and (5) 5.0 μg of compound (II); (b) additions of (3) 0.0, (4) 2.5 and (5) 5.0 μg of compound (III); (c) additions of (3) 0.0, (4) 2.5, and (5) 5.0 μg of compound (IV). Scans (1) and (2) in each case are for the reagent blanks and the urine extracts, respectively.

The specificity of the urine assay was tested by adding various amounts of (II), (III) and (IV) to urine samples containing a known amount of compound (I). The solutions were extracted by the method described above, and then subjected to polarography. Figure 2 shows the effects of successive additions of compounds (II), (III) and (IV) at different concentration levels to 7.5×10^{-6} M compound (I) in urine samples. The peak

TABLE 2

Comparison of methods for the plasma concentrations of ciclazindol in patients undergoing treatment at 100 mg (base) per day. Plasma level is expressed as μg of (I) per ml

Patients	Polarographic assay			Chromatographic assay		
	2 weeks	4 weeks	6 weeks	2 weeks	4 weeks	6 weeks
B.F.	3.3	2.6	6.6	3.2	3.2	5.3
J.B.	1.3	1.0	1.2	0.9	1.2	1.4
F.L.	2.2	2.3	2.4	2.0	2.1	1.9
M.P.	1.9	2.2	2.5	2.1	2.2	2.4
V.B.	2.8	2.6	2.7	2.7	2.9	2.6
H.W.	2.3	2.9	NS	2.4	2.6	2.2
L.J.	3.4	4.6	3.6	3.4	4.4	3.1
G.C.	4.3	2.7	2.3	4.3	2.8	2.5
M.Y.	1.3	1.5	1.1	0.9	1.3	1.3
A.C.	2.1	2.8	2.4	2.3	2.1	2.0

height is seen to be unaffected by the co-extracted biological impurities and possible metabolites, thus establishing the specificity of the urine assay.

Application of the method to clinical samples

Plasma samples obtained from patients after oral administration of ciclazindol at 100 mg (base) per day were analysed by d.p.p. and the results compared with those obtained by gas—liquid chromatography (g.c.). The g.c. method [13] involves extraction of the drug from the plasma, concentration and silylation with 8% BSA in dimethylformamide followed by g.c. with an unheated flame ionization detector. A similar extraction procedure was also applied to the plasma samples prior to polarography. Table 2 shows that the results obtained by d.p.p. and g.c. are in good agreement. The correlation coefficient [22] between the polarographic and chromatographic assays is 0.95.

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Analytica Chimica Acta, 98 (1978) 111—114

POLAROGRAPHIC INVESTIGATIONS OF SOME COPPER CHELATES OF 3-ARYLAZOPENTANE-2,4-DIONES

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SUMMARY

The polarographic curves of the copper chelates of twelve substituted 3-arylazopentane-2,4-diones exhibit single 4e diffusion-controlled irreversible waves between pH 2 and 11. The two azo groups in the compound are equivalent so that only a single 4e wave is obtained; no copper reduction wave is observed. The polarographic behaviour differs from that of *p*-bisazobenzene. There is a linear correlation between the $E_{1/2}$ values and the Hammett substituent constants.

The complexes of acetylacetone and other 1,3-diketones with metals have proved of inestimable value in the purification and separation of metals [1], because of their high volatility and solubility in apolar solvents. The study of such copper chelates is of particular interest because of their pharmaceutical uses [2].

This paper presents a study of the polarographic reduction of copper chelates of 3-arylazopentane-2,4-diones (soluble in 40% methanol) and the effect of various substituents on their $E_{1/2}$ values. The polarographic mechanism involves reduction of the ligand rather than the central atom; this is not frequently encountered, although some azo complexes have been studied [3], particularly for copper complexes. A relationship between the half-wave potentials and Hammett substituent constants is also discussed.

EXPERIMENTAL

Chemicals and solutions

Twelve copper chelates of 3-arylazopentane-2,4-diones, having as substituents H, 2-CH_3, 3-CH_3, 4-CH_3, 2-OCH_3, 3-OCH_3, 4-OCH_3, 2-OC_2H_5, 4-OC_2H_5, 2-Cl, 3-Cl and 4-Cl, were synthesized [4] and their purity was checked by t.l.c.

Britton—Robinson buffers [5] in the pH range 2—11 were prepared by adding suitable amounts of 0.2 M sodium hydroxide solution in a stock B.R. buffer solution (pH 1.8) composed of a mixture of (A.R.) boric acid, phosphoric acid and glacial acetic acid.

The stock solutions (10^{-3} M) of the copper chelates of the 3-aryl-azopentane-2,4-diones were prepared in methanol (A.R.).

Apparatus and procedures

Polarograms were recorded at 30 ± 0.1°C with a Cambridge pen recording polarograph. The capillary characteristics were 1.215 $mg^{2/3} s^{-1/2}$. A saturated calomel reference electrode was used. The number of electrons (n) involved in the reduction was determined for the parent compound — the copper chelate of 3-phenylazopentane-2,4-dione — by the method of DeVries and Kroon [6] with a mercury pool cathode. Nejedly's method [7] was used to determine the temperature coefficient.

Polarographic measurements were carried out in a mixture of 4.0 ml of buffer, 2.0 ml of methanol (which was necessary to keep these compounds in solution), 1.0 ml of 1 M KCl, 2.0 ml of the sample solution and 1.0 ml of gelatin solution (0.05%). Oxygen was removed by passing hydrogen for 5 minutes.

RESULTS AND DISCUSSION

All these compounds at the 2.0×10^{-4} M level are reduced in a single 4e wave; no other wave was observed, except the hydrogen wave at −1.6 V. As reduction of the azo linkage occurs more readily than reduction of copper, the wave was assigned to the reduction of —N=N—; typical polarographic curves are shown in Fig. 1. The temperature coefficient was 1.0—1.4% deg^{-1}. The wave heights were proportional to the square root of the mercury reservoir height as well as to the concentration of depolarizer (0.5×10^{-4}—3.0×10^{-4} M). The current is thus diffusion-controlled. The characteristics of these waves are given in Table 1.

The dependence of wave height and half-wave potential on pH was investigated in buffer solutions of pH 2.0—11.0. For all the compounds studied, the wave height was independent of pH; the half-wave potential depended on pH and shifted towards more negative potentials with increase in pH. The $-E_{1/2}$ vs. pH relationship was linear with a slope of 40—44 mV/pH (Table 1). The half-wave potentials of all these compounds shifted to negative potential with increasing concentration of depolarizer. This suggests the irreversible nature of the waves and the possible role of adsorption [8].

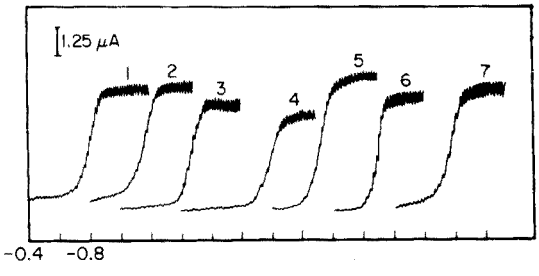

Fig. 1. Typical polarographic curves of copper chelates of 3-arylazopentane-2,4-diones. (1) R = 2-CH_3, (2) R = 4-OCH_3, (3) R = 3-CH_3, (4) R = 4-CH_3, (5) R = 4-OCH_3, (6) R = 3-CH_3 (7) R = 4-OC_2H_5. Curves (1)—(4), (7) at $c = 2.0 \times 10^{-4}$; (5), (6) at 2.5×10^{-4} M; curves (1)—(6) at h = 70 cm; (7) at h = 80 cm; each curve starts at −0.4 V. Curves (1), (2), (5), (6), (7), at pH 2.7; curve (3) at pH 4.8; curve (4) at pH 7.2.

TABLE 1

Characteristics of some copper chelates of 3-arylazopentane-2,4-diones at pH 2.7 (C = 2.0 × 10^{-4} M; a and b are from the relation $-E_{1/2} = a(\text{pH}) + b$.)

No.	R	$-E_{1/2}$ (V)	$\Delta E_{1/2}$ (V)	i_d (μA)	α_n	I	a	b
I	H	0.75	0.00	3.500	0.686	14.40	40	0.70
II	2-CH_3	0.80	−0.05	3.750	0.686	15.43	40	0.73
III	3-CH_3	0.76	−0.01	3.625	0.653	14.91	44	0.70
IV	4-CH_3	0.77	−0.02	3.500	0.630	14.40	41	0.69
V	2-OCH_3	0.70	0.05	3.625	3.630	14.91	40	0.65
VI	3-OCH_3	0.74	0.01	3.750	0.653	15.43	40	0.65
VII	4-OCH_3	0.78	−0.03	3.750	0.630	15.43	40	0.71
VIII	2-OC_2H_5	0.70	0.05	3.560	0.686	14.65	40	0.65
IX	4-OC_2H_5	0.78	−0.03	3.560	0.630	14.65	44	0.71
X	2-Cl	0.70	0.05	3.500	0.630	14.40	44	0.60
XI	3-Cl	0.71	0.04	3.370	0.686	13.86	44	0.66
XII	4-Cl	0.72	0.03	3.500	0.630	14.40	38	0.65

Controlled-potential electrolysis of a 2 × 10^{-4} M solution of the parent compound in Britton—Robinson buffer pH 2.70 gave n = 4. The following general equation can be proposed for the reduction of these compounds:

$$(R.C_6H_4{-}N{=}N{-}C_5H_6O_2)_2Cu + 4e + 4H^+ \rightarrow (R.C_6H_4{-}NH{-}NH{-}C_5H_6O_2)_2Cu$$

This mechanism is in accord with the observed pH-dependence of the half-wave potentials. Similar mechanisms for the reduction of azo groups have been proposed by other workers [9—11]. Tachi [12] and Nygard [13] have reported that reduction of azobenzene gives a reversible 2e wave at low concentration and low pH. That a single irreversible 4e wave was observed for the present compounds may be attributed to the two bulky acetyl substituents in the molecule. In contrast to earlier work [3], there is no similarity between the mechanisms for the compounds studied and bis-azobenzene. In the present case, both the azo groups are equivalent, and can be reduced in one step; with bisazobenzene, stepwise reduction occurs.

Effect of substituents

For application of the Hammett equation, the half-wave potential in the absence of surface-active substances or at the same concentration of surface-active substances were compared. In all the compounds, the values of the transfer coefficient (α) were similar and the values of $dE_{1/2}/d\text{pH}$ from the $E_{1/2}$ vs. pH curves are more or less constant (Table 1). The conditions for the application of the Hammett equation are thus fulfilled [14].

The linear correlation between the half-wave potential and Hammett substituent constant is shown in Fig. 2. The values of σ_{o-x} used are from o-benzoic acids [15]. It has been found that values for o-derivatives, viz., 2-methyl, 2-methoxy, 2-ethoxy and 2-chloro, deviate from the linear plot obtained for m- and p-substituents. The deviation of ortho derivatives can

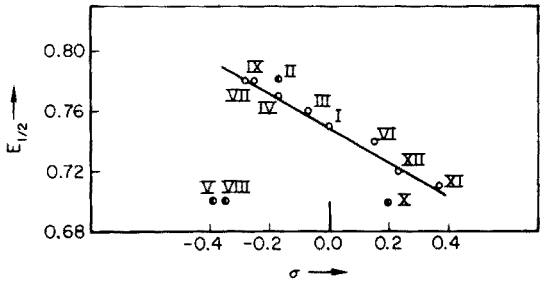

Fig. 2. Relationship between the half-wave potentials of the copper chelates of 3-aryl-azopentane-2,4-diones in B.R. buffer pH 2.7 and the Hammett substituent constant. •, *Ortho* derivatives.

be explained on the basis of steric hindrance to coplanarity [16]. The observed value (0.11 V) of the specific reaction constant (ρ), based on measurements of half-wave potentials of the twelve substances selected to cover a wide range of σ values, was in good agreement with the values reported earlier for azobenzene (0.10 V) and other similar systems [14]. The value of ρ was found to remain constant in the pH range 2.0—11.0. Similar behaviour has been reported for other azo compounds [14]. The positive value of the specific reaction constant indicates a nucleophilic mechanism. The linear relationship of the $E_{1/2}$ values and the Hammett substituent constants also indicates that the substituents appreciably affect reduction of the azo groups by polar and mesomeric effects.

The magnitude of the polarographic ortho shift (Δ_0) [14] expressed by the relation $\Delta_0 = (E_{1/2})_{o-x} - (E_{1/2})_{p-x}$ for methyl, methoxy, ethoxy and chloro substituents was found to be positive. This suggests that *o*-substituted copper chelates are more easily reducible than their *p*-derivatives.

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Analytica Chimica Acta, 98 (1978) 115—119

TITRIMETRIC DETERMINATION OF SILVER, CHLORIDE AND BROMIDE IN GLASSES

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SUMMARY

Titrimetric methods are described for the determination of total silver, free silver or free halide (Cl, Br and I), and bromide (or iodide) in glasses. Total silver is titrated potentiometrically with standard bromide solution after hydrofluoric—sulfuric acid sample decomposition followed by sodium hydrogensulfate fusion for volatilizing hydrogen halide. Free silver is determined similarly on a separate sample without the fusion step. For glasses containing excess of halide, free halide is titrated potentiometrically with standard silver(I) solution after dissolution of the sample in ice-cold hydrofluoric—nitric acid. Total bromide (or iodide) is determined by iodometric titration after oxidation to bromate (or iodate) with hypochlorite solution. The methods have been applied to a wide range of complex glass compositions and results are compared with values obtained by controlled-potential coulometry and x-ray fluorescence analysis.

Many methods, including gravimetry, spectrophotometry and titrimetry [1—3], have been reported for the determination of chloride in siliceous materials. In this laboratory, halides (Cl, Br and I) in glasses are usually determined by potentiometric titration with standard silver nitrate solution after dissolution of the sample in cold hydrofluoric acid [3]. Similarly, glasses can be analyzed for silver by titrating with standard bromide solution after decomposing the sample. However, the analyses of many complex modern glasses [4, 5] are complicated by the presence of silver, chloride and bromide. This paper describes analytical procedures developed for the titrimetric determination of total silver, free silver or free halide (Cl, Br and I) and total bromide (and/or iodide).

EXPERIMENTAL

Apparatus

Potentiometric titrations of silver and halide were carried out either manually with a Corning Model 12 pH meter or instrumentally with a Metrohm E436 Potentiograph in conjunction with a silver indicator electrode and an Orion 90-01 single-junction Ag/AgCl reference electrode. The filling solution of the reference electrode was 1 N KNO_3 saturated with AgCl.

Procedures

Determination of total and free silver. To obtain total silver, accurately weigh sufficient ground sample (ca. 0.5 g) to contain >0.3 mg Ag and transfer the sample to a 75-ml platinum dish. Dissolve the sample with 7 ml of 29 M HF and 6 ml of 9 M H_2SO_4 on a steam bath and then evaporate for 2—3 h. After volatilizing the water and hydrofluoric acid, transfer the platinum dish to a hot plate and gradually fume to dryness. Fuse the residue with 6 g of $NaHSO_4$ (dish covered) over a Fisher burner and dissolve the melt in 5 ml of 9 M H_2SO_4 plus 20 ml of distilled water by warming gently. Quantitatively transfer the solution to a 150-ml beaker, dilute to about 100 ml, and titrate the silver with either 0.01 or 0.05 M standard KBr solution.

For free silver, omit the fusion step and titrate the silver subsequent to the volatilization of HF.

Determination of free halides (Cl, Br, I). Accurately weigh a suitable amount of finely ground sample (ca. 200 mesh) to contain about 0.01—0.5 meq of free halides. Prepare an acid mixture by adding 10 ml of 29 M HF, 6 ml of 16 M HNO_3 and 10 ml of distilled water into a 100-ml Teflon beaker or platinum vessel. If more than 15% PbO is present in the sample, also add 10 ml of 3% (w/v) Na_2SO_4 solution. Carefully transfer the sample to the acid mixture cooled in an ice bath and then decompose the sample by mixing well for about 30 min with a magnetic stirrer. Maintaining the ice bath to cool the sample, add 10 ml of cold methanol and titrate the halide ions with either 0.01 or 0.05 M standard silver nitrate solution.

Determination of total bromide. Weigh accurately about 1 g (0.5—5 mg Br) of finely ground sample (100-200 mesh) into a 75-ml platinum dish, add 10 ml of saturated silver acetate solution (for a total halide of less than 3.0 meq) and 10 ml of 29 M HF. Stir and digest on a steam bath until the sample has decomposed and the precipitate coagulates. Add 15 ml of distilled water or, if PbO is present in the sample, add 15 ml of 5 M HNO_3 and stir well. Filter the precipitate on a fine-porosity Selas porcelain filtering crucible and wash it several times with 1% HF solution. Place the Selas crucible in a 400-ml Vycor brand beaker and add 15 ml of 0.1 N $Na_2S_2O_3$ solution. Rinse the platinum dish with 5 ml of 0.1 N $Na_2S_2O_3$ solution plus 1 ml acetic acid to dissolve any residual silver halide and transfer the solution to the beaker. Add 2 drops of 0.04% methyl red indicator solution and 6 M NaOH solution dropwise until a salmon color results. Add 1.0 g of $NaH_2PO_4 \cdot H_2O$ and 25.00 ml of 5% NaOCl solution (Baker Analyzed reagent grade). Boil the solution for 1 min and add 5 ml of saturated sodium formate solution slowly with the beaker partially covered. Cool, remove the watch glass and withdraw the Selas crucible. Wash the residual solution from the watch glass and crucible into the beaker. Dilute to 200 ml, add 1.000 g of KI, 12 ml of 9 M H_2SO_4 and several drops of 0.5% Na_2MoO_4 solution. Titrate the liberated I_2 immediately with standard 0.01 N $Na_2S_2O_3$ solution using 2 ml of 1% starch solution near the endpoint. Run a reagent blank for

correction. (Sodium thiosulfate and starch solutions are freshly prepared every week.)

Calculation. When free silver is present, the difference between total silver and free silver is the amount of silver bound to halide (Cl + Br). Use this value and the bromide obtained experimentally to calculate percent chloride. When free halide is present, use the total silver obtained experimentally to calculate the amount of halide bound as silver halide. From the bound halide, free halide, and bromide, calculate the total percent chloride present.

RESULTS AND DISCUSSION

Under optimum conditions, titrations with silver nitrate solutions can be used for the stepwise titration of iodide, bromide, and chloride [6]. However, for analyses of complex glasses with the amount of bromide much smaller than that of chloride, the stepwise titration was impractical. Therefore, direct potentiometric titration was utilized to titrate total bromide and chloride, and bromide was determined separately by a redox titration.

The procedure developed for bromide was based on the method of Kolthoff and Yutzy [7, 8] in which the bromide is oxidized to bromate with a solution of hypochlorite. Bromate is then determined iodometrically after removal of the excess of hypochlorite. If iodide is present, it will be oxidized to iodate and the sum of iodide and bromide obtained. Since iodide can be determined separately in the presence of chloride and bromide by other methods [8], bromide can be calculated by difference. The redox method for bromide is considerably more sensitive than the direct titration with silver(I) and is applicable to the determination of small amounts of bromide (or iodide) in the presence of chloride. It is, however, more time-consuming (5 h vs. 1 h) and requires a blank correction. In a typical analysis, the blank amounted to about 0.6 ml of 0.01 N sodium thiosulfate solution. Thus, accurate weighing and pipetting of reagents are recommended in the procedure to obtain accurate blank correction. The filtering crucible was reusable after cleaning for several analyses. The accuracy and precision of the method were estimated by analyzing standard KBr solutions. Five determinations of 4.00 mg Br yielded a mean recovery of 3.97 mg with standard deviation of 0.03 mg. For triplicate analyses of 4.79 mg Br, the mean recovery and standard deviation were 4.67 mg and 0.05 mg, respectively.

In the determination of free halide, acid decomposition and titration were completed in an ice-cold solution to avoid loss of halide and also to improve the potentiometric end-point. Results obtained for chloride in four NBS SRM glasses are shown in Table 1. The No. 89 glass contained 17.5% PbO which did not interfere with the titration. However, with glasses having greater than 20% PbO, lower results were obtained because of the formation of lead chlorofluoride [1]. This interference was more evident when the solution was allowed to stand longer before the titration, indicating a gradual formation of PbClF in the acidic medium. The problem was circumvented by

TABLE 1

Determination of chloride in NBS SRM glasses

SRM No.	Glass type	% Cl		
		Certified	Present method[b]	
			Mean	Std. dev.
89	Lead—barium	0.05	0.052	0.001
91	Opal	0.014[a]	0.017	0.001
93	High-boron	0.036	0.034	0.001
93a	High-boron	0.06$_0$	0.064	0.002

[a]Cl analyzed by three laboratories: 0.01, 0.014 and 0.017%. [b]Results obtained on 4 determinations.

adding Na_2SO_4 solution to precipitate lead as $PbSO_4$ during the acid dissolution. Filtration of the precipitate was not necessary prior to the halide titration. For analysis of high vanadium glass, the sample was decomposed by a carbonate—borate fusion and the melt dissolved with hot distilled water. The cooled solution was acidified and the vanadium reduced with ascorbic acid (ca. 50 mg) just before the titration. The addition of ascorbic acid satisfactorily overcame the difficulty of an erratic end-point. In addition to the above approach for eliminating the interfering effect of vanadium, pyrohydrolysis [9] was also briefly investigated. Halides were steam-distilled

TABLE 2

Determination of silver, chloride and bromide in glasses by the proposed method, x-ray fluorescence and controlled-potential coulometry

Sample	% Ag			% Cl			% Br		
	Present method	X.r.f.	C.p.c.	Present method	X.r.f.	C.p.c.	Present method	X.r.f.	C.p.c.
1	0.17	0.17	0.18	0.24	0.23	—	0.14	0.12	—
2	0.15	0.16	0.15	0.24	0.23	—	0.19	0.16	—
3	0.19	0.17	—	0.29	0.27	—	0.16	0.17	—
4	0.14	0.14	—	0.76	0.67	—	0.11	0.08	—
5	0.13	0.12	—	0.46	0.43	—	0.18	0.13	—
6	0.12	0.11	—	0.57	0.52	—	0.17	0.12	—
7	0.48	0.43	0.45	—	—	—	—	—	—
8	0.47	0.41	—	—	—	—	—	—	—
9	0.14	—	—	0.60	0.59	—	0.18	—	—
10	—	—	—	—	—	—	0.48	—	0.51
11	—	—	—	—	—	—	1.04	—	1.07
12[a]	1.10	—	1.09	0.54	—	0.56	—	—	—

[a]Sample contained excess of Ag; total Ag is given under % Ag and free % Ag given under % Cl.

and separated as the respective hydrogen halides. The limited data obtained indicated that although chloride was quantitatively recoverable, some bromide was lost, probably through partial oxidation to bromine. The presence of vanadium might have promoted the reaction.

The entire procedure was tested by analyzing the NBS SRM 93 glass spiked with known amounts of silver, chloride and bromide. Results obtained on triplicate analyses of sample containing 5.40% Ag, 3.54% Cl and 0.40% Br were 5.44 ± 0.03% (standard deviation), 3.48 ± 0.01% and 0.39 ± 0.01%, respectively. Numerous complex glass compositions were analyzed by the present methods. Some results are shown in Table 2 and compared with values obtained by controlled-potential coulometry [10, 11] and x-ray fluorescence analysis. It should be noted that in controlled-potential coulometry, halide was obtained indirectly by reacting with known amounts of silver and measuring the excess of silver. Therefore, the procedure is only applicable to total halide and does not distinguish between chloride and bromide. The x-ray fluorescence method is sensitive to glass composition and requires calibration standards established by the proposed methods.

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Analytica Chimica Acta, 98 (1978) 121—126

SIMULTANEOUS MICRODETERMINATION OF CARBON, HYDROGEN, MERCURY, CHLORINE, BROMINE AND SULPHUR IN ORGANIC COMPOUNDS

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SUMMARY

A simple, reliable method for the simultaneous microdetermination of carbon, hydrogen, mercury, chlorine (or bromine) and sulphur in organic compounds is described. The intermediate storage technique is used. Sulphur and the halogens are absorbed on electrolytic silver and determined gravimetrically or titrimetrically. Mercury is absorbed on gold wire, and is weighed as the metal or desorbed and determined by a mercury-8-hydroxyquinoline method.

Organomercury compounds have many applications as bactericides, algicides, fungicides, herbicides, and — in a less lethal vein — even as diuretics [1]. Although analyses for such substances in trace amounts is probably of greater current interest, analysis of the relatively pure materials themselves is of considerable importance. The aim of the work reported here was to determine simultaneously carbon, hydrogen, mercury and a halogen or sulphur in different combinations, by means of the intermediate storage technique in organic microanalysis [2]. The compound is decomposed in oxygen at a relatively high temperature in an empty tube, the halogen and sulphur are absorbed on silver, mercury is absorbed on pure gold and carbon and hydrogen are determined in the usual manner. Mercury can then be determined either by weighing the gold absorption tube or by a procedure based on the formation of a mercury—8-hydroxyquinoline complex [3, 4]. The halogen or sulphur is determined by weighing the silver absorber or by desorption followed by titration.

EXPERIMENTAL

Apparatus

The combustion apparatus is a standard Heraeus micro combustion unit with a 165-mm long main furnace and a 65-mm long movable furnace. An additional heater (90-mm long) is connected for the intermediate storage of halogen, sulphur and mercury. The combustion tube and the absorption tubes, all made of quartz, are shown in Fig. 1. Care must be taken that there

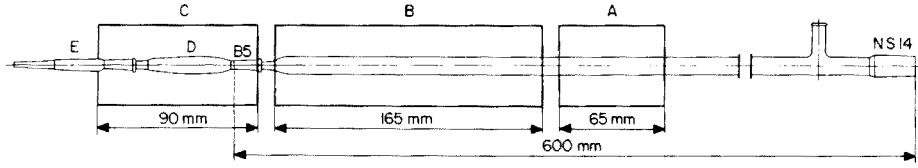

Fig. 1. Combustion and absorption apparatus. A, Movable furnace; B, main furnace; C, additional heater; D, absorption tube packed with electrolytic silver; E, absorption tube packed with gold wire.

is no cold zone between the furnace and the additional heater, to prevent condensation.

Oxygen, nitrogen, and hydrogen are fed through copper lines via solenoid valves (Type 135 A 04, Lucifer Ltd., Uster, Switzerland) as reported previously [2]; a single flowmeter with a needle valve (0.6—6 l h^{-1}) is used for all gases. All gases are purified by passing through soda asbestos and anhydrone between the flowmeter and the combustion tube.

For the potentiometric titrations, a 100-ml beaker is fitted with a rubber stopper through which are placed a silver amalgam electrode, a salt bridge, and the tip of a 5-ml microburette, which is long enough to immerse in the sample solution. A saturated calomel reference is used.

Reagents

All reagents used were of analytical-reagent quality except where otherwise mentioned. Electrolytic silver needles were prepared from silver nitrate in the usual way.

Procedure

Wash and dry the quartz tubes. Pack the absorption tube for halogens and sulphur with electrolytic silver needles and the absorption tube for mercury with pure gold wire. Pack the absorption tubes for carbon dioxide and water with anhydrone and soda-asbestos in the usual way. Use a tube filled with manganese dioxide and anhydrone for absorption of nitrogen oxides. The absorbers for CO_2, nitrogen oxides and water are placed after the silver absorber, in the conventional manner.

After the apparatus has been set up, heat the furnaces to their optimal temperatures (950°C for the main furnace, 850°C for the movable furnace and 550°C for the additional heater). Adjust the gas pressures so as to produce flows of 50 ml min^{-1} for oxygen and nitrogen, and 30 ml min^{-1} for hydrogen, at a single setting of the needle valve.

Weigh a sample of 2—5 mg in a platinum boat, and place it in the tube, 40—50 mm before the burner. Secure the ground-glass cap with a spring clip, place the movable furnace over the tube and burn the sample within 10 min in a stream of oxygen. Detach and weigh the carbon dioxide and water absorption tubes in the conventional manner.

For the determination of mercury, there are four possibilities.

Method (i). Detach the gold absorption tube and weigh it.

Method (ii). Switch the gas flow to nitrogen, and attach the gold absorption tube directly to the combustion tube. Move the combustion back so that the gold absorption tube is heated in the main furnace. Absorb the sublimed mercury in 5 ml of concentrated nitric acid in a test tube; this requires 4 min with a nitrogen flow of 15 ml min^{-1}. Heat on a hot-plate with addition of distilled water to expel nitrogen oxides completely. Cool the solution, and add exactly 2.00 ml of a 1% (w/v) solution of oxine in 95% ethanol. Add ammonia solution dropwise until a heavy precipitate appears and then add 1 ml of a 10% (w/v) ammonium or sodium acetate solution to adjust the pH to 6—7. Heat the solution to 50°C and allow to cool for 20 min. Filter, wash the precipitate thoroughly with 95% ethanol (25 ml) until the washings are colorless, dry to constant weight at 90°C and weigh as (C_9H_6ON) HgOH.

Method (iii). To the filtrate and washings from Method (ii), add 5 ml of 5 M hydrochloric acid followed by 1 ml of 10% (w/v) potassium bromide solution. Add an accurately measured excess of 0.05 N potassium bromate solution, stopper the flask, shake, leave it in the dark for ca. 5 min. Add 4 ml of 10% potassium iodide solution, and titrate the liberated iodine with a 0.02 N sodium thiosulphate solution to a starch end-point. Standardize the thiosulphate solution against the bromate solution.

Method (iv). Dissolve the dry precipitate from Method (ii) in 5 ml of 5 M hydrochloric acid and treat it as described for Method (iii). For Methods (iii) and (iv) carry out blank determinations under the same experimental conditions.

Determination of chlorine, bromine or sulphur

These elements can be determined gravimetrically by weighing the silver absorption tube. For a titrimetric determination, connect the silver tube directly to the combustion tube in the additional heater, and attach a Teflon joint secured with Viton O-rings (MF-Kupplung DE, Serve Technik, D-6909 Malsch). Attach a piece of polyethylene tubing (1 mm i.d.) to the Teflon joint, and dip the end into a mixture of 5 ml of distilled water and 0.5 ml of hydrogen peroxide (100 vol.) in a 100-ml Erlenmeyer flask; the peroxide can be omitted for the determination of halogen. Switch the valve to the nitrogen flow; after 2 min, energize the second valve to start the hydrogen flow. Allow reduction of 5 min for chlorine, 10 min for bromine, and 5 min for sulphur. Pass nitrogen for a further 2 min to remove all hydrogen from the tube, as well as the last traces of hydrogen halide and sulphur dioxide. For the determination of sulphur, add 20 ml of ethanol and a few drops of 1% thorin solution and titrate with a standard 0.02 M barium perchlorate solution [5]. The blank is zero. For the chloride or bromide determination, titrate with 0.02 M mercury(II) perchlorate solution in 80% ethanolic medium in the presence of diphenylcarbazone indicator [6]. Simultaneous determinations of sulphur and chloride or bromide are

possible by titrating with barium perchlorate to thorin indicator in 80% ethanolic medium, and then adding nitric acid (0.5 ml of 0.5 M) and titrating with mercury(II) perchlorate solution to the diphenylcarbazone end-point.

RESULTS AND DISCUSSION

Determination of mercury

Mercury can be determined gravimetrically as the metal, but its determination as the 8-hydroxyquinoline complex was also studied. There have been various reports on the complexes and addition compounds that may be formed between mercury(II) and oxine [7], hence a detailed examination was made. Elemental analysis of the complex was consistent with the formula $(C_9H_6ON)HgOH$. Absorption measurements in the range 280—450 nm in a 1:1 chloroform—ethanol medium showed definite complex formation, with maximum absorption at 300 nm and a weak band at 390 nm; the maximum for oxine itself lies at 286 nm. The infrared spectrum of the complex showed the characteristic bands of oxine at 1577 cm^{-1} and 1495 with weak peaks at 1563 cm^{-1} and a band at 780—740 cm^{-1} which may indicate a quinazoline structure [8].

Some potentiometric titrations were done to confirm the stoichiometry of the reaction. A 10^{-3} M mercury(II) nitrate solution was first standardized by titration with 0.01 M EDTA in presence of hexamine buffer and methylthymol blue indicator [9]. Known volumes of this solution were mixed with sodium nitrate to give ionic strength 1.0, 1 ml of ammonia liquor was added to give pH 6—7, and the solutions were titrated with 0.01, 0.02 or 0.03 M oxine solution. A silver amalgam indicating electrode was used with a saturated calomel reference electrode connected via a salt bridge [9]. Sharp potential breaks were obtained at a metal : ligand ratio of 1:1 (Fig. 2).

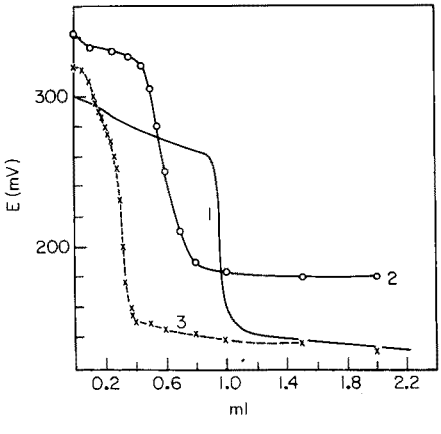

Fig. 2. Potentiometric titrations of 10^{-3} M mercury(II) solution with (1) 0.01 M oxine, (2) 0.02 M oxine, (3) 0.03 M oxine.

TABLE 1

Determination of mercury in organomercury compounds

Compound	Weight	Hg calc. (%)	Hg found (%)			
			(i)	(ii)	(iii)	(iv)
$C_4H_6O_4Hg$	5.120	62.95	62.8	63.2	63.1	63.0
	3.980		62.9	63.4	63.2	63.1
	4.330		62.9	63.2	62.7	63.2
$C_2S_2N_4H_8Cl_2Hg$	3.750	47.3	47.3	47.2	47.0	47.4
	2.885		47.2	47.6	47.1	47.7
	5.460		47.3	47.8	47.0	47.5
$C_{12}H_{10}Hg$	4.155	56.55	56.5	56.7	56.6	56.5
	3.340		56.7	56.3	56.7	56.7
	5.050		56.3	56.5	56.4	56.4
C_6H_5HgCl	4.825	64.05	64.0	64.2	64.2	64.1
	2.750		64.0	64.3	64.0	64.2
	3.425		63.8	64.4	64.2	64.5
$C_{14}H_{14}Hg$	5.085	52.4	52.3	52.6	52.2	52.5
	2.380		52.6	52.7	52.2	52.6
	3.265		52.5	52.7	52.5	52.6

Calculation of the results obtained by method (ii) on the basis of the composition $(C_9H_6ON)HgOH$ gave satisfactory results (Table 1), in good agreement with the values obtained by methods (i), (iii) and (iv).

The results obtained by the intermediate storage technique for a range of organic mercury compounds show average errors of ±0.11, ±0.23, ±0.17 and ±0.16%, respectively for methods (i)—(iv).

Simultaneous determinations

The proposed general procedure provides an easy, reliable way of determining carbon, hydrogen, mercury, chlorine (or bromine) and sulphur simultaneously in organic compounds by the intermediate storage technique. Chlorine, bromine, or sulphur can be determined gravimetrically by weighing the silver absorption tube. Alternatively, the oxides of sulphur and silver halides can be removed from the silver by reduction in a stream of hydrogen; the gases are easily absorbed in water and can then be titrated as indicated in the Experimental part. Typical results are summarized in Table 2. Large numbers of other samples have also been analyzed under routine conditions with good results. Chlorine, bromine and sulphur determined gravimetrically gave average errors of ±0.11, ±0.18, ±0.14%, respectively; with the titrimetric methods, the average errors were ±0.09, ±0.06, ±0.18%, respectively.

TABLE 2

Simultaneous microdetermination of carbon, hydrogen, sulphur, chlorine and/or bromine (Gravimetric (G) and titrimetric (T) results are given for the heteroelements.)

Compound	Weight	Calc. %				Found %					
		C	H	Cl or Br	S	C	H	Cl or Br		S	
								G	T	G	T
4-Chlorobenzoic acid	3.250	53.7	3.2	22.7		53.6	3.4	22.60	22.70		
$C_7H_5ClO_2$	4.875					53.6	3.3	22.97	22.88		
	2.840					53.9	3.4	22.5	22.60		
4-Bromobenzoic acid	2.320	41.8	2.5	39.8		41.9	2.7	40.0	39.7		
$C_7H_5BrO_2$	4.020					41.8	2.6	39.9	39.8		
	5.010					41.8	2.8	39.6	39.9		
Phenothiazine	3.295	72.3	4.6		16.1	72.4	4.8			16.0	16
$C_{12}H_9NS$	3.500					72.2	4.7			16.3	16
	4.100					72.5	4.8			16.0	16
Mercury(II) acetate	5.120	15.1	1.9			15.0	2.0				
$C_4H_6O_4Hg$	3.980					14.8	2.2				
	4.330					15.1	2.0				
Dichloro(bis thiourea)mercury	3.750	5.71	1.9	16.8	15.1	5.6	2.2		16.8		15
$C_2S_2N_4H_8Cl_2Hg$	2.885					5.7	2.0		16.7		14
	5.460					5.6	2.1		16.9		15
Diphenylmercury	4.155	40.6	2.8			40.7	2.8				
$C_{12}H_{10}Hg$	3.340					40.5	3.0				
	5.050					40.8	3.0				
Phenylmercury chloride	4.825	23.0	1.6	11.3		23.1	2.0	11.3	11.4		
C_6H_5HgCl	2.750					22.8	1.7	11.5	11.3		
	3.425					23.0	1.8	11.6	11.6		
Bis(*p*-tolyl)mercury	5.085	43.9	3.7			43.8	3.9				
$C_{14}H_{14}Hg$	2.380					43.5	3.9				
	3.265					43.9	3.8				

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Analytica Chimica Acta, 98 (1978) 127—131

SPECTROPHOTOMETRIC STUDIES OF THE COMPLEXES OF GROUP IIIA METAL IONS WITH SOME PHENYLAZO-8-QUINOLINOL DYES

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SUMMARY

A new rapid precise method for the spectrophotometric microdetermination of group IIIA metal ions with 5-phenylazo-8-quinolinol (I) and 5-(*p*-chlorophenylazo)-8-quinolinol (II) is given. The optimum conditions favouring the formation of the complexes are reported, as are the compositions of the complexes, their stabilities, and the ranges for obedience to Beer's law. Spectrophotometric titration methods for Al, Ga and In with EDTA in presence of ligand I or II as indicator are also described.

5-(*p*-Ethoxy, *o*-carboxy and *p*-hydroxyphenylazo)-8-quinolinol have been used in identification of Hg^{2+}, Sr^{2+}, Be^{2+}, Ag^{+} and Mg^{2+} [1]. The *m*-nitro and *p*-nitro derivatives are suitable reagents for the colorimetric determination of chloride [2]. 5-(*p*-Sulphophenylazo)-8-quinolinol can be used for Ca^{2+} determination [3]. A systematic spectrophotometric study of the Al^{3+}, Ga^{3+}, In^{3+}, and Tl^{+} complexes with 5-phenylazo-8-quinolinol (I) and 5-(*p*-chlorophenylazo)-8-quinolinol (II) is reported in this paper.

EXPERIMENTAL

Chemicals and solutions

The chemicals were all of the highest purity available. The dyes were prepared as previously described [4]. Twice-distilled water (glass apparatus) was used.

Standard 0.001 M solutions of Al^{3+}, Ga^{3+}, In^{3+} and Tl^{+} were prepared by diluting previously standardized stock 0.05 M solutions [5, 6]. The 0.001 M solutions of (I) and (II) were prepared by dissolving accurately weighed amounts of the dye in ethanol. Modified Britton—Robinson universal, acetate and hexamine buffer solutions [7] were used.

Spectrophotometric determination of Group IIIA metal ions

When (I) is used as the chromophoric reagent, solutions containing up to 8.0, 28, 35 and 50 μg of Al, Ga, In and Tl, respectively, are used; when reagent (II) is used, solutions containing up to 3.5, 15, 30 and 40 μg of the four cations, respectively, are used. To each solution are added 6 ml of acetate buffer (in 20% ethanol) of the recommended pH value and 1 ml of

10^{-3} M (I) or 1.4 ml of 10^{-3} M (II). These solutions are diluted to 10 ml with water. The absorbances are measured at 440 nm for Al—I, Ga—I and In—I, 400 nm for Tl—I, 450 nm for Al—II, Ga—II and In—II, and at 360 nm for Tl—II complexes against the appropriate reagent blank. Calibration curves are prepared in the same manner.

Spectrophotometric titration of Al^{3+}, Ga^{3+} and In^{3+} with EDTA and I or II as indicator

The conditions for such titrations are given in Table 1. For a titration, the metal ion solution was mixed with 6 ml of acetate buffer (in 20% ethanol) and 1 ml of 10^{-3} M (I) or 1.4 ml of 10^{-3} M (II). This mixture was titrated with 0.001 M EDTA, the absorbance being measured at 440 nm. Appropriate dilution corrections were applied before the titration curve was plotted.

RESULTS AND DISCUSSION

Spectrophotometric determinations

The optimum conditions were studied for the spectrophotometric determination of Al^{3+}, Ga^{3+}, In^{3+} and Tl^{+} with reagents I and II. The optimal pH values for development of the orange Al^{3+}, Ga^{3+} and In^{3+} complexes and the yellow Tl^{+} complex are shown in Table 2. Acetate buffers were the most suitable media. The absorption spectra of reagents I and II and their complexes at the recommended pH values against water and against ligand show that the complexes of Al, Ga and In give absorption maxima at wavelengths well separated from the maxima of the reagents. In contrast, the complexes of Tl^{+} absorb at the same wavelength as the corresponding ligand, i.e. at about 380 nm, but with higher absorptivity (Table 2). Representative curves are shown in Fig. 1. Solutions are best mixed in the order metal—buffer—dye in all cases.

All complexes are formed instantaneously and the absorbances remain stable for about 30 min; thereafter precipitation starts. Limits of obedience to Beer's law and the absorptivities of the complexes are shown in Table 2.

TABLE 1

Optimum conditions for the spectrophotometric titrations

Metal	Indicator	pH	Optimum concentration range (μg/10 ml)
Al	I	4.0	5.4—21.6
	II	3.5	8.1—21.6
Ga	I	3.5	14.0—42.0
	II	3.5	27.9—69.8
In	I	3.5	23.0—92.0
	II	3.0	23.0—92.0

TABLE 2

Optimal pH, range for Beer's law and absorptivities of the complexes

Metal	Ligand I				Ligand II			
	pH	λ(nm)	ppm	ε (× 10^4 l mol^{-1} cm^{-1})	pH	λ(nm)	ppm	ε (× 10^4 l mol^{-1} cm^{-1})
Al	4.0	450	0.1—1.0	2.16	3.5	440	0.08—0.45	3.02
Ga	3.5	450	0.3—2.8	2.10	3.5	450	0.2—2.1	1.38
In	3.5	480	0.5—4.0	1.84	3.0	450	0.5—4.0	2.18
Tl	3.5	400	1.0—6.5	0.73	3.0	360	0.5—5.3	0.51

Investigation of the complexes by the conventional molar ratio, continuous variations and slope ratio methods showed the results listed in Table 3. The logarithmic values of the stability constants of the complexes as calculated from the molar ratio and continuous variations methods [8] are listed in Table 3.

A systematic study of the influence of foreign ions can be summarized as follows:

For aluminium complexes. Li^+, Na^+, K^+, Ca^{2+}, Ba^{2+}, Sr^{2+}, Mn^{2+}, Cr^{3+}, Cl^-, Br^-, I^-, NO_3^-, CN^-, CH_3COO^-, ClO_4^-, PO_4^{3-}, and phthalate have no influence when present in 20-fold amounts relative to aluminium. Fe^{2+}, Fe^{3+}, Cu^{2+}, Co^{2+}, Pb^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+}, UO_2^{2+}, Tl^+, Bi^{3+}, Ga^{3+}, In^{3+}, Th^{4+}, Zr^{3+}, F^-, citrate, tartrate, oxalate, EDTA and CDTA interfere.

For gallium complexes. Li^+, Na^+, K^+, Pb^{2+}, Zn^{2+}, Cd^{2+}, Mn^{2+}, Ca^{2+}, Ba^{2+}, Sr^{2+}, Cr^{3+}, Zr^{3+}, Cl^-, Br^-, I^-, NO_3^-, ClO_4^-, CH_3COO^-, SO_4^{2-}, and phthalate do not interfere (20-fold). Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, UO_2^{2+}, Tl^+, Bi^{3+}, Al^{3+}, In^{3+}, Th^{4+}, F^-, CN^-, PO_4^{3-}, citrate, tartrate, oxalate, EDTA and CDTA interfere.

For indium complexes. Li^+, Na^+, K^+, Pb^{2+}, Zn^{2+}, Cd^{2+}, Mn^{2+}, Ca^{2+}, Ba^{2+}, Sr^{2+}, Cr^{3+}, Th^{4+}, Cl^-, Br^-, I^-, F^-, NO_3^-, CN^-, ClO_4^-, SO_4^{2-}, PO_4^{3-}, CH_3COO^-, citrate, tartrate, oxalate and phthalate have no influence (20-fold). Fe^{2+}, Fe^{3+}, Cu^{2+},

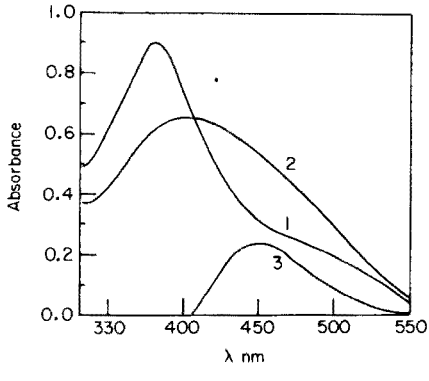

Fig. 1. Absorption spectra of 5-phenylazo-8-quinolinol and its complex with aluminium at pH 4. (1) Dye (4 × 10^{-5} M) against water. (2) 1:1 complex (4 × 10^{-5} M) against water. (3) 1:1 complex (4 × 10^{-5} M) against dye.

TABLE 3

The apparent stability constants of the metal ion complexes with ligands (I) and (II)

Complex	Mole ratio	n	$\log \beta_n$	Complex	Mole ratio	n	$\log \beta_n$
Al—I	1:1	1	4.37	Al—II	1:3	3	11.91
Ga—I	1:2	2	9.43	Ga—II	1:2	2	8.15
In—I	1:1	1	3.77	In—II	1:2	2	7.86
	1:3	3	13.97				
Tl—I	1:2	2	8.03	Tl—II	1:2	2	7.81

Co^{2+}, Ni^{2+}, Tl^{+}, UO_2^{2+}, Zr^{3+}, Bi^{3+}, Al^{3+}, Ga^{2+}, EDTA and CDTA should not be present.

For thallium(I) complexes. Li^{+}, Na^{+}, K^{+}, Cd^{2+}, Ca^{2+}, Ba^{2+}, Sr^{2+}, Pb^{2+}, Zn^{2+}, UO_2^{2+}, Cr^{3+}, Zr^{3+}, F^{-}, Br^{-}, Cl^{-}, CN^{-}, NO_3^{-}, SO_4^{2-}, PO_4^{3-}, ClO_4^{-}, CH_3COO^{-}, tartrate, oxalate, phthalate, EDTA and CDTA do not interfere; Fe^{2+}, Fe^{3+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Bi^{3+}, Al^{3+}, Ga^{3+}, In^{3+}, Th^{4+}, I^{-}, CrO_4^{2-}, and citrate interfere.

The proposed spectrophotometric determinations of Al, Ga, In and Tl, which are applicable in the presence of a variety of cations and anions, are simple and rapid. With ligand I, the standard deviations (18 determinations) in determining 4.0, 15, 25 and 40 μg of Al^{3+}, Ga^{3+}, In^{3+} and Tl^{+}, respectively, are 0.18, 0.33, 0.16 and 0.15%, respectively. With ligand II, the corresponding standard deviations are 0.23, 0.26, 0.16 and 0.17%, respectively. These data show that the present method is very precise.

Spectrophotometric titrations of Al^{3+}, Ga^{3+} and In^{3+} with EDTA

Each of these metal ions can be determined by direct spectrophotometric titration with EDTA solutions in the presence of ligands I and II as indicators. The conditions for such determinations are summarized in Table 1. All titration curves, except that of Al with ligand II, show a sharp end-point intersection with maximum sharpness in the case where indium is titrated with ligand II as indicator. Representative curves are shown in Fig. 2. These

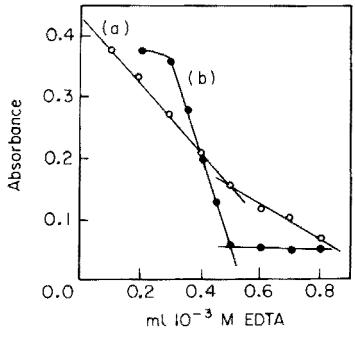

Fig. 2. Spectrophotometric titration of 0.5 ml of 10^{-3} M Al (a) and In (b) with ligand II as indicator.

results can be explained by comparing the values of the stability constants of those metal ions both with EDTA [9] ($\log K_1$ = 16.1, 20.3 and 25 for the Al, Ga and In complexes, respectively) and with ligands I and II as calculated in the present work (Table 3). The comparison shows that the difference between $\log K_1$ of the EDTA complex and $\log \beta_n$ (where n is the maximum number of I or II molecules per metal ion) has the highest value (16.89) in the case of the In—II complex and a minimum value (4.19) in the case of the Al—II complex. In other cases, the differences between the values amount to about 10.

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Analytica Chimica Acta, 98 (1978) 133—136

Short Communication

POLYURETHANE FOAM OF THE POLYETHER TYPE AS A SOLID POLYMERIC EXTRACTANT FOR COBALT AND IRON FROM THIOCYANATE MEDIA

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Polyurethane foam immobilizing 1-(2-pyridylazo)-2-naphthol (PAN) has been used for the separation and preconcentration of trace elements [1]. During that study, it was noticed that unloaded polyurethane foam of the polyether type can extract cobalt from aqueous thiocyanate solutions.

Several investigators [2—8] have used untreated polyurethane foam for the absorption and recovery of several inorganic and organic compounds from aqueous solution. However, Gesser et al. [9] were the first to report that open-cell polyurethane foam of the polyether type is a convenient solid substitute for liquid ethyl ether in extraction systems; they investigated the separation of gallium and iron from aluminium in acid chloride solutions.

The present work gives further proof that open-cell polyether-type polyurethane foam can act as a solid ether solvent which extracts the thiocyanate complexes of cobalt and iron from aqueous liquid phases.

Experimental

Reagent and materials. All reagents used were of standard analytical purity except where otherwise mentioned. Polyurethane foam, a polyether of open cell type (Greiner K.G. Schaumstoffwerk-Kremsmünster, Austria) and an open-cell polyester polyurethane foam (PPI-800 nFR; Eurofoam, Damstraat, 92000 Wetteren) were compared. These foams were cut, washed and dried as previously described [1].

Stock cobalt(II) and iron(III) solutions were prepared from analytical-grade cobalt(II) chloride and iron(III) chloride, respectively. All solutions were standardized by conventional methods [10]. Solutions containing microgram amounts of cobalt and iron were prepared daily by diluting the stock solutions. All cobalt and iron solutions were then spiked with cobalt-58 and iron-59, respectively.

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Column preparation. Glass columns of 25-mm diameter and 12-cm length were used; 5 g of the dried unloaded foam was packed in the column, as previously described [11], to produce a 5-cm bed height.

Instrumentation. For activity measurements, a NaI(Tl) well crystal and an energy-selective counting device (type NK-107/B; Gamma, Budapest) were employed.

Results and discussion

In acidic media, cobalt(II) and iron(III) form, respectively, blue and red thiocyanate complexes, which are readily extracted with diethyl ether [12]. In the present investigation, the extraction of these complexes was tested in both batch and column experiments.

Rate of extraction of cobalt and iron from thiocyanate media. In separate experiments, 0.1 g of polyurethane foam (polyether type) was mixed with 10 ml of aqueous acidic (0.1 N HCl or H_2SO_4) 0.5 M thiocyanate solution containing 2 μg of cobalt or iron in a 100-ml stoppered flask. The flasks were shaken mechanically for 1—60 min. After each shaking period, the radioactivity of 2 ml of the aqueous solution was measured and the concentration of cobalt or iron was determined. The amount of metal ion extracted on the foam was then calculated by difference. As can be seen from Fig. 1, the rate of extraction of cobalt is slightly higher than that of iron, but both extraction rates are quite fast. These results suggested the possibility of using unloaded polyurethane foam of the polyether type in column operations for the collection of trace amounts of these metal ions from aqueous thiocyanate media at reasonable flow-rates.

Cobalt and iron were not extracted at all from acidic thiocyanate solutions on polyurethane foam of the polyester type. This proves that the high

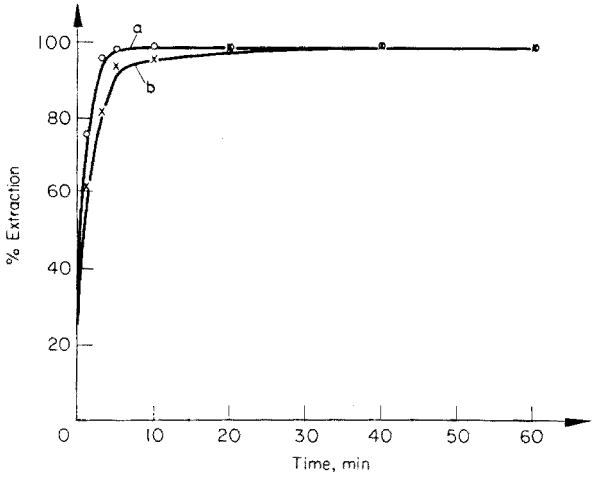

Fig. 1. Rate of extraction of cobalt and iron from 0.5 M acidic thiocyanate solution. (a) Cobalt. (b) Iron.

efficiency of extraction with the polyether-type foam is mainly due to absorption by the polyether, i.e., the thiocyanate complexes dissolve in the polyether instead of being adsorbed on its surface. The relatively high absorption capacity found [9] in the extraction of gallium from aqueous hydrochloric acid solution onto the polyether foam also confirms such a mechanism. Similar results have been obtained for the extraction of gold from aqueous cyanide media on unloaded polyurethane foam of polyether type [13].

Effect of flow-rate on the extraction efficiency for cobalt and iron. Cobalt or iron (2 μg) in 20 ml of 0.2 M or 0.5 M potassium thiocyanate solution, respectively (0.1 N in HCl or H_2SO_4) were allowed to pass through the polyether

TABLE 1

Effect of flow-rate on the extraction of 2 μg of cobalt or iron from 0.2 M or 0.5 M thiocyanate solutions, respectively, on unloaded polyether foam columns

Flow-rate (ml min^{-1})	Average metal ion extracted on foam[a] ($\bar{x}$, %)	Relative accuracy of the mean (%)	Standard deviation (s)
10	99.6[b]	−0.4	0.23
	99.2[c]	−0.8	0.36
20	99.7[b]	−0.3	0.23
	99.5[c]	−0.5	0.36
40	99.6[b]	−0.4	0.24
	99.6[c]	−0.4	0.47

[a]Average of 5 determinations. [b]Cobalt. [c]Iron.

TABLE 2

Collection of various concentrations of cobalt or iron from 0.2 M or 0.5 M thiocyanate solutions, respectively, on unloaded polyether foam columns at flow-rates of 20 ml min^{-1}

Amount of metal ion (μg)	Average metal ion extracted on foam[a] ($\bar{x}$, %)	Relative accuracy of the mean (%)	Standard deviation (s)
0.2	99.7[b]	−0.3	0.23
	99.4[c]	−0.6	0.30
2.0	99.7[b]	−0.3	0.24
	99.5[c]	−0.5	0.30
20.0	99.5[b]	−0.5	0.22
	99.4[c]	−0.6	0.33
200.0	99.5[b]	−0.5	0.21
	99.3[c]	−0.7	0.38

[a]Average of 5 determinations. [b]Cobalt. [c]Iron.

TABLE 3

Preconcentration of 1 μg of cobalt or iron per litre on unloaded polyether foam columns at flow-rates of 30 ml min^{-1}

Metal ion	Average metal ion extracted on foam[a] ($\bar{x}$, %)	Relative accuracy of the mean (%)	Standard deviation (s)	Confidence limit $\bar{x} \pm ts/\sqrt{n}$, $t = 0.95$
Cobalt	95.9	−4.1	0.85	95.9 ± 0.8
Iron	96.1	−3.9	0.67	96.1 ± 0.6

[a]Average of five determinations.

polyurethane foam column at various flow-rates (10—40 ml min^{-1}). The extraction efficiency was high (Table 1); more or less complete extraction was obtained even at flow-rates of 40 ml min^{-1}. For iron, 0.5 M thiocyanate solution was used, because iron was not completely extracted at lower thiocyanate concentrations.

Collection of cobalt or iron from 0.2 M or 0.5 M thiocyanate solutions, respectively. Collection of various concentrations of cobalt and iron from acidic thiocyanate solutions on the unloaded polyether foam columns was examined at flow-rates of 20 ml min^{-1}. Complete recoveries of cobalt and iron were obtained at concentrations of 0.2—200 μg (Table 2). Again, these results show the high capacity of the foam material for these thiocyanate complexes.

The preconcentration of 1 μg of cobalt or iron from 1 l of aqueous thiocyanate solutions was also tested. Satisfactory results were obtained (Table 3) at flow-rates of 30 ml min^{-1}.

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Analytica Chimica Acta, 98 (1978) 137—139

Short Communication

SEPARATION OF BACITRACIN A AND BACITRACIN F BY ISOELECTRIC FOCUSING IN GEL

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The bacitracins are a group of peptide antibiotics produced by different strains of *Bacillus licheniformis* and *B. subtilis*. Bacitracin A, the microbiologically active main component, as well as bacitracin B and the inactive oxidation products, bacitracin F (from A and from B) are well known [1, 2]. For the separation of bacitracins, countercurrent distribution [3], zone electrophoresis [4], column chromatography [5], high-pressure liquid chromatography [6] and differential pulse polarography [7] are either time-consuming or require expensive apparatus.

The present report describes the separation and determination of bacitracin A and bacitracin F by isoelectric focusing in gel.

Experimental

Acrylamide gels for isoelectric focusing were prepared as described by Wrigley [8]. The following solutions were used. (A) Carrier ampholyte solution, LKB Ampholine, 40% solution, pH range 3.5—10. (B) Acrylamide solution, containing 30 g of acrylamide and 1 g of *N,N'*-methylene bisacrylamide dissolved in water to 100 ml. (C) Catalyst solution, containing 1.0 ml of *N,N,N',N'*-tetramethylethylenediamine and 14 mg of riboflavin dissolved in water to 100 ml. Glass tubes (5 mm i.d., 100 mm long) were partially filled with 0.03 ml of A, 0.3 ml of B, 0.08 ml of C and 0.82 ml of water and photopolymerized for 30 min in front of a fluorescent lamp. Isoelectric focusing was done at 20°C in a disc electrophoresis apparatus (Buchler). The bacitracin samples (0.1—2 mg) were applied in a 20% sucrose solution and covered with a protecting layer (0.05 ml) of 10% sucrose and 1% Ampholine. The lower anodic chamber contained 5% phosphoric acid and enclosed the gel in the glass tube. The upper cathodic chamber contained 5% ethylenediamine. The focusing was carried out at a constant voltage of 150 V for 2 h. After isoelectric focusing, the bands were visualized as white precipitates by removing the gel columns from the glass tube and placing them in 14% trichloroacetic acid for 30 min. The bands were photographed in front of a black background. Isoelectric points were measured with a pH meter

by slicing an unstained gel column and eluting the sliced sections with 1.0 ml of water overnight.

Purified bacitracin A was kindly provided by Professor Lyman C. Craig (The Rockefeller University). The sample was stored as a powder (containing a few per cent moisture) for one year at 4°C before use.

Results and discussion

With 1% carrier Ampholine, pH 3.5—10, the pH range in the gel extended from 4.0 to 8.5 (see Fig. 1). Isoelectric focusing of bacitracin A (0.5 mg) was done as described above. By precipitation with trichloroacetic acid (TCA), four bands were observed at pH 7.1, 6.8, 6.5 and 6.0 (see Fig. 1). With carrier Ampholine, pH 6—8, no additional bands were observed. The main band (at pH 7.1) possesses microbiological activity (against the test organism *Micrococcus flavus*) before TCA precipitation, and represents bacitracin A. Extraction and refocusing of the individual bands at pH 7.1, 6.8, 6.5 and 6.0 resulted in single bands at the corresponding pH. This suggests that the several bands may have been formed during the storage of the sample and not through the isoelectric focusing procedure. The band at pH 6.8 possessed weak microbiological activity. An active transformation product of bacitracin has also been reported by others [6]. The bands at pH 6.5 and 6.0 were microbiologically inactive. By oxidation of bacitracin A to bacitracin F [1] the band at pH 7.1 decreased in size, whereas the band at pH 6.0 increased. The pH 6.0 band was assumed to represent bacitracin F. Additionally, two diffuse bands which appeared in the lower pH region might be other transformation products of bacitracin A.

By focusing commercial bacitracin lots produced by our company, the bands at pH 7.1, 6.8, 6.5 and 6.0 were observed (see Fig. 1). When greater amounts of the lots were focused, two weak bands appeared in the lower pH region. The band at pH 7.1 probably represents closely related bacitracins such as bacitracin A and bacitracin B [5]. The bands at pH 6.0 which were microbiologically inactive are probably bacitracin F (from A and from B).

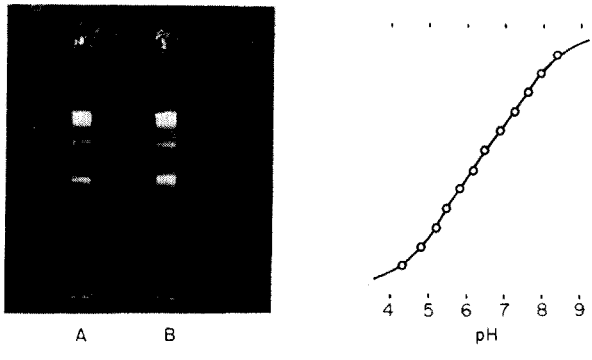

Fig. 1. Isoelectric focusing in gel of 0.5 mg of bacitracin A (A) and 0.5 mg of a commercial bacitracin lot containing 62 i.u./mg (B). pH in an unstained gel column was measured in sliced sections (o—o).

When 0.1—2 mg of bacitracin A was applied on the gel columns, the width of the band at pH 7.1 was approximately proportional to the sample amount, and was not affected by enrichment with bacitracin F. Similar results were obtained for the band at pH 6.0 (bacitracin F).

The method has been used to demonstrate synthesis de novo of bacitracin A from the constituent amino acids [9] in a cell-free system. Disc isoelectric focusing in gel is a simple and promising method for the separation of groups of bacitracins.

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Analytica Chimica Acta, 98 (1978) 141—144

Short Communication

THE SYNERGIC INFLUENCE OF 1,10-PHENANTHROLINE ON THE EXTRACTION OF MANGANESE WITH DIBENZOYLMETHANE AND 2-THENOYLTRIFLUOROACETONE

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β-Diketones such as dibenzoylmethane (HDBM) and 2-thenoyltrifluoroacetone (HTTA) form inner chelates with heavy metals, including manganese(II), which can be extracted with apolar organic solvents [1, 2]. Applications of these compounds, especially HDBM, are limited by the long time needed to reach equilibrium. Increased effectiveness and improved kinetics in different extraction systems can be achieved by introduction of a synergic factor; neutral organophosphorus compounds, ketones, alcohols, amines, etc., have been used [3, 4].

This communication reports the results of investigations on the synergic effect of 1,10-phenanthroline on the extraction of manganese(II) with benzene solutions of HDBM and HTTA. The synergic properties of 1,10-phenanthroline have already been shown in the extraction of nickel with dithizone [5] and of lanthanides with HTTA [6].

Experimental

Reagents. A manganese(II) solution (10^{-3} M) was prepared from anhydrous manganese(II) sulphate. A 10^{-2} M solution of 1,10-phenanthroline (POCh, Poland) was obtained by dissolution of the monohydrate. Dibenzoylmethane (B.D.H., England) and 2-thenoyltrifluoroacetone (ChEMIPAN, Warsaw) were used as 10^{-2} M solution in benzene.

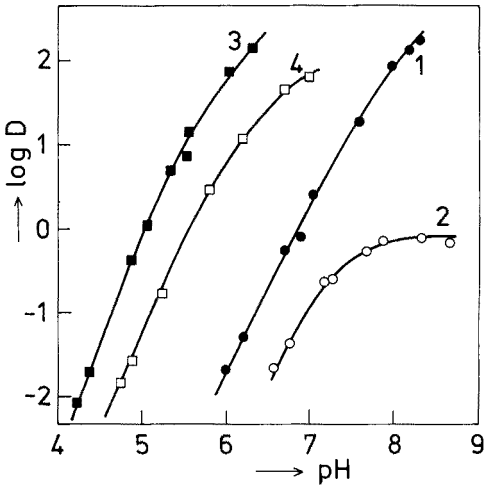
Determination of the distribution coefficients of manganese. The aqueous solution containing 2×10^{-5} M manganese(II) and 1,10-phenanthroline (known concentration), adjusted to a given pH value and at ionic strength (I) of 0.1 (NaCl) was shaken for 20 min with an equal volume of HDBM or HTTA solution. After phase separation, the pH value was measured and the manganese was determined in both phases. The spectrophotometric methods applied were the formaldoxine and PAN methods [7], after evaporation and mineralization. On the basis of these results the distribution coefficient values were calculated.

Results and discussion

The dependence of the distribution coefficient of manganese(II) on the pH of the aqueous phase in extractions with HDBM and HTTA in benzene, with and without 1,10-phenanthroline, is shown in Fig. 1. The improvement in the extraction efficiency in the presence of 1,10-phenanthroline is quite clear.

With 1,10-phenanthroline present the extraction equilibrium with both β-diketones was achieved much more quickly. For the extraction of manganese, attainment of equilibrium required more than 210 min with HDBM and about 150 min with HTTA. The presence of 1,10-phenanthroline in these systems shortened the equilibration times to 20 and 15 min, respectively.

The dependence of the distribution ratios of manganese on the initial concentration of the extractant in the organic phase (log—log plot), at constant pH value and constant 1,10-phenanthroline concentration, is shown in Fig. 2. For both extractants the values of the slopes are 2. In these investigations, the equilibrium concentrations of HDBM or HTTA in the organic phase were practically the same as the initial ones, because dissociation of the reagents was insignificant at the pH values selected, and because their initial concentration was much higher than that of manganese.



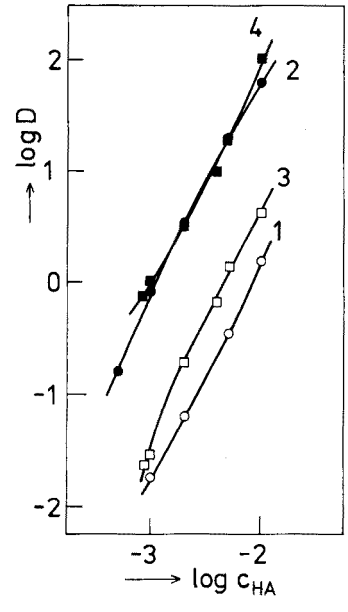


Fig. 1. Extraction of manganese(II) with HDBM or HTTA in benzene as a function of pH. Curve 1: [HDBM] = 1×10^{-2} M, [phen] = 2×10^{-3} M. Curve 2: [HDBM] = 1×10^{-2} M, [phen] = 0. Curve 3: [HTTA] = 1×10^{-2} M, [phen] = 2×10^{-3} M. Curve 4: [HTTA] = 1×10^{-2} M, [phen] = 0.

Fig. 2. Dependence of the distribution coefficients of manganese(II) on the equilibrium concentration of HDBM. Curves 1 and 2 and HTTA. Curves 3 and 4, in benzene, in the presence of 1,10-phenanthroline (2×10^{-3} M). Curve 1, pH 7.0. Curve 2, pH 7.9. Curve 3, pH 5.3. Curve 4, pH 6.0.

The effects of the initial concentration of 1,10-phenanthroline in the aqueous phase at constant HDBM and HTTA concentrations and unchanged pH, are shown in Fig. 3. The values of the slopes are close to 1 for the lower concentrations of phenanthroline. At the higher concentrations of 1,10-phenanthroline, its complex-forming power towards manganese in aqueous systems plays a part. The equilibrium concentrations of 1,10-phenanthroline in the organic phase were very similar to the initial concentrations, because the amounts used in complex formation were insignificant compared to the bulk concentration, and protolysis was negligible at the selected pH value.

These results (Figs. 1—3) indicate that the compounds extracted are $Mn(DBM)_2phen$ and $Mn(TTA)_2phen$; the extraction can be described as follows:

$$Mn^{2+} + 2HA_o + phen_o \rightleftharpoons MnA_2phen_o + 2H^+ \quad (1)$$

where subscript "o" denotes the organic phase. The equilibrium constant (extraction constant) is

$$K_{ex} = [MnA_2phen]_o\,[H^+]^2/[Mn^{2+}]\,[HA]_o^2\,[phen]_o \quad (2)$$

Since the protolysis constant of phenanthroline (K_a) is 1.05×10^{-5} [8] and the partition constant for benzene and water (P_{phen}) is 3.81 [9], then if complexation of phenanthroline with manganese is neglected, the equation for the equilibrium concentration of phenanthroline in the organic phase is

$$[phen]_o = [phen]/\left(1 + \frac{1}{P_{phen}} + \frac{[H^+]}{P_{phen} \cdot K_a}\right) \quad (3)$$

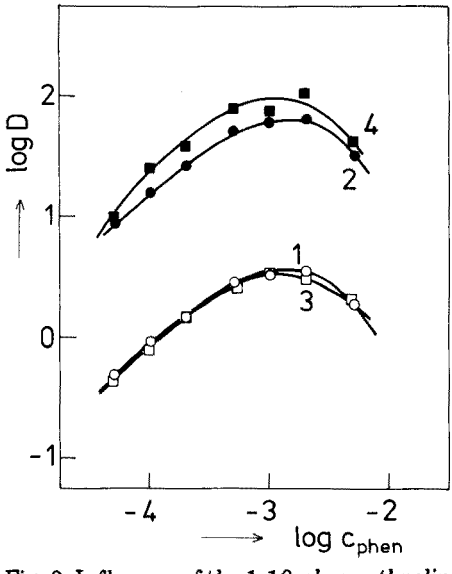


Fig. 3. Influence of the 1,10-phenanthroline concentration on the extraction of manganese(II) with HDBM and HTTA in benzene. Curve 1: [HDBM] = 2×10^{-3} M, pH 7.9. Curve 2: [HDBM] = 1×10^{-2} M, pH 7.9. Curve 3: [HTTA] = 2×10^{-3} M, pH 6.0. Curve 4: [HTTA] = 1×10^{-2} M, pH = 6.0.

where [phen] denotes the initial concentration of phenanthroline.

The equilibrium concentration of the chelating agent in the organic phase is

$$[HA]_o = [HA] / \left(1 + \frac{1}{P_{HA}} + \frac{K_{HA}}{P_{HA}[H^+]}\right) \quad (4)$$

The partition constants for the benzene—water system are $P_{HDBM} = 10^{5.35}$ and $P_{HTTA} = 10^{1.62}$, and the dissociation constants are $K_{HDBM} = 10^{-9.35}$, $K_{HTTA} = 10^{-6.35}$ [1]. Manganese in the aqueous phase hydrolyses and is complexed with phenanthroline. Hydrolysis of manganese is insignificant at pH values below 9 [10]. The stability constants of manganese(II) complexes with phenanthroline are $\beta_1 = 10^{3.88}$, $\beta_2 = 10^{7.04}$ and $\beta_3 = 10^{10.11}$ [11]. If it is assumed that manganese is present in the organic phase as MnA_2phen, then the extraction constant can be described by

$$K_{ex} = \{D[H^+]^2 (1 + \beta_1[phen] + \beta_2[phen]^2 + \beta_3[phen]^3)\} / [HA]_o^2 [phen]_o \quad (5)$$

On the basis of the data given in Figs. 1—3, and from eqns. (3—5), the extraction constants were found to be $K_{ex} = 10^{-6.30}$ for $Mn(DBM)_2phen$ and $K_{ex} = 10^{-2.57}$ for $Mn(TTA)_2phen$, at ionic strength 0.1 (NaCl).

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Analytica Chimica Acta, 98 (1978) 145—146

Short Communication

A RAPID METHOD FOR THE DETERMINATION OF PALLADIUM BY THE RING-OVEN TECHNIQUE

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The Weisz ring-oven technique [1] offers unique possibilities for the analysis of very dilute solutions. This communication describes a simple method for the determination of palladium in microgram amounts, based on the orange colour formed with rubeanic acid.

Experimental

Reagents and apparatus. The palladium solution used (0.3566 mg Pd ml^{-1}) was standardized gravimetrically [2].

Automatic capillary pipettes (1-μl and 2-μl capacity; Karl Kolb Scientific and Technical Supplies, Buchschlag, Frankfurt), Whatman filter paper No. 41, and a Weisz ring oven with a working temperature of 110°C, were used.

Procedure. A known volume of palladium solution was applied to the centre of the filter paper and washed into the ring zone with 0.2 M hydrochloric acid in the usual way. Immediately, the paper was sprayed with rubeanic acid solution (0.1% w/v in 96% ethanol) whereupon an orange-red ring appeared; 8—10 washings sufficed. A standard scale was obtained from standard solutions, and unknown solutions were analyzed in the usual way [1]. Palladium in unknown solutions was also determined by the Segment Technique [3—6].

Results and discussion

Under the above conditions, the sharp orange-red ring is formed immediately after spraying rubeanic acid on the paper. The results obtained by the standard-scale method are shown in the Table; the maximum error is 6.5%. The shelf life of the standard scale, checked as recommended by Weisz [1], was found to be only one day. In view of this instability, the Segment Technique [3—5] was tested. The results (Table 1) show that the average error is decreased somewhat, and that the Segment Technique is suitable for the range 357 μg to 1.43 mg of palladium.

The effects of diverse ions on the determination of palladium by the segment method were investigated [5]. There was no interference from up

TABLE 1

Determination of palladium

Pd taken (μg)	Standard scale		Segment Technique	
	Pd found (μg)	Error (%)	Pd found (μg)	Error (%)
357	333	−6.5	346	−2.9
535	505	−5.5	505	−5.5
891	891	± 0.0	865	−2.9
1160	1130	−2.6	1130	−2.6
1430	1400	−2.1	1400	−2.1

to 8-fold amounts of mercury, tellurium and rhodium; the amounts of platinum, gold, ruthenium, osmium and rubidium which could be tolerated were 11, 4, 3, 9 and 15-fold, respectively, relative to the weight of palladium. There was no interference from large amounts of iron(III).

The method reported is quick and sufficiently accurate for assays of palladium in various routine situations.

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Analytica Chimica Acta, 98 (1978) 147—149

Short Communication

SPEKTRALPHOTOMETRISCHE BESTIMMUNG VON BLEI(II)-, SOWIE DIALKYLBLEI- UND TRIALKYLBLEIVERBINDUNGEN IN GERINGEN KONZENTRATIONEN

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Um Pb^{2+}, R_3Pb^+ und R_2Pb^{2+} (R = Alkylrest) in geringen Konzentrationen nebeneinander zu bestimmen, kann man deren Dithizonkomplexe [1, 2] aus der gepufferten wässrigen Lösung in CCl_4 oder $CHCl_3$ extrahieren und die Konzentrationen aus den Extinktionswerten bei 424, 500 und 540 nm mit Hilfe eines Gleichungssystems ermitteln [1]. Wir konnten in analoger Weise auch Tri- und Diarylbleiverbindungen und Pb^{2+} nebeneinander bestimmen [3, 4], und zwar ebenso wie Alkylverbindungen bis herab zu etwa 10 μg Pb l^{-1}. Dabei zeigte sich daß bei den Alkyl- und bei den Arylverbindungen die Analysenfehler am kleinsten waren (relative Standardabweichung bei 50—100 μg Pb ca. 1,8%), wenn die Konzentrationen der drei nebeneinander zu bestimmenden Verbindungen ungefähr gleich waren. Wichen die Konzentrationen jedoch stärker voneinander ab (schon um den Faktor 2—3), so waren die Fehler wesentlich größer. Ein weiterer Nachteil dieses Verfahrens ist die Lichtempfindlichkeit der Dithizonkomplexe. Für unsere Untersuchungen über die Biomethylierung von Pb^{2+} und Organobleiverbindungen [5] benötigten wir aber ein Analysenverfahren, mit dem wir die dabei in unterschiedlichen und zeitlich veränderlichen Konzentrationen beteiligten Bleispezies R_3Pb^+, R_2Pb^{2+} und Pb^{2+} nebeneinander bestimmen konnten.

Ergebnisse und Diskussion

Die spektralphotometrischen Einzelbestimmungen von Pb^{2+} [6] und R_2Pb^{2+} [7] mit 4-(2-Pyridylazo)-resorcin (PAR) lassen sich dahingehend erweitern, daß Pb^{2+}, R_2Pb^{2+} und R_3Pb^+ nebeneinander bestimmt werden können, und zwar auch dann mit hoher Genauigkeit, wenn die Einzelkomponenten in unterschiedlichen Konzentrationen vorliegen.

Die 1:1-Komplexe, die PAR sowohl mit Pb^{2+} als auch mit R_2Pb^{2+} bei pH 9—10 bildet, weisen beide bei 520 nm ein Absorptionsmaximum auf. Durch Zusatz von EDTA wird jedoch der Pb^{2+}-Komplex, nicht aber der R_2Pb^{2+}-Komplex entfärbt. R_3Pb^{2+} bildet mit PAR unter den gleichen Bedingungen keinen Komplex [7]. Mißt man daher die Gesamtextinktion bei 520 nm und

zieht davon die nach Zusatz von EDTA gemessene Restextinktion bei 520 nm, die dem R_2Pb^{2+}-Gehalt entspricht, ab, so erhält man den Gehalt an Pb^{2+}. Zerstört man in einer zweiten Probelösung die Organobleiverbindungen mit Jod (z.B. in Anlehnung an [1]) und ermittelt dann nach Reduktion überschüssigen Jods und Komplexierung mit PAR spektralphotometrisch den Gesamtbleigehalt, so ergibt die Differenzbildung mit den Pb-Gehalten aus der ersten Lösung den Gehalt an R_3Pb^+. Begleitelemente, die die Bleibestimmung mit PAR stören, können durch Extraktion des Bleis mit Isobutylmethylketon abgetrennt werden [6].

Wenn Pb^{2+} und R_2Pb^{2+} in sehr stark unterschiedlichen Konzentrationen vorliegen, ist es nicht sinnvoll, das Probenvolumen zur genaueren Erfassung der Komponente, die in geringer Konzentration vorhanden ist, zu vergrößern, weil hierbei die Genauigkeit durch die hohe Gesamtextinktion beeinträchtigt werden kann. In diesem Falle ist es vorteilhaft, die Komponente mit der höheren Konzentration in einer aus wenig Probenmenge hergestellten Lösung so wie oben beschrieben zu bestimmen, hingegen die Komponente mit der niedrigen Konzentration in einer aus einer größeren Probenmenge hergestellten Lösung in der folgenden, abgewandelten Weise zu messen: 1. Liegt Pb^{2+} in großem Überschuß vor, so gibt man zu der Lösung (hohe Konzentration), in der R_2Pb^{2+} bestimmt werden soll, das EDTA vor der Messung hinzu. 2. Liegt R_2Pb^{2+} im großem Überschuß vor, so mißt man gegen eine identische, aber mit EDTA versetzte — durch den R_2Pb^{2+}—PAR— Komplex intensiv rot gefärbte — Vergleichslösung und erhält dadurch unmittelbar den Extinktionswert für Pb^{2+}.

Die spektralphotometrische Bestimmung geringer Mengen der flüchtigen Tetraalkylplumbane kann — nach Absorption in methanolischer J_2-Lösung und Überführung in Pb^{2+} — statt mit Dithizon [8] zeitsparender mit PAR erfolgen.

Experimenteller Teil

Reagenzien. Es kamen analysenreine Reagenzien zur Anwendung. Die Lösungen wurden unter Verwendung von destilliertem Wasser hergestellt und mit PAR auf Verunreinigung durch Pb^{2+} überprüft.

Reduzierende Pufferlösung, aus 20 g KCN, 40 g Zitronensäure, 200 g Na_2SO_3, 1300 ml Wasser und 1200 ml konz. NH_3-Lösung in Anlehnung an [1].

Bestimmung von Pb^{2+} und R_2Pb^{2+}. Die Probelösung, deren Volumen 30 ml nicht übersteigen darf, sollte 10—250 μg Pb enthalten. Sie wird mit 5 ml PAR-Lösung (2×10^{-3} M 4-(2-Pyridylazo)-resorcin, Mononatriumsalz, im Wasser), 5 ml 1 M NH_4NO_3-Lösung und 2 ml einer 2,5%igen NH_3-Lösung versetzt und auf 50 ml aufgefüllt. Die Extinktion der Lösung wird gegen eine Blindlösung bei 520 nm gemessen, die in analoger Weise hergestellt wurde. Danach setzt man solange EDTA zu (einige mg), bis keine weitere Farbänderung mehr erfolgt und mißt erneut bei 520 nm. Den Gehalt an den jeweiligen Verbindungen erhält man durch Multiplikation der Extinktionswerte mit Faktoren aus Eichkurven. Diese wurden mit Eichlösungen bestimmt, die auf die

beschriebene Weise aus Pb^{2+} und R_2Pb^{2+}-Standardlösungen hergestellt worden waren. Aus 14 Proben, die 10 μg Pb enthielten, ergab sich eine Standardabweichung von 0,135 μg Pb.

Bestimmung des Gesamtbleigehaltes. Es werden bis zu 20 ml Probenlösung verwendet, denen 10 ml Methanol zur Erhöhung der Löslichkeit von J_2 zugesetzt werden. Nach Zugabe von 10 ml KJ · J_2-Lösung (33,4 g KJ, 47 g J_2 und 100 ml Wasser) wird unter häufigem Umschütteln 30 min stehen gelassen; dann werden 30 ml reduzierende Pufferlösung zugegeben. Bei der Reduktion erwärmt sich die Lösung etwas. Nach dem Abkühlen gibt man 10 ml 1 M NH_4NO_3-Lösung und 5 ml PAR-Lösung zu, füllt auf 100 ml auf und mißt wie oben beschrieben. Als Blindlösung dient dieselbe Lösung, nachdem ihr in der Küvette etwas EDTA zur Komplexierung des Pb^{2+} zugesetzt worden war.

Aus 10 Proben, die 10 μg Pb als R_3Pb^+ enthielten, ergab sich eine Standardabweichung von 0,7 μg Pb.

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Analytica Chimica Acta, 98 (1978) 151—155

Short Communication

THE EFFECT OF THE SOLVENT IN THE NITRATE-SELECTIVE ELECTRODE

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Different types of nitrate-selective electrode have been suggested in the literature, but those based on the tris(bathophenanthroline)nickel-II nitrate ion-association complex are the most widely used, because they possess relatively good selectivity. This exchanger, dissolved in 2-nitro-*p*-cymene, was also used in the wick design of electrode [1]. The life-time of these electrodes was approximately 6 weeks. Attempts to use this solvent-exchanger composition for the preparation of PVC-based electrodes as described by Davies et al. [2], generally failed; in a relatively short time the electrodes lost their sensing properties, probably because of segregation of the membrane components, primarily connected with the solvent used. In an attempt to find a better solvent, 2-nitrophenyloctyl ether and 2-nitrophenylphenyl ether were examined; these solvents were proposed by Jaber et al. [3] for the preparation of a barium-selective electrode.

Experimental

2-Nitrophenyloctyl ether was synthesized [4]. 2-Nitrophenylphenyl ether was kindly donated by Dr. J. D. R. Thomas, UWIST. Tris(bathophenanthroline)nickel(II) nitrate was prepared in a suitable solvent [5].

The wick electrode was modified compared to the previous construction (Fig. 1A) [1]. The PVC electrode was equipped with a solid silver rod in contact with the PVC membrane (Fig. 1B). The membranes were prepared as recommended by Davies et al. [2]. All measurements were made with a PHM 64 meter (Radiometer) and a Servograph REC 61 recorder.

Results and discussion

In comparative measurements, six modifications of electrodes were investigated; two electrode constructions were studied, each with three different solvents: 2-nitro-*p*-cymene (NC), 2-nitrophenyloctyl ether (NPOE) and 2-nitrophenylphenyl ether (NPPE). In many respects, the characteristics of these electrodes were similar. The slopes of the response vs. activity plots were always initially 55—60 mV/pNO_3; the response time at concentrations

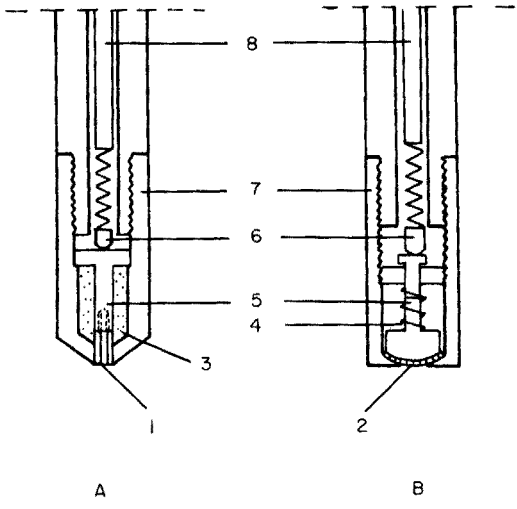

Fig. 1. (A) Cross-section of the wick electrode. (B) Cross-section of the PVC electrode. 1, Porous wick; 2, membrane based on polyvinyl chloride matrix; 3, fibrous reservoir of liquid exchanger; 4, spring; 5, silver contact; 6, cable ending; 7, Teflon cap; 8, cable.

above 10^{-4} M was less than 1 min, or for lower concentrations, 2—3 min; and the potential stability was better than 0.5 mV. During one day the potential drift was less than 1 mV. The most significant improvement was the prolonged lifetime of electrodes with NPOE and NPPE as solvents. Both wick and PVC electrodes were useful for more than 10 months, without essential change in linearity and slope.

The linear range of the calibration curves for the electrodes with 2-nitro-*p*-cymene extended to pNO_3 4.3, but when NPOE or NPPE was used, the calibration plots were linear as far as pNO_3 5.0. However, this extension of the range is not really significant in practical applications, because with the buffering system containing silver sulphate and phosphate pH buffer used in most procedures, the practical linear range is defined by the selectivity of the electrode and not by the sensitivity of the extraction characteristics. It seems most probable that the hydrogensulphate ion exerts the greatest effect in this respect; the estimated selectivity coefficient for this ion corresponds to $\log K_{NO_3, HSO_4} = -1.9$.

The selectivity of the electrodes is influenced by the dielectric constant of the medium. In the case of the three solvents used, the dielectric constant varies from 17.7 for 2-nitro-*p*-cymene to 28.3 for NPPE. This change has only a small effect on the selectivity coefficients (Table 1), which were determined at different concentration levels in mixed solutions.

The pH effect on the electrode response is quite similar for all types of electrodes (Fig. 2). In the pH range 2—8, the response is not influenced by pH.

The membranes prepared with the use of the ether solvents have not only

TABLE 1

Selectivity coefficients

Interfering ion	Concn. (M)	$-\log K_{NO_3X}$		
		NC	NPOE	NPPE
NO_2^-	1×10^{-4}	—	0.15	0.5
	5×10^{-4}	0.7	0.5	1.0
	1×10^{-3}	0.7	1.1	1.0
	1×10^{-2}	1.1	1.0	—
Cl^-	5×10^{-4}	1.0	1.2	1.0
	1×10^{-3}	1.2	1.3	1.1
	1×10^{-2}	2.1	2.0	2.0
HCO_3^-	1×10^{-3}	1.0	1.1	1.4
	1×10^{-2}	1.5	1.7	2.4
SO_4^{2-}	1×10^{-2}	2.7	3.7	3.3
	5×10^{-2}	3.1	3.4	3.5
$H_2PO_4^-$	1×10^{-2}	2.2	3.3	3.2
	5×10^{-2}	3.3	3.7	3.4
	1×10^{-1}	—	4.0	3.7

longer life-times but also better mechanical properties. To check this, the mechanical resistance of the membranes was investigated. The limit of determination of the membrane, in agreement with the Hooke Law, as well as the resistance to membrane destruction, increases 2—3 times (Fig. 3). This helps greatly in handling the membranes during the electrode assembly.

A comparison of the solvent properties (Table 2) indicates that there are differences in viscosity and in the water content, as determined by the Karl Fischer method in a solvent equilibrated with water. These factors change in parallel with the improvement in electrode properties and may be considered responsible for these effects. It is interesting to note that the solubility parameter changes irregularly, in contrast to the observation of Nielsen and Hansen [9]. This is probably due to the drastic changes in the structure of the solvents in the present case; Nielsen and Hansen [9] investigated compounds of similar structure.

The PVC nitrate-selective electrodes were used successfully for the determination of nitrate in peat soils, after extraction with 1% K_2SO_4. Because neither chloride nor nitrite was found in the samples, only boric acid (3 g dm^{-3}) was added and the extractant was adjusted to pH 3 with H_2SO_4, to eliminate the effect of hydrogencarbonate. The soil (40 cm^3) was extracted with 100 cm^3 of the solution for 30 min. No difference was observed for filtered and unfiltered samples when a standard addition technique was used. The results compare favourably with those obtained colorimetrically, after reduction on an activated cadmium column and reaction with sulphanilamide and *N*-naphthalenediamine (Table 3).

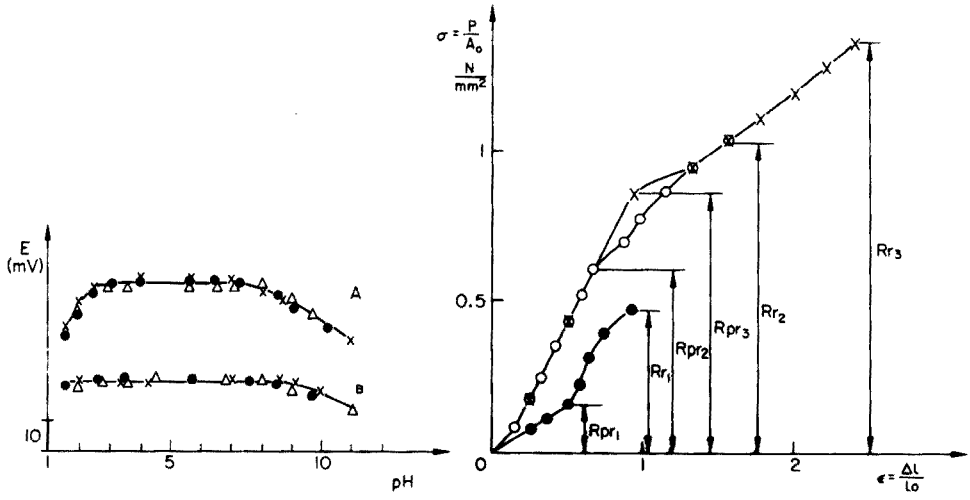

Fig. 2. Response of the nitrate electrodes at various pH values. (A) 10^{-4} M KNO_3; (B) 10^{-3} M KNO_3. Membranes based on (•) NC, (△) NPOE and (×) NPPE.

Fig. 3. Resistance to membrane destruction. Membranes based on PVC and (•) NC; (○) NPOE; (×) NPPE. R_{pr} is the limit of proportionality, and R_r the tear resistance of the membrane.

TABLE 2

Properties of solvents

Solvent	2-Nitro-*p*-cymene	2-Nitrophenyl-octyl ether	2-Nitrophenyl-phenyl ether
Boiling point (°C) [3]	259	290 (dec.)	280 (dec.)
Viscosity at 25°C [3]	3.24	12.8	16.1
Dielectric const. [3]	17.7	23.5	28.3
Density	1.08 [6]	1.04[a]	1.26 [7]
Water solubility (% w/w)	0.72	0.60	0.56
Solubility parameter[b]	10.2	9.6	11.1

[a]Determined at 20°C. [b]Calculated as described by Small [8].

TABLE 3

Determination of nitrate (mg N dm^{-3}) in peat soils

Sample	Colorimetric	Potentiometric	Std. dev. (n = 6)
1	23.0	22.0	0.046
2	6.0	6.7	0.063
3	7.2	7.4	0.043
4	137	136	0.070

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Analytica Chimica Acta, 98 (1978) 157—161

Short Communication

THE DETERMINATION OF MOBILE NITROGEN IN VANADIUM STEELS BY THE EXTRACTION METHOD WITH HYDROGEN

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Nitrogen is present in steels in the atomic form in solid solution, and combined as nitrides. When hydrogen is passed over fine millings at 500°C, the atomic nitrogen is removed as ammonia [1]. Any iron or manganese nitrides decompose at that temperature and also form ammonia with hydrogen. The nitrogen removed at that temperature is known as mobile nitrogen. The more stable nitrides formed with boron, silicon, aluminium, titanium, zirconium, vanadium, niobium and tantalum are not decomposed in hydrogen at 500°C; this nitrogen is known as combined nitrogen. Certain properties of steel, such as strain aging and toughness are influenced not only by the total nitrogen content, but also by the ratio of mobile to combined nitrogen.

The hydrogen extraction method has not been considered to be reliable for steels containing vanadium or niobium where there is more than 20 ppm of mobile nitrogen [1]. The results obtained for mobile nitrogen in these steels by this method are lower than the differences between the total nitrogen contents by a vacuum fusion method, and the combined nitrogen contents determined after the dissolution of the steels in a bromine—methyl acetate mixture [2]. The iron matrix is dissolved in the bromine—ester mixture, and the atomic nitrogen released, while the stable nitrides remain unattacked. Any iron and manganese nitrides present in the steel are also soluble in the bromine—ester mixture. However, very small particles of stable nitrides may not be retained on the filter, thereby giving a low combined nitrogen result [2].

Fisher and White [1] stated that the mobile nitrogen results for vanadium steels containing more than 20 ppm of mobile nitrogen are low, because of further precipitation of vanadium nitride at 500°C. It was hoped that the apparent mobile nitrogen content of a steel could be increased as the hydrogen extraction temperature was decreased until the mobile nitrogen result was identical with the mobile nitrogen result obtained by the difference method. The effect of the cooling rate of the steel after heat treatment was also investigated, as this is of major importance in considering the mechanical properties of steel.

TABLE 1

Mobile nitrogen contents of the steels by the hydrogen extraction method at different temperatures
(Duplicate results are given.)

Steel[a]	250°C	350°C	400°C	440°C	500°C	650°C
SG1	<5	86, 80	86, 78	85, 89	66, 63	<5
BSC1	<5	40, 39	42, 38	39, 40	33, 33	<5

[a]The compositions of the steels (%) are:

Steel	C	Si	Mn	S	P	Ni	Cr	Cu	Sn	V
SG1	0.08	0.24	1.51	—	—	—	—	—	—	0.16
BSC1	0.24	0.24	1.45	0.018	0.011	0.046	0.037	0.067	0.009	0.084

SG1 is a vacuum-melted experimental steel, and BSC1 is a commercial cast from British Steel Corporation.

Experimental

The steels SG1 and BSC1 (Table 1) were heated and held at 1100°C and 1150°C, respectively, for 30 min, and then quenched in iced water to obtain a high mobile nitrogen content. Steel SG1 was also cooled at controlled rates between 3.6 and 120°C min^{-1} from 1100°C.

The mobile nitrogen content was determined by the hydrogen extraction method of Headridge and Long [3], at different temperatures between 250°C and 650°C; the collection time (3—5 h) was continued until cessation of ammonia production, which was determined by the ion-selective electrode.

The combined nitrogen content was determined by bromine—methyl acetate dissolution followed by filtration on glass fibre discs, conversion of the insoluble nitrides to ammonium ion by fuming sulphuric acid, followed by steam distillation of ammonia after addition of alkali and spectrophotometric determination of ammonia as indophenol blue [4]. The total nitrogen content was determined by vacuum fusion with a Balzer Exhalograph EN2200.

Results and discussion

Mobile nitrogen determinations. The mobile nitrogen results are shown in Table 1, which also shows the chemical compositions of the steels. The times for the complete extraction of mobile nitrogen were approximately 4.75, 3.5, 3.0 and 3.0 h at 350, 400, 440, 500°C, respectively. The results obtained at 500°C are significantly below those obtained at lower temperatures.

Total and combined nitrogen determinations. The combined nitrogen contents of the steels obtained by the bromine—ester method and the apparent mobile nitrogen contents by difference are shown in Table 2. There is a very large discrepancy between the mobile nitrogen as calculated by difference and the values obtained directly. From Table 1, the results for mobile nitrogen by the extraction method are virtually the same at 350°C, 400°C and 440°C. If precipitation of vanadium nitride were occurring during the extraction of atomic nitrogen, the apparent mobile nitrogen content would be expected to increase as the temperature of extraction decreased. This is noticed only between 500 and 440°C. Hence it appears that the mobile nitrogen results are reliable at the lower temperatures, and that results by the difference method are grossly in error.

At 1100—1150°C, much of the nitrogen in steels SG1 and BSC1 should be in solution [5], and vanadium nitride precipitated during quenching would consist of extremely fine particles, which could have passed through the glass-fibre filters or have been at least partially dissolved in the bromine—ester mixture. There has been some controversy about vanadium nitride recovery in past papers [6, 7].

Very little mobile nitrogen was recovered at 250°C and 650°C (Table 1). A temperature of 250°C is too low for appreciable reaction between atomic nitrogen and hydrogen on a steel surface [8]. Precipitation of vanadium nitride through reaction between atomic nitrogen and elemental vanadium gives a very low mobile nitrogen value at 650°C. In both these steels there is an excess of vanadium over nitrogen on an atomic basis.

Steels SG1 and BSC1 were also heated in argon at 350°C for 1 h before the mobile nitrogen contents of the steels were determined. The results obtained were 80 ppm and 39 ppm, respectively, which is in good agreement with the values of 83 and 40 ppm. Clearly no significant precipitation of vanadium nitride occurred during the heating in argon. These results and those of Table 1 show that the hydrogen extraction method is reliable, if the temperature of extraction is between 350°C and 440°C. The difference method for mobile nitrogen in vanadium steels seems to be seriously in doubt when steels contain very fine particles of this nitride.

Effect of cooling rate on mobile nitrogen values. The mobile nitrogen values as determined by the hydrogen extraction method at 440°C for the steel SG1, cooled at five different rates, are shown in Table 3. The range of equivalent cooling rates from that of air-cooled large section sizes down to water-quenched small sections was investigated; this covers all the

TABLE 2

The distribution of nitrogen (ppm) in the steels

Steel	Total N	Combined N (bromine—ester)	Mobile N (by difference)
SG1	360, 380	65, 68	303
BSC1	160, 170	50, 50	115

TABLE 3

Effect of cooling rate on mobile nitrogen values

Cooling rate from 1100°C (°C min^{-1})	8,000[a]	120	36	12	3.6
Equivalent cooling of	20-mm bar in water	13-mm bar in air	50-mm bar in air	120-mm bar in air	380-mm bar in air
Mobile nitrogen extracted at 440°C (ppm)	87	12	9	5	<5

[a]Water-quenched.

commercial heat treatment conditions that are likely to be encountered in practice.

In view of the known effects of mobile nitrogen on mechanical properties, these results are very significant. After rapid quenching, 87 ppm of nitrogen are retained in solid solution, whereas at slower cooling rates, the mobile nitrogen content is progressively decreased because of precipitation of vanadium nitride on cooling. At the slowest cooling rate, there is virtually no mobile nitrogen in the steel, which means that all the nitrogen is combined as vanadium nitride. These results also emphasize the ability of vanadium to remove all the nitrogen efficiently from solid solution by controlled heat treatment. Other elements such as aluminium and silicon remove only part of the nitrogen from solid solution under similar heat treatment conditions [9]. However, it must be pointed out that steel SG1 has a much higher total nitrogen content than vanadium steels encountered commercially.

In both steels, the temperatures of 1100 and 1150°C, although sufficiently high for the solution of a significant amount of vanadium nitride, are not high enough to dissolve all the vanadium nitride. However, it is not known how much vanadium nitride precipitates on cooling, even with the rapid cooling rates produced by water quenching. Thus the combined nitrogen values will consist of undissolved vanadium nitride at the solution temperatures (1100 or 1150°C), and vanadium nitride which precipitates on cooling. The variation between 87 and <5 ppm nitrogen in solid solution shows the significance of cooling rate, which has a very important effect on the mechanical properties of steel, such as toughness and creep strength.

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Short Communication

POLAROGRAPHIC CHARACTERISTICS OF METAL IONS IN VARIOUS SUPPORTING ELECTROLYTES†

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A previous paper [1] tabulated the polarographic characteristics of a number of common metal ions in 9 different supporting electrolytes containing thiocyanate, hydrazine, pyridine, and other complexing agents; two further papers [2, 3] dealt similarly with 13 supporting electrolytes containing citrate, tartrate, malonate, and oxalate. Systematic data like these are useful in designing procedures for practical analysis by polarography, differential pulse polarography, and related techniques, and they also serve to reveal peculiarities of behavior that may deserve more detailed investigation. The present communication gives similar data on 5 new supporting electrolytes.

Data are presented on the half-wave potentials and other parameters characterizing the polarographic waves of most of the common heavy metal ions in five supporting electrolytes: 0.1 M sodium citrate—0.1 M sodium cyanide—0.1 M sodium hydroxide, 0.1 M hydrazine dihydrochloride—0.2 M potassium iodide—0.4 M sodium acetate, saturated hydroxylammonium chloride, 1 M hydrochloric acid saturated with phenol, and 0.2 M ammonia—0.2 M ammonium thiocyanate.

The d.c. polarographic apparatus and experimental techniques employed have been described [1]. Definitions of the symbols and abbreviations [2] that appear in the Tables are as follows. "> 0" denotes a wave which begins at a potential so positive that it merges with the anodic wave caused by dissolution of the mercury electrode, and carries no implication as to whether $E_{1/2}$ would actually be positive or negative if it were possible to measure its value. A value of $E_{1/2}$ in parentheses denotes an anodic wave. "NR" signifies that no wave is obtained; "w.-d." and "i.-d." mean well-

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and ill-defined, respectively, the criterion being the degree of accuracy with which the height of the wave could be measured. A wave described as "very w.-d.", for example, is suitable for precise polarographic determination; a "very i.-d." wave, however, merely serves to interfere with other waves, and is useless for quantitative purposes. Descriptions between these are necessarily matters of personal judgement.

TABLE 1

Polarographic data in 0.1 M sodium citrate—0.1 M sodium cyanide—0.1 M sodium hydroxide

Element and oxidation state	$E_{1/2}$ (V vs. SCE)	Oxidation state of product	$E_{3/4}-E_{1/4}$ (mV)	Notes
Ag(I)	>0	0	—	Very w.-d.
As(III)	NR			
As(V)	NR			
Bi(III)	-0.69_4	0	—	Small max. at −0.72 V
Cd(II)	-1.04_7	0	—	Large max. at −1.28 V
Ce(IV)	−1.22	III	−284	Small, i.-d.
Ce(IV)[a]	−1.44	III	−317	Small, i.-d.
Co(II)	−1.240	0	−129	Very w.-d.
Cr(III)	NR			
Cr(VI)	−0.87	III	−104	Very w.-d.
Cu(II)	NR			
Fe(II)	NR			
Fe(III)	NR			
Mn(II)	−1.670	I (?)	−38	Very small, w.-d.
Mo(VI)	NR			
Ni(II)	−1.422	0	−54	W.-d.
Pb(II)	>0	0	—	Very w.-d.
Sb(III)	−1.103	0	−79	W.-d.
Sb(V)	NR			Apparently quant. pptn.
Sn(II)[a]	−1.121	0	−72	W.-d.
	(−0.802)	IV	+54	I.-d.
Sn(IV)	−1.25	II	−56	W.-d.
	−1.81	0	−146	I.-d.
Te(IV)	-0.96_4	0	−87	I.-d., rising portion deformed
Te(IV)[a]	−1.19	0	−32	I.-d., rising portion deformed
Te(VI)	−1.50	?	−92	I.-d. with maxima at −1.27 and −1.66 V
Te(VI)[a]	−1.509	?	−132	W.-d.
U(VI)	−0.933	?	−93	W.-d., partial pptn.
	-1.60_4	?	−62	I.-d.
V(IV)	−1.82	II	−110	Very i.-d.
V(V)	−1.79	II	−100	Very i.-d.
W(VI)	NR			
Yb(III)	NR			
Zn(II)	−1.71	0	—	Very small

[a]With 0.002% Triton X-100.

TABLE 2

Polarographic data in 0.1 M hydrazine dihydrochloride—0.2 M potassium iodide—0.4 M sodium acetate (pH 5.5)

Element and oxidation state	$E_{1/2}$ (V vs. SCE)	Oxidation state of product	$E_{3/4}-E_{1/4}$ (mV)	Notes
Ag(I)	NR	—	—	Apparently quant. pptn.
As(III)	−0.773	0	−45	Adsorption wave
	−0.9	−III	—	Very large
As(V)	NR			
Bi(III)	NR			Apparently quant. pptn.
Cd(II)[a]	−0.547	0	−33	W.-d.
Ce(III)	NR			
Ce(IV)	—	—	—	Rapidly reduced to Ce(III), q.v.
Co(II)	NR			
Cr(III)[b]	−0.929	II	—	Fairly w.-d.
Cr(VI)[c]	NR			
Cu(I)	−0.30	0	—	Partially pptd.
Cu(II)	—	—	—	Rapidly reduced to Cu(I), q.v.
Fe(II)	NR			
Fe(III)	−1.40	II	−61	Very i.-d.
In(III)[a]	−0.635	0	−52	W.-d.
Mn(II)	NR			
Mo(VI)	−0.68	?	—	Drawn out and i.-d.
Ni(II)	−1.086	0	—	Drawn out and i.-d.
O_2(0)	−0.010	(H_2O_2)	—	W.-d.
Pb(II)	−0.44	0	—	Drawn out, partial pptn.
Sb(III)	−0.422	0	−20	W.-d.
Sb(V)	NR			
Sn(II)	−0.501	0	−26	W.-d.
Sn(IV)	NR			
Tl(I)	NR			Apparently quant. pptn.
U(VI)	−0.48			W.-d.
Yb(III)	NR			
Zn(II)	−0.988	0	−44	W.-d.

[a]With 0.0008% Triton X-100, added to suppress a maximum. [b]Added as a stock solution of $KCr(SO_4)_2$. [c]Added as a stock solution of K_2CrO_4.

TABLE 3

Polarographic data in saturated (8.58 M) hydroxylammonium chloride[a]

Element and oxidation state	$E_{1/2}$ (V vs. SCE)	Oxidation state of product	$E_{3/4}-E_{1/4}$ (mV)	Notes
Ag(I)[c]	>0	0	—	Very w.-d.; max. only partially suppressed by Triton X-100
As(III)[c]	-0.52_0	0	−58	Fairly w.-d.
	−0.73	—III	−25	Fairly w.-d.; small rounded max. not suppressed by Triton X-100
	−0.94	(H_2)	−90	Very i.-d., asymmetrical ($E_{1/2}-E_{1/4}$ = −29 mV), large max. not suppressed by Triton X-100
As(V)	NR			
Bi(III)	−0.178	0	−18	Very w.-d.
Cd(II)	-0.71_4	0	−30	W.-d.
Ce(III)	NR			
Ce(IV)	—	—	—	Rapidly reduced to Ce(III), q.v.
Co(II)	NR			
Cr(III)	−0.81	II	−90	I.-d.
Cr(VI)	−0.58	?	−137	Abnormally small (incomplete redn.), very i.-d.
Cu(I)	-0.33_5	0	−53	Very w.-d.; small rounded max. suppressed by 0.002% Triton X-100
Cu(II)	—	—	—	Rapidly reduced to Cu(I), q.v.
Fe(II)	NR			
Fe(III)	—	—	—	Rapidly reduced to Fe(II), q.v.
I(V) (IO_3^-)	(−0.22)		+39	Small, i.-d.
In(III)	-0.61_7	0	−20	Very w.-d.
Mo(VI)	-0.17_8	V + H_2	−139	I.-d. to −0.7 V, w.-d. thereafter; maxima and min. not suppressed by 0.006% Triton X-100 but wave height decreases rapidly as the Triton is added
Ni(II)	−0.67	0	−130	I.-d.
Os(VIII)	−0.73	?	−59	Two very i.-d. waves of approximately equal height; addn. of 0.0004% Triton X-100 increases height of max. at −1.0 V
	−0.87	?	−57	
Pb(II)	−0.538	0	−29	Very w.-d.
Sb(III)[c]	−0.817	0	−18	Very w.-d.; large max. suppressed by Triton X-100
Sb(V)	−0.18	0	−22	Very small, i.-d.

TABLE 3 (continued)

Element and oxidation state	$E_{1/2}$ (V vs. SCE)	Oxidation state of product	$E_{3/4}-E_{1/4}$ (mV)	Notes
[Sb(V)]	−0.37	0(?)	−103	Σi_1 is nearly the same as for Sb(III) at the same concn.
Sn(II)[d]	-0.51_7	0	−27	Very w.-d.; large max. suppressed by Triton X-100
Sn(IV)	-0.16_1	II	−59	Fairly w.-d.
	−0.51	0	−21	W.-d., $\Sigma i_1 = 2.1\ i_1$; both waves become irregular on adding Triton X-100
Ti(IV)[b]	-0.8_4	III + NH_4^+(?)	−430	Very large, very i.-d.
	−1.16	III + N_2(?)	−39	$\Sigma i_1 = 2.5\ i_1$
U(VI)	>0	V + IV	—	Fairly w.-d.
	−0.59	IV	−129	I.-d.
	−0.89	III	−70	Very i.-d.
V(IV)	−0.78	II(?)	−161	Very i.-d.
V(V)	−0.47	II	−149	Fairly w.-d., asymmetrical ($E_{1/2}-E_{1/4} = -59$ mV)
W(VI)[c]	−0.28	III(?)	−52	I.-d.
	−0.49	(H_2)	−85	Very large and i.-d.; large rounded max. at −0.73 V not suppressed by Triton X-100
Yb(III)	NR			
Zn(II)	−1.05	0	−35	Very i.-d.

[a]The solution contained approximately 0.012 M hydrochloric acid, present as an impurity. [b]With 0.002% Triton X-100. [c]With 0.004% Triton X-100. [d]With 0.006% Triton X-100.

TABLE 4

Polarographic data in 1 M hydrochloric acid saturated with phenol[a]

Element and oxidation state	$E_{1/2}$ (V vs. SCE)	Oxidation state of product	$E_{3/4}-E_{1/4}$ (mV)	Notes
As(III)	−0.83	0	−70	Fairly i.-d.
As(V)	NR	—	—	
Bi(III)	−0.095	0	−33	W.-d.
Cd(II)	−0.641	0	−35	W.-d.
Ce(IV)	NR	—	—	
Co(II)	NR	—	—	
Cr(III)	NR	—	—	
Cr(VI)	>0	—	—	Very small
	−1.0		−100	
Cu(II)	>0	I	—	
	−0.2	0	—	Large rounded max. not suppressed by 0.002% Triton X-100
Fe(II)	NR	—	—	

TABLE 4 (continued)

Element and oxidation state	$E_{1/2}$ (V vs. SCE)	Oxidation state of product	$E_{3/4}-E_{1/4}$ (mV)	Notes
Fe(III)	>0	—	—	W.-d., very small
I(V) (IO_3^-)	>0	—	—	W.-d., very small
In(III)	NR	—	—	
Mn(II)	NR	—	—	
Mo(VI)	−0.26	IV	−83	
	−0.9	III	—	Very i.-d.
Ni(II)	NR	—	—	
Os(VIII)	>0	—	—	W.-d., rounded max. at −0.76 V
Pb(II)	−0.434	0	−31	W.-d.
Sb(III)	−0.139	0	−20	W.-d.
Sb(V)	NR	—	—	
Sn(II)	NR	—	—	
Sn(IV)	NR	—	—	
Te(IV)	−0.480	—	−130	W.-d., acute max. at −0.81 V
Ti(IV)	NR	—	—	
Tl(I)	−0.489	0	−52	
U(VI)	−0.37	—	—	W.-d.
V(IV)	−1.14	—	−30	I.-d.
V(V)	>0	—	—	
W(VI)	NR			
Yb(III)	NR			
Zn(II)	NR			

[a]The solubility of phenol in 1 M hydrochloric acid is 13.1 ± 0.1 wt. %.

TABLE 5

Polarographic data in 0.2 M ammonia—0.2 M ammonium thiocyanate

Element and oxidation state	$E_{1/2}$ (V vs. SCE)	Oxidation state of product	$E_{3/4}-E_{1/4}$ (mV)	Notes
Ag(I)	>0	0	—	Very w.-d.
As(III)	−1.62	−III	−17	Fairly i.-d.
As(III)[b]	−1.62	−III	−39	I.-d.
As(V)	NR			
Bi(III)	NR			Apparently quant. pptn.
Cd(II)	−0.707	0	−26	Very w.-d.
Ce(III)	NR			
Ce(IV)	(−0.295)	?	−36	W.-d., some pptn.
Co(II)	−1.01	0	−44	Fairly i.-d. to −1.3 V, fairly w.-d. thereafter
Co(II)[a]	−0.985	0	−42	Deep min. at −1.12 V, w.-d. thereafter
Cr(III)	−1.373	II	−44	W.-d., some pptn.
	−1.566	0		I.-d., irregular max.

TABLE 5 (continued)

Element and oxidation state	$E_{1/2}$ (V vs. SCE)	Oxidation state of product	$E_{3/4}-E_{1/4}$ (mV)	Notes
Cr(III)[b]	−1.381	II		W.-d., some pptn.
	−1.569	0		Very i.-d., max. at −1.63 V
Cr(VI)	−0.326	III	−54	W.-d.
	−1.419	II	−25	Fairly w.-d., max. at −1.68 V
Cu(II)	>0	I	—	W.-d.
	−0.429	0	−73	W.-d., max. at −0.67 V
Cu(II)[b]	>0	I	—	W.-d.
	−0.452	0	−73	W.-d.
Fe(II)	(−0.334)	III	+51	W.-d., some pptn.
	−1.387	0	—	Small max. on rising portion
Fe(III)	NR			Apparently quant. pptn.
In(III)	NR			Apparently quant. pptn.
Mn(II)	−1.514	0	−22	W.-d., max. at −1.55 V
Mn(II)[b]	−1.525	0	−34	W.-d., max. suppressed
Mo(VI)	−1.676	IV(?)	−86	I.-d.
Ni(II)	−0.870	0	−39	Two maxima
Ni(II)[b]	−0.889	0	−62	W.-d., max. suppressed
Pb(II)	−0.488	0	−37	W.-d.
Sb(III)	−0.810	0	−32	W.-d., some pptn.
Sb(V)	NR			
Te(VI)	−1.337	0	−63	W.-d., small max. at −1.41 V
Te(VI)[a]	−1.344	0	−94	W.-d., max. suppressed
Tl(I)	−0.474	0	−46	W.-d., max. at −0.56 V
Tl(I)[b]	−0.478	0	−58	W.-d.
V(VI)	−0.989	?	−151	W.-d., some pptn.
V(IV)	(−0.287)	V	+84	W.-d.
	−1.158	III	−42	W.-d., $i_{l,c} = -i_{l,a}$
V(V)	−1.042	III	−126	W.-d.
	−1.312	II(?)	−57	W.-d., $i_{l,2} > 2i_{l,1}$
W(VI)	−1.616	?	−339	i_l corresponds approximately to $n = 1$
Yb(III)	NR			
Zn(II)	−1.209	0	−6	W.-d., large max.
Zn(II)[b]	−1.251	0	−42	W.-d., max. suppressed

[a]With 0.004% Triton X-100. [b]With 0.002% Triton X-100.

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Short Communication

COULOMETRIC PREPARATION OF STANDARD NITROGEN DIOXIDE GAS MIXTURES BY ELECTROLYSIS OF MOLTEN NITRATE

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Standard nitrogen dioxide gas mixtures are very important for calibration purposes. The gravimetric preparation of standard gas mixtures is of fundamental importance, but the method is limited practically by both the sensitivity of the balance and the nature of the gas. The reactive nature of nitrogen dioxide makes its storage and transference troublesome, because of adsorption on, and/or reaction with, containers or connections. The permeation tube method is of practical value for the preparation of standard nitrogen dioxide mixtures, but the tube cannot be kept permanently, and there is sometimes no security that it is in good condition. Strictly speaking, there is no accurate method for the preparation of standard nitrogen dioxide gas mixtures.

In the method reported here, definite amounts of nitrogen dioxide are prepared continuously in a carrier gas by electrolysis of molten nitrate [1, 2]. The dioxide is produced at the anode and transferred to the carrier gas without dissolution in the melt. The current efficiency for the generation is practically 100% over quite a wide range of current density, if water in the melt is removed completely. Thus, standard gases containing various concentrations of nitrogen dioxide can be produced by controlling the electrolytic current and the flow rate of argon carrier gas.

Experimental

Apparatus. The apparatus for the electrogeneration of nitrogen dioxide and its absorption in 0.1 M sodium hydroxide solution is shown in Fig. 1. The generating cell, made of Pyrex glass, can be used about 10 times. The anode is a round platinum foil (2.5-cm diameter) or a coiled platinum wire, depending on the current density. The cathode is a coiled platinum wire (5 cm long). A constant current source with a vacuum tube and an electric stop-clock with a synchronous motor were used. The overall error for the measurement of electricity was within 0.5%.

Reagents. All reagents were of analytical grade. Immediately before use, sodium and potassium nitrates were dehydrated by pulverizing, heating in

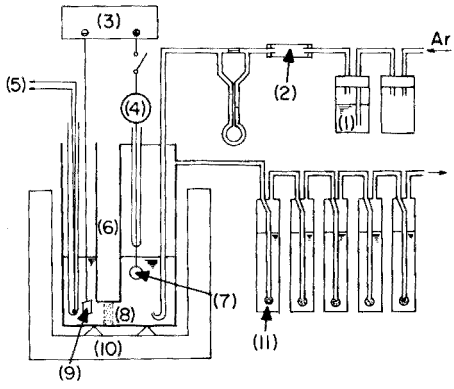

Fig. 1. Apparatus for electrogeneration and absorption of nitrogen dioxide. (1) H_2SO_4. (2) NaOH. (3) Constant-current source. (4) Milliammeter. (5) Thermocouple. (6) Pyrex glass cell. (7) Anode. (8) Fritted glass disc. (9) Cathode. (10) Heater. (11) Fritted glass bulbs.

an oven overnight (more than 10 h) at 150°C, and then dehydrating more completely in a vacuum oven for more than 3 h at the same temperature.

Procedure. The dried eutectic mixture of sodium and potassium nitrates was fused in the cathodic and anodic compartments of the cell at 250 (±1)°C under a dried argon stream (200 ml min^{-1}), and about 1 g of vacuum-dried silver nitrate was added to the catholyte. The gas was then introduced into the anolyte for about 3 h to complete the dehydration. The absorbing bottles, each containing 50 ml of 0.1 M sodium hydroxide solution, were connected as shown in Fig. 1. The constant-current electrolysis was then begun under a constant flow of argon for a definite time interval, the nitrogen dioxide produced being absorbed in the sodium hydroxide solution. After the electrolysis, the flow of argon was continued for 20 min to expel residual dioxide from the cell compartment.

The nitrite produced in the absorbing solution was determined by the ordinary sulfanilamide—*N*-1-naphthylethylenediamine dihydrochloride method [3, 4]. In some experiments, nitrate was titrated: the absorbent was acidified with sulfuric acid, nitrite was oxidized to nitrate with permanganate solution, and total nitrate was determined by the method of Kolthoff et al. [5] with molybdate as catalyst.

Results and discussion

Preliminary experiments indicated that the current efficiency for the anodic generation of nitrogen dioxide by the reaction $2NO_3^- \rightarrow 2NO_2 + O_2 + 2e^-$, was decreased appreciably by traces of water in the melt [1, 2]. Quantitative results could be obtained after the drying procedure recommended. The melt, however, contained some reducible materials as impurities, and slightly low results were sometimes obtained in the first generation with a new melt. This problem could be avoided by preliminary

electrolysis of the melt; passage of only 2—3 coulombs sufficed to remove the interference.

The effect of current density on the generation efficiency of nitrogen dioxide was first investigated, with the results shown in Table 1. The decreased efficiency for current densities below 4 mA cm^{-2} is probably due to residual water and/or impurities in the melt. The lower efficiency at the high current densities may be caused by some dissolution of the anode. Densities between 4 and 30 mA cm^{-2} were most satisfactory.

The results obtained for generation of the dioxide in the argon carrier at concentrations above 600 ppm are summarized in Table 2. In these results, the blank correction (see below) was not applied, because it was negligibly small compared with the amounts of dioxide generated. The addition of silver nitrate to the catholyte was effective in simplifying the cathodic reaction [2]; without this addition, sodium oxide deposited on the surface of the cathode and increased the cell resistance remarkably. Slightly low results were obtained for generation of the highest concentration of the dioxide at a fast flow rate of argon. This is probably caused not

TABLE 1

Relation between current density and current efficiency (Ar flow rate: 100 ml min^{-1})

Current density (mA cm^{-2})	No. of detns.	Current efficiency (%)	s_r (%)	Current density (mA cm^{-2})	No. of detns.	Current efficiency (%)	s_r (%)
0.82	1	98	—	42.2	3	99.2	1.7
2.11	4	97	2.1	55.7	2	98.5	1.1
4.12	4	99.9	2.0	121	1	97	—
10.5	8	100.0	2.1	140	1	98	—
21.1	5	100.1	2.5	167	1	97	—
29.5	8	99.4	1.8	190	1	97	—

TABLE 2

Electrolytic generation of nitrogen dioxide into an argon stream

Ar flow rate (ml min^{-1})	Current (mA)	Time of electrolysis (s)	No. of detns.	Concn of NO_2 produced (ppm)	s_r (%)	Generation efficiency (%)
200	9.11	10990	1	621[a]	—	98
200	10.00	1000	6	682	2.4	97.9
200	12.50	800	9	846	0.4	97.2
100	7.10	180	1	987	—	98
100	23.8	82.6	2	3302	0.6	99.4
100	60.0	130.4	1	8360	—	98
100	62.0	125.5	2	8635	0.04	100.4
200	429	402.0	2	16850[a]	5.6	98.8

[a]Titrimetric method was used.

by decreased current efficiency but by incomplete absorption of nitrogen dioxide in the sodium hydroxide solution. When the nitrite in each 50 ml of absorbing solution was determined separately by the spectrophotometric method, a trace amount of nitrite could sometimes be detected even in the solution in the fifth absorber when both the total amount of generated dioxide and the flow rate of argon were increased. However, the amount of nitrite in the fifth bottle was usually less than 0.5% of the total nitrite. When a sixth absorber was added, nitrite was detected in it only once in 5 experiments; even so, the amount was less than 0.3% of total nitrite, and could be neglected.

In order to obtain more dilute gas mixtures containing nitrogen dioxide, the electrolysis current was decreased, but the argon flow rate was kept

TABLE 3

Blank generation of nitrogen dioxide into argon at a flow rate of 200 ml min^{-1} without electrolysis

Time interval (min)	NO_2 found		NO_2 concn. in argon (ppm)
	(μg)	($\mu g\ h^{-1}$)	
60	28	28	1.10
120	37	19	0.75
180	56	19	0.76
240	103	26	1.04
300	116	23	0.91
	Mean	23	0.91

TABLE 4

Electrolysis generation of low concentrations of nitrogen dioxide in argon at a flow rate of 200 ml min^{-1}

Current (mA)	Time of electrolysis (s)	No. of detns.	Concn. of NO_2 produced (ppm)	s_r (%)	Generation efficiency (%)	
					Uncorrected	Corrected[a]
0.200	5000	2	13.5	0	105	98
0.500	2000	2	33.8	0.8	103	97
0.700	1429	2	48.5	1.1	105	100
1.10	910	3	75.8	0.8	104	99
2.52	400	2	178	1.0	105	102
2.50	800	2	174	1.5	103	100
2.50	2000	1	176	—	101	100
2.50	4000	2	174	5.6	100	99
5.00	2000	5	349	2.7	100.5	100.1
7.00	1428	6	483	2.0	99.4	98.7

[a]Corrected for blank generation.

constant at 200 ml min^{-1}. In this case, nitrite could not be detected in the fifth absorber. The blank generation of nitrogen dioxide from the melt caused a problem for the diluted gas; this blank may arise from decomposition of the melt and/or from impurities (hydrocarbons, etc.) in the argon carrier [6]. An example is shown in Table 3; obviously this blank value must be added to the value calculated from the electricity involved for a specified time interval.

Table 4 shows results for the generation of dilute gas mixtures. The results were quite satisfactory when the blank correction was applied, and gas mixtures down to 13 ppm could be produced. Owing to the limitations of the apparatus and of the confirmatory methods available for nitrite, more dilute gases were not investigated, but production of gases containing only a few ppm of nitrogen dioxide should be possible by modification of the apparatus.

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Short Communication

TITRIMETRIC DETERMINATION OF SULFATE IN MINERAL WATERS

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The need to analyze large numbers of water samples for sulfate has led to the development of analytical methods faster than the barium sulfate gravimetric or turbidimetric procedures. Some of these include spectrophotometric [1—3], polarographic [4, 5], and titrimetric methods [6, 7]. This communication describes a procedure that utilizes back-titration of excess of barium with standard sulfate, in presence of nitrosulfonazo-III indicator [8]. The method is faster and more precise than the turbidimetric procedure over the same range of sulfate concentration.

Experimental

Reagents and materials. Standard solutions include barium chloride (1.00×10^{-3} M) and sodium sulfate (2.60×10^{-3} M). A 1.0×10^{-4} M solution of nitrosulfonazo-III (2,7-bis-(4′-nitro-2′-sulfophenylazo)-1,8-dihydroxy-naphthalene-3,6-disulfonic acid, sodium salt; Aldrich Chemical Co.) was prepared by dissolution of the salt in doubly-distilled water. A pH meter, magnetic stirrer, micropipet, fluorescent lamp, ion-exchange resin (Dowex 50-X8, 20-50 mesh, H^+-form) and acetone are also required. Standard addition of sodium sulfate to a soil extract was utilized for method evaluation; a 25-ml aliquot of this extract was diluted to 50 ml during the preparation of the test solutions. The major components of aqueous soil extracts are listed in Table 1.

Procedure. Mix a 10.00-ml aliquot of the sample with 10 g of the ion-exchange resin. Transfer a 5.00-ml aliquot of the supernatant liquid (filter or decant, avoiding fines) to a beaker, add 5.00 ml of the $BaCl_2$ solution, and adjust the pH to 1.90. At this point, add 1.0 ml of the indicator solution and 10 ml of acetone, and titrate the resulting mixture with the standard sulfate solution. Titrate rapidly initially but slowly near the end-point to permit the development of a faint pink color in the blue solution. Determine a blank by mixing 5.00 ml of distilled water with 5.00 ml of $BaCl_2$ solution, adjusting the pH, adding the indicator and acetone, and titrating with standard sulfate.

The use of a discontinuous titration is convenient for end-point detection when more than one sample is being titrated. The use of a fluorescent lamp and comparison with a solution that has been titrated to the equivalence

TABLE 1

Major components of aqueous soil extracts

Component	Concentration	Component	Concentration
Calcium	2.53×10^{-3} M (101 mg l^{-1})	Hydrogencarbonate	5.72×10^{-4} M
Magnesium	1.28×10^{-3} M (31 mg l^{-1})	Nitrate	1.15×10^{-3} M
Sodium	6.38×10^{-4} M	Sulfate	3.35×10^{-3} M (322 mg l^{-1})
Potassium	1.37×10^{-4} M		
pH = 7.68		Specific conductance = 790 μmho cm^{-1}	

point are helpful in assigning the end-point. The sample should have a sulfate concentration range of 20—90 mg l^{-1} for best results, thus dilution of a mineral water sample may be necessary.

Results and discussion

Ground waters, surface waters and aqueous soil extracts from the coal mining area of the Northern Great Plains are often very high in sulfate (>500 mg l^{-1}) as well as other components such as sodium and calcium. The proposed method was developed to analyze these waters for sulfate.

Analysis of 32 sodium sulfate solutions which had a sulfate concentration range of 10—140 mg l^{-1} exhibited a positive bias of 2.42% with a standard deviation of 4.38%. The positive bias results from a slightly premature assignment of the end-point in the titrations, and probably reflects the magnitude of the color difference that the eye can detect.

Standard addition studies were made to evaluate the method with a natural sample. The values listed in Table 2 are for single determinations; the least-squares correlation between sulfate observed and added is 0.997, with an intercept value of 164 mg l^{-1} which is 1% above the expected amount.

The removal of calcium and magnesium by ion exchange is critical since

TABLE 2

Sulfate analysis for standard addition study

Sulfate added (mg l^{-1})	Sulfate found (mg l^{-1})	Dilution 1:*x*	Recovery (%)	Sulfate added (mg l^{-1})	Sulfate found (mg l^{-1})	Dilution 1:*x*	Recovery (%)
0[a]	162	5	100.6	217[c]	378	5	100.0
217	392	5	103.4	327[c]	487	8.33	99.8
327[b]	487	8.33	99.8	434[c]	596	10	100.2
434	579	10	97.3	545[c]	704	10	99.7
545	719	10	101.8	653[c]	813	12	99.8
635	819	10	102.9				

[a]Turbidimetric analysis gives 171 mg l^{-1}. [b]Turbidimetric analysis gives 460 mg l^{-1}. [c]These samples were treated with Dowex 50-X8 before addition of Na_2SO_4.

these ions cause a premature indicator change. At least ten samples can be analyzed per hour with results comparable to or better than those obtainable with the conventional turbidimetric procedure. The procedure is not affected by phosphate, chloride, nitrate and fluoride in amounts up to 200 mg l^{-1}, although these anions can result in slightly different indicator colors. The presence of these anions has caused problems in other titrimetric procedures [6, 7].

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Analytica Chimica Acta, 98 (1978) 181—184

Short Communication

INFLUENCE OF AMERICIUM ON PLUTONIUM DETERMINATIONS

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Normally the americium generated together with plutonium during the irradiation of uranium is eliminated by purification. But during the storage of plutonium, regeneration of americium-241 occurs by the decay of 14-y plutonium-241. Some of the plutonium currently available, produced in early high burn-up irradiations, contains up to several percent americium. The question of the influence of americium on the accuracy of plutonium determinations therefore arises. For safety and economic reasons a high accuracy is required, hence separations prior to the determination, which always include the risk of additional errors, should be avoided.

Americium may interfere on account of its mass, its redox behaviour and by radiolysis. The chemical methods actually employed for precise plutonium determination [1] can be divided into: gravimetric methods, and redox methods based on the Pu^{4+}/Pu^{3+} and Pu^{6+}/Pu^{4+} couples. Thus all possible interfering effects of americium may be classified into nine groups which result from combinations of each kind of interference with each kind of method. Some of these influences are self-evident, e.g. that of americium on the gravimetric determination of plutonium. Since the difference between the redox potentials of americium ions (1.74 V for the Am^{5+}/Am^{3+}-couple and 1.69 V for the Am^{6+}/Am^{3+}-couple) and the Pu^{4+}/Pu^{3+}-couple (0.97 V) is large enough to prevent any interference, one other group of hypothetical disturbances can be neglected. This investigation is restricted to the influence of the americium redox behaviour on the Pu^{6+}/Pu^{4+}-methods, and the radiolysis effects on all kind of redox methods.

In the methods based on the Pu^{6+}/Pu^{4+} couple, plutonium is oxidized to the hexavalent state by silver(II) oxide in a nitric acid and/or sulfuric acid medium. The excess of oxidant is destroyed either by heating [2, 3] or by addition of sulfamic acid [4]. Then the plutonium is titrated directly with iron(II) [2], or iron(II) is added in excess and the excess is titrated with cerium(IV) [3] or potassium bichromate [4]. Americium may interfere in

its higher oxidation states which may be produced [5] during the oxidation, and these would react similarly to plutonium. The only important product of radiolysis is hydrogen peroxide which can influence the oxidation, oxidize the reductant, or decrease the stability of plutonium(VI). Finally americium may be oxidized by the cerium(IV) or dichromate giving a false end-point for the titration.

In the methods exploiting the Pu^{4+}/Pu^{3+}-couple, the plutonium is reduced to the trivalent state in a Jones reductor [6, 7], by titanium(III) chloride [9] or electrically by constant potential coulometry [10]. Subsequently the plutonium is titrated by cerium(IV) [6, 8], dichromate [7] or coulometrically. Interference by americium is possible via its radiolytic product (hydrogen peroxide) or by consumption of oxidant leading to high plutonium results.

Experimental

Influence on Pu(VI)/Pu(IV) methods. A solution (5 ml) containing 3.5 mg of americium, and 1 N in both sulfuric and nitric acids, was treated with silver(II) oxide under the oxidation conditions for plutonium. After the elimination of the excess of oxide by heating, higher valencies of americium could not be detected. The same result was obtained after destruction of the excess of oxidant by sulfamic acid.

After the addition of cerium(IV) sulfate to an americium(III) solution in a molar ratio of Am/Ce = 1/10, Am(V) and Am(VI) could not be observed. In these experiments, the valency of the americium was determined in a Beckman DK-2 spectrophotometer by measuring the peaks at 503 nm (Am (III)), 715 nm (Am(V)) and 995 nm (Am(VI)). The detection limits were 20 μg, 130 μg, and 60 μg, respectively. A 3×10^{-3} M solution of americium-241, 1 M in sulfuric acid, became 1.0×10^{-2} M in hydrogen peroxide during one month. After treatment of 5 ml of this solution under the plutonium oxidation conditions with silver(II) oxide, titration with cerium(IV) solution showed no hydrogen peroxide. The limit of detection of this titration was 5×10^{-5} mmol. In a solution containing 8 mg of plutonium(VI) and 0.8 mg of americium, the rate of autoreduction over an interval of 2 days was not higher than that in a pure plutonium solution. In order to confirm these surprising results, plutonium solutions containing increasing amounts of americium were analyzed by the silver oxidation—cerimetric titration procedure [3]. The results are shown in Table 1.

Influence on Pu(IV)/Pu(III) methods. A 0.05 M hydrochloric acid solution (10 ml) containing 0.4 mmol of hydrogen peroxide were passed through a Jones reductor (15 mm i.d., bed height 70 mm, grain diameter 1 mm). The effluent did not contain hydrogen peroxide, when titrated with cerium(IV) solution as described above. In 50 ml of a solution, which was 1 N in both nitric and sulfuric acids, and contained 0.4 mmol of hydrogen peroxide, hydrogen peroxide could not be detected after addition of 1 ml of titanium-(III) chloride solution (15%) and a waiting period of 5 min (detection limit 5×10^{-5} mmol).

TABLE 1

Influence of Am on Pu titration (AgO method)

Pu added (mg)	Am added (mg)	Pu found (mg)	Difference (mg)
25.238	0.126	25.223	−0.015
25.238	0.252	25.250	−0.012
20.025	0.601	20.016	−0.009
20.025	1.00	20.019	−0.006
20.025	2.00	20.028	−0.003
20.025	4.00	20.025	±0.000

To determine the influence on the coulometric method [10], 0.04 mmol of hydrogen peroxide was added to solutions containing 19.37 mg of plutonium in sulfuric acid medium. Current integrals corresponding to 34.8 mg of plutonium were found during the reduction at 0.27 V vs. SCE. During the oxidation at 0.67 V vs. SCE, only 17.11 mg of plutonium could be detected. In both cases, the electrolysis was long and high residual currents were obtained. After pre-reduction for 3 h at 0.27 V vs. SCE to eliminate hydrogen peroxide, 19.32 mg of plutonium were found during the oxidation step. For the copper(I) chloride method [9], the authors indicate that 0.44 mmol of hydrogen peroxide has no appreciable effect.

Conclusions

In the most important titrimetric methods employed for the determination of plutonium, up to at least 10% americium (referred to the amount of plutonium) does not interfere. In the methods based on the Pu^{6+}/Pu^{4+} couple, americium is not oxidized to higher valency states during the usual silver oxide oxidation. Any hydrogen peroxide present in older solutions is destroyed during this oxidation. In the presence of 10% americium (with respect to the plutonium content), no significant rise in the autoreduction rate of the hexavalent plutonium could be observed. In titrimetric methods based on the Pu^{4+}/Pu^{3+} couple, the hydrogen peroxide is reduced during the reduction step. During the oxidation step, americium is not oxidized by an excess of oxidant. In the constant-potential coulometric method, hydrogen peroxide causes high results during the reduction and low results during the oxidation step. The interference during the oxidation can be prevented by a prolonged pre-reduction.

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Analytica Chimica Acta, 98 (1978) 185—191

Book Reviews

Peter A. Rock (Ed.), *Special Topics in Electrochemistry*, Elsevier, Amsterdam, 1977, viii + 224 pp., price $39.50, Dfl. 97.—.

Publishing symposium papers poses a problem: they are usually less exhaustive than review articles prepared primarily for publication, offer less complete coverage than textbooks, including multi-author volumes, and are in general less well-documented than either of the former. They offer two types of advantage — more personal treatment and information on work in progress. The positive and negative aspects should be carefully compared before the literature is extended by such progress reports. Decisions concerning the publication of such volumes should keep in mind the probable accessibility of such volumes. Information published therein is easily retrieved if such volumes form part of a journal (as a special issue) or part of an established series (e.g. the Delahay—Tobias series in the case of electrochemistry or the Advances in Chemistry Series). Libraries often have standing orders for such series, but publication of proceedings outside such series can result in difficult accessibility, particularly at a time of fiscal stringency in libraries.

The volume under review is based for the most part on papers presented at the Symposium entitled "Teaching of Electrochemistry" held at the ACS meeting (1976) in San Francisco. The aim is to bridge the gaps in several sub-fields of contemporary electrochemistry caused by recent rapid expansion of research efforts. This volume contains contributions on advanced electrochemical energy systems (L. R. McCoy), photovoltaic phenomena (H. Gerischer, Berlin-Dahlen), electrochemical synthesis (C. K. Mann and M. R. Asirvatham), on cells without liquid junction and selective sensors (P. A. Rock), mechanism of electrochemical oscillations (J. Kaizer), electrochemistry of nerves (W. J. Moore) and three papers on theory and applications of electron transfer at electrodes and in solution (R. A. Marcus).

As can be expected, the approach, handling and level differ widely. High accolades are deserved by H. Gerischer and R. A. Marcus; Marcus is one of the rare breed of theoreticians who can make even complex problems understandable to an average chemist. At the other end is the contribution by C. K. Mann which has a nondescriptive title, contains numerous inaccuracies and half-truths, uses unorthodox nomenclature and shows a choice of topics reflecting personal interest more than the importance of individual problems. The treatment in this chapter is very elementary. The reviewer was surprised that the chapter on electrochemistry of nerves did not have more reference to the work of R. N. Adams and others concerning chemical processes accompanying nervous activity.

It can be argued that electroanalytical problems have recently received sufficient publicity and therefore can be omitted, but the complete exclusion

of voltammetric methods from a symposium on teaching of electrochemistry is surprising. The analytical chemist will find most attractive the chapter on species-selective electrodes, even though this subject has recently been extensively treated in a number of reviews and the scope of this chapter is only slightly wider than chapters of modern textbooks. Otherwise the analyst might use the volume as a primer on some aspects of electrochemical theory.

P. Zuman

C. W. Fuller, *Electrothermal Atomization for Atomic Absorption Spectrometry*, The Chemical Society, London, 1977, viii + 127 pp., £6.75, $13.50.

Electrothermal atomizers (such as the Massmann furnace and the carbon rod) are without doubt one of the most fascinating and rapidly advancing aspects of analytical atomic spectroscopy. Their much greater sensitivity than flames has led to their widespread use for all types of samples. It is only within the last three or four years, however, that some of the more fundamental aspects of electrothermal atomization have received attention. The time is ripe, therefore, for a comprehensive account of the state-of-the-art, in both fundamental and applied aspects. The present monograph admirably supplies these requirements. Within a mere 111 pages of text, Dr. Fuller deals first with the history and design of the various electrothermal atomizers, including detailed descriptions of the commercial devices. This is followed by an exposition of atomization processes, from both thermodynamic and kinetic considerations, and by comments on the operation of the devices, sample handling and instrumental parameters (optics, gases, background correction, calibration and interferences). Finally, analytical conditions for each element are specified (in alphabetical order of the element) and applications to various types of samples are described (mainly in tabulated form). The book concludes with 372 references (some as late as 1976) and a subject index.

This book is much needed. It is well-presented, and its conciseness does not detract from its readability, clarity or the amount of information presented. In timeliness, and size, it is reminiscent of the appearance of Elwell and Gidley's monograph on atomic absorption spectrometry fifteen years ago, when the whole subject was in its infancy, and should be equally successful and influential. The information it contains will be invaluable to those engaged in applied work, and those investigating more basic problems; it clarifies those areas where knowledge is lacking and will undoubtedly inspire many further advances. Yet the text is small enough to be bought, read and understood by students, and analytical chemists working in other fields, and is heartily recommended to them (especially if it becomes available as a paperback).

A. Townshend

K. Oikawa, *Trace Analysis of Atmospheric Samples*, Kodansha, Tokyo and Halstead Press (J. Wiley), New York, 1977, price $28.60, £16.90.

Contrary to the indications given by the title, this work is not concerned with the whole range of trace analysis of atmospheric samples, but only with the analysis of suspended particulate matter, especially for metals. The introduction implies that gaseous pollutants are of secondary importance.

The book is divided into four chapters, the first of which deals, in less than 3 pages, with the physical and chemical properties, including the sources, of suspended particulates. Chapter 2 considers sampling, an extremely important aspect of atmospheric analysis. The various components of sampling equipment are discussed in reasonable detail, but certain aspects are dismissed too briefly. Sampling sites, for example, are described in seven lines, with no mention of how to carry out an air-quality survey. In relation to low-volume samplers, the orientation of the sampling head and the particle size range collected by such systems are not mentioned. Dust-fall sampling is also described and the British Standard Deposit gauge is mentioned, but without reference to the Standard itself. However, the author particularly mentions the disadvantages of glass bowls, and may not have read the Standard, which clearly describes plastic bowls. Once again, surveys and collection efficiencies in relation to particle size are not described. Chapter 3 covers many aspects of sample pretreatment, while analytical methods for metals themselves are described in Chapter 4.

On the whole, the book offers a useful collection of information on the sampling and analysis of suspended particulate matter, but must be used with care by those not familiar with the field. Errors are numerous, some factual and many seemingly arising from translation from the original text. The absence of details on surveys means that the book is not as useful as it might otherwise have been to those investigating air pollution, without specialized knowledge. Better and more comprehensive texts on this topic are available and are likely to be of more use to those concerned with air quality from a practical aspect.

R. S. Barratt

C. E. Roland Jones and Carl A. Cramers (Eds.), *Analytical Pyrolysis*, Elsevier, Amsterdam, 1977, x + 424 pp., price Dfl. 96.00, U.S. $39.25.

This book presents the texts of the papers presented at the Third International Symposium on Analytical Pyrolysis, held in Amsterdam in September 1976. The texts of 34 papers are presented, together with an Appendix which, "for reasons beyond the Editors' control", gives only the authors' abstracts of a further 15 papers which were read at the Symposium. The

wide range of topics covered includes Automation, Special Techniques, Microbiology, Forensic Science and Pharmacology, Pyrolysis Mass Spectrometry, Soil Chemistry, Biochemistry and Geochemistry, Laser Pyrolysis, Pyrolysis Reaction Mechanisms, and Polymers, which constitute the largest single section (8 contributions, dealing with paints, polystyrenes, vinyl copolymers and fluorine polymers). The contributions on "Automation of a Thermogravimetry Apparatus with a Laboratory Computer" (Dickens, Pummer and Flynn, National Bureau of Standards, Washington, D.C.) and on "An Interfaced Vapor Phase Instrumental System for Thermal Analysis and Pyrolysis" (Uden, Henderson and Lloyd, University of Massachusetts) can possibly be singled out for their merit without creating any invidious comparisons: the editors of this book consider that Pyrolysis Mass Spectrometry opens up exciting new prospects and makes the combination of pyrolysis with gas chromatography a little less inevitable.

This book has been produced by the direct reproduction of typed manuscripts: once again we have the situation where some manuscripts have reproduced acceptably and others have not; the criticism made recently in this journal (Vol. 94, p. 221) of another book is therefore renewed. All those active in this area of analytical science will wish to read this book if they were unable to attend the Symposium; there is a great deal of development still to come in this field, and the decision to hold a Fourth Symposium in Analytical Pyrolysis in Budapest in 1979 seems to be justified.

D. M. W. Anderson

L. C. Thomas and G. J. Chamberlain, *Colorimetric Chemical Analytical Methods*, 8th edn., Tintometer Ltd., Salisbury (distributed by J. Wiley, London) 1977, xli + 625 pp., price £20.00, $34.40.

This book — which is labelled 8th edition, 1974, but has a 1977 publication date — provides a weighty (2.5 kg) and expensive reminder that the Lovibond Comparator is still regarded in some circles as the epitome of analytical elegance. At a recent environmental meeting, a speaker could not say which analytical method had been used to produce some distinctly peculiar results; his technician had merely selected a colorimetric method, and he did not know the basis of the method nor what the possible interferences might be. From just such a collection of recipes as is presented in this book might the choice have been made!

Recipes are given for assays of various organic and inorganic species and for quality tests; atmospheric pollution has a special section. The procedures vary from the ancient (e.g. precipitation of calcium as its phosphate, or of potassium as the cobaltinitrite, followed by colorimetry) to the inane (e.g. sodium with manganese uranyl acetate). Little or nothing is said about

the limitations, ranges or accuracy of the methods. The entire production is given a spurious air of reliability and modernity by the introduction of lists of modern references which do not involve Lovibond apparatus.

These recipes were printed originally in loose-leaf format, so that only the recipe needed for a routine works assay with the Lovibond equipment had to be purchased. To present the recipes in book format cannot be justified on any scientific or practical basis. At a time when analytical methods are being applied more and more by people with less and less analytical training or understanding, it is dismaying that reputable publishers should lend their imprimatur to books such as this.

A. M. G. Macdonald

Gessner G. Hawley (Ed.), *The Condensed Chemical Dictionary*, 9th edn., Van Nostrand-Reinhold, New York, 1977, xiii + 957 pp., price £26.35.

The first edition of this dictionary was published in 1919 for the benefit of chemical industries. The 9th edition maintains its usefulness as a compendium of technical data. The information listed largely consists of technical descriptions of chemicals, materials and processes, definitions of chemical phenomena and terminology, and identification of trademarked chemical products. The type of information given can be illustrated by two examples: for dichlorodifluoromethane, the data listed are alternative names, formula, properties, derivation, grades, type of container for the commercial product, hazardous properties, uses and shipping regulations; for POPDA, the reader is simply referred to polyoxypropylenediamine. It is hardly surprising in a book which has existed for so long that there is a splendid disregard of accepted present-day nomenclature; the mixture of Celsius and Fahrenheit temperatures is confusing. Some of the definitions are overly simplistic: to attempt to explain what a glass electrode is and does without mentioning hydrogen ion concentration, pH or alkali metals cannot be satisfactory. Nonetheless, this dictionary is a mine of information for those requiring details of tradenames and industrial uses of chemicals.

C. W. Fuller (Ed.), *Annual Reports on Analytical Atomic Spectroscopy, reviewing 1976, Vol. 6*, The Chemical Society, London, 1977, viii + 282 pp., price £18.00, $36.00.

The relentless expansion of atomic spectroscopic techniques of analysis, and likewise the continuing exponential growth of the literature, is mirrored in this review of 1976. The number of references quoted has increased from 1573 last year to 1682 in this report (and from 1092 in 1971). The overall presentation remains as in previous years, with a limited amount of descrip-

tive text, mainly concentrated on the more fundamental aspects, and the majority of the information in tabular form, for ease of reference. The continued up-dating of the tables of commercially available instrumentation for emission and absorption measurements is valuable, and the methodology, arranged in alphabetical order of element for types of sample, provides a permanent record of applications reported in 1976. The inclusion of information divulged at lectures and discussions is a further feature which adds to the timeliness of this production.

The Editor, and the Chairman of the Editorial Board are both retiring after what must have been a very time-consuming two-year stint, but, as the Editor comments in his foreword "this encourages the continuation of an enthusiastic approach". This is certainly true, and this very worthwhile enterprise will continue to maintain its high standards.

Beat Meyer, *Sulfur, Energy and Environment*, Elsevier, Amsterdam, 1977, xii + 448 pp., price Dfl. 97.00, U.S. $39.60.

Only 14 of this book's pages are devoted to analytical chemistry; these are used to give a general introduction to analytical methods for sulphur and to the more specialized analytical literature. Although the approach and contents of this book are not strongly analytical in character, it is, nevertheless, an interdisciplinary book that offers a lot of important subsidiary information that is useful for analytical chemists, particularly those with strong environmental interests. This is also a particularly good book for students; it is written in a clear and interesting way, with a great deal of information in Tables and Figures, and some 1600 references are cited — with many citations of literature published in 1975 and 1976. The general standard of scholarship is high, and the quality of production is excellent: only the spelling of Döbereiner (spelt in a triad of ways — two of them incorrectly) could be criticized. This book is strongly recommended to libraries which cater for broad interests in environmental science.

R. S. Asquith (Ed.), *The Chemistry of Natural Protein Fibres*, Plenum Press, New York and London, 1977, xx + 417 pp., price U.S. $42.00.

This book is similar to that reviewed above in the sense that it contains a great deal of interesting information for analytical chemists without purporting to be an analytical text; the book is, of course, essential reading for all those concerned with the chemistry and applications of silk, keratins, and wool. The 10 chapters, each written by a British, German or Australian expert, cover a wide range of science and technology. The first chapter, by J. C. Fletcher and J. H. Buchanan, entitled "The Basis of Protein Chemistry",

gives an excellent account of the characterization of proteins by physical methods, of amino acid analysis, and of amino acid sequencing; this chapter alone would have been sufficient basis for this book to be recommended to students requiring a broad, general introduction to this specialized field, but, as happens all too frequently these days, the cost will restrict sales to other than the more specialized libraries.

Analytica Chimica Acta, 98 (1978) 192

Announcements

5th Symposium on Recent Developments in Activation Analysis

St. Catherine's College, Oxford, 17th—21st July, 1978

The increase in interest over recent years in analytical techniques exploiting sample irradiation with neutrons, charged-particles or γ-photons has resulted in investigations being carried out by scientists of various disciplines — analysts, biologists, engineers, metallurgists, physicists, etc. Contact between the several disciplines has not always been sufficiently good to permit developments in one field to be exploited by investigators in another and opportunities are clearly needed for workers in the various fields to meet and discuss topics of mutual interest. The 5th Symposium on Recent Developments in Activation Analysis, which is organised in conjunction with the Central Bureau for Nuclear Measurements of the Joint Research Centre of the European Communities, is intended to provide such an opportunity. The informal nature of previous Symposia will be preserved to promote a free exchange of views between scientists with similar interests. As before, the subject matter of the Symposium will not be limited to experimental methods which demand measurement of induced radioactivity; techniques such as elastic scattering, neutron capture and other methods based on counting prompt radiation such as x-rays or particles emitted as a result of nuclear reaction processes will also be considered.

The technical programme of the Symposium will be designed to cover practical and theoretical aspects of analytical techniques exploiting sample irradiation with neutrons, charged-particles and γ-photons.

Further details can be obtained from: Mr. C. H. Gill (Symposium Secretary), Marconi Elliott Avionic Systems Ltd., Neutron Division, Elstree Way, Borehamwood, Herts WD6 1RX, England;
or from Dr. J. Pauwels, Central Bureau for Nuclear Measurements, Steenweg naar Retie, B-2440 Geel, Belgium.

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Short Communications

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CONTENTS

(continued on inside of cover)