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Methods of Surface Analysis

edited by **A.W. CZANDERNA,** Professor of Physics, Department of Physics and Institute of Colloid and Surface Science, Clarkson College of Technology, Potsdam, N.Y., U.S.A.

METHODS AND PHENOMENA, Volume 1

1975. 496 pages. US $62.50/Dfl. 150.00. ISBN 0-444-41344-8

This book elucidates the methods and procedures used to obtain the elemental composition of surfaces and of the underlying bulk and to identify species attached to a surface. The composition-in-depth profile, which depends on using ion etching with surface analysis, is also treated.
First, the effect of sputtering on the results obtained by the most widely used methods of surface analysis is considered. Then an overview is presented of the strengths and weaknesses of the various methods as related to the multiplicity of important parameters such as the influence of the sample on the results, detection sensitivity, resolution, matrix effects, signal to noise, time to obtain the data, the potential for quantitative analysis, etc. The following chapters present details on ion scattering (ISS), X-ray photoelectron spectroscopy (ESCA), Auger spectroscopy (AES), secondary ion mass spectrometry (SIMS), AES combined with SIMS, the atom probe field ion microscope, field ionization mass spectrometry and infrared reflection-absorption spectroscopy. Although the emphasis is on experimental methods and procedures, a generous presentation of typical results and applications is also given.
The book not only provides an overview to prospective scientists and technologists who plan to use surface analytical techniques, but also presents refreshing ideas to active workers in the fields of physics, chemistry, materials, electronic devices, engineering science, catalysis and corrosion. The volume is particularly timely since instruments for SIMS, XPS, AES, ISS and the atom probe have become commercially available in recent years and the application of these methods is expanding rapidly.

CONTENTS: The aspects of sputtering in surface analysis methods (G.K. Wehner). A comparison of the methods of surface analysis and their applications (D. Lichtman). Low energy ion scattering spectrometry (T.M. Buck). Surface analysis by x-ray photoelectron spectroscopy (W.M. Riggs and M.J. Parker). Auger electron spectroscopy (A. Joshi, L.E. Davis and R.W. Palmberg). Secondary ion mass spectrometry (J.A. McHugh). The use of Auger electron spectroscopy and secondary ion mass spectrometry in the microelectronic technology (J.M. Morabito and R.K. Lewis). The atom-probe field ion microscope (E.W. Müller). Field ion mass spectrometry applied to surface investigations (J.H. Block and A.W. Czanderna). Infrared reflection-absorption spectroscopy (H.G. Tompkins).

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Vibrational Spectra and Structure

A SERIES OF ADVANCES, VOL. 4

edited by **JAMES R. DURIG,** Department of Chemistry, University of South Carolina, Columbia, South Carolina

1975. 316 pages. US $31.25/Dfl. 75.00. ISBN 0-444-41380-4

One of the greatest needs of science today is for competent people to critically review the recent literature in conveniently small areas and to evaluate the real progress that has been made, as well as to suggest fruitful avenues for future work. The current volume, number 4 in a series now published by Elsevier Scientific Publishing Company, attempts to fulfill these goals. Chapter 1 reports on the complementary infrared and Raman matrix isolation studies of similar chemical systems, and the usefulness of the newer laser-Raman technique is demonstrated relative to the well-established infrared matrix methods. Recent studies on the vibrational spectra and structure of plastic crystals are reviewed in Chapter 2, while Chapter 3 describes methods and applications of intramolecular force field calculations. Chapter 4 continues the theme of Chapter 1 with an example of the valuable use of infrared and Raman matrix isolation techniques. This section reviews the characterization of the products of metal atom-molecule cocondensation reactions studied by these methods. The techniques described should provide the inorganic and organometallic chemist with new chemical pathways for the synthesis and stabilization of chemical species which would have been difficult if not impossible to prepare and study by conventional chemical procedures. Physical chemists, spectroscopists, physicists, and other research scientists who use vibrational spectroscopy in their work should find this volume of immense value.

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Pharmaceutical applications of Thin-Layer and Paper chromatography

edited by **KAREL MACEK,** *Medical Faculty, Charles University, Prague*

1972, xvi + 744 pages, Dfl. 250.00
ISBN 0-444-40939-4

Chromatography is the most widely used modern procedure in analytical chemistry today. With an increasing awareness of the importance of the applications of paper and thin-layer chromatography in the fields of pharmaceutical research, production and control, it has become necessary to survey the possibilities of these methods with their associated literature, and to present this information in a useful form.
With this in mind, the editor provides an introduction to the techniques, the evaluation, and the applications of paper and thin-layer chromatography. Selected procedures such as the preparation of samples, detection methods, choice of solvent systems and sorbents, and the principles of quantitative analysis, are discussed generally and the book is richly tabulated. An appendix outlines the preparation of more than 150 detection reagents. On the basis of these data, readers with an understanding of the principles of the techniques described can solve any analytical problems they may encounter in drug analysis.

CONTENTS: Introduction. Techniques of paper and thin-layer chromatography. Radioactive compounds. Combination of TLC and PC with other chromatographic techniques. Combination of PC and TLC with some spectroscopic methods. Identification of organic compounds by PC and TLC. Documentation of chromatograms. Laboratory for PC and TLC. The tasks of paper and thin-layer chromatography. Synthetic drugs. Steroids. Cardiac glycosides and their genins. Saponins. Peptide and protein hormones. Alkaloids. Vitamins. Antibiotics. Plant extracts. Auxiliary compounds. Investigation of the fate of drugs. Detection reagents. Author index. Subject index. List of substances chromatographed.

CONTRIBUTORS: V. Betina, J. Davídek, I. M. Hais, K. Hiller, J. Janák, G. Katsui, B. P. Lisboa, M. Luckner, K. Macek, L. Nover, V. Rábek, G. Székely, H. D. Woitke.

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Analytica Chimica Acta, 80 (1975) 209—214

A NOVEL ENZYME ELECTRODE METHOD FOR THE DETERMINATION OF NITRITE BASED ON NITRITE REDUCTASE

CHIH-HEN KIANG, S. S. KUAN and G. G. GUILBAULT

Department of Chemistry, University of New Orleans, New Orleans, Louisiana 70122 (U.S.A.)

(Received 19th June 1975)

SUMMARY

The enzyme, nitrite reductase, can be extracted and purified from spinach leaves; the freeze-dried preparation is completely stable for at least 4 months if kept in a freezer. The enzyme catalyzes the reduction of nitrite to ammonia in the presence of reduced methyl viologen as electron donor. An assay of nitrite can be based on the measurement of the ammonia formation, with an air-gap electrode as sensor. Nitrite in the 10^{-4} M—$5 \cdot 10^{-2}$ M range can be accurately determined with either soluble or immobilized enzyme, but the latter is stable for at least 3 weeks, is less susceptible to interferences during assay, and can be used repeatedly for about a hundred runs. These advantages make the method very simple, valuable and economical for the routine analysis of nitrite ion.

The toxicity of nitrite to the human body and animals has been studied frequently. Nitrite is usually determined colorimetrically after reaction with various coupling reagents. A chromatographic separation of nitrite and/or its derivatives, followed by electrochemical [1] or spectroscopic [2] detection, has been recently developed. However, most of these methods are subject to interference from turbidity and color or from diverse ions present in the samples. Therefore, the development of a new method without these interferences is desirable.

Recent uses of enzymes as analytical reagents have clearly demonstrated that this is a powerful tool in analytical chemistry because of unmatched advantages of specificity and sensitivity. For this reason, an attempt was made in this laboratory to determine nitrite through an enzymatic approach. The nitrite was first selectively and quantitatively reduced to ammonium ion by nitrite reductase, partially purified from spinach leaves, in the presence of methyl viologen as the electron donor [3]

$$NO_2^- + 6\,e^- + 8\,H^+ \xrightarrow[\text{Reductase}]{\text{Nitrite}} NH_4^+ + 2\,H_2O$$

The ammonium ion generated in the reaction was sensed by a newly developed air-gap electrode [4, 5] which has the unique advantage of freedom from any interference in the reaction solution. In this way a totally specific and sensitive method was developed for the assay of nitrite.

EXPERIMENTAL

Materials

Fresh spinach leaves of spinacea oleracea were obtained from a local market. The midribs and petioles of leave tissues were removed before use.

Methyl viologen (Sigma Chemical Co., St. Louis, Mo.) was dissolved in 0.75 M Tris buffer, pH 7.0, to give a final concentration of 0.015 M.

The electrolyte solution was a $5 \cdot 10^{-3}$ M NH_4Cl—10^{-2} M NaCl mixture saturated with wetting agent (Victawet 12, Stauffer Chemical Co., U.S.A.).

The following chemicals were used without further purification: bovine albumin (Sigma Chemical Co., St. Louis, Mo.); DEAE-Cellulose (General Biochemicals, Chagrin Falls, Ohio); Sephadex G-25 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.); glutaraldehyde (Sigma Chemical Co., St. Louis, Mo.).

All other chemicals were of analytical grade.

Apparatus

The air-gap electrode used in all studies was the same as described by Hansen and Růžička [5]. A digital pH meter (Corning digital 110) and a recorder (Heath Model Eu-205-11) were used with the electrode.

Procedures

Purification of enzyme

For analytical purposes, the enzyme was only partially purified up to the DEAE-Cellulose chromatography step. The procedure was carried out essentially the same as described by Ho and Tamura [6] except that 0.01 M phosphate buffer, pH 8.0, was used instead of Tris buffer. The main nitrite reductase fractions eluted from the DEAE column were combined and dialyzed against this phosphate buffer overnight. The dialyzed extract was then applied to a Sephadex G-25 column previously equilibrated with the 0.01 M phosphate buffer for the removal of NH_4^+ contaminant. Finally, the enzyme solution was freeze-dried and stored in a freezer until use. All fractionation procedures were conducted at 0—5° C unless otherwise indicated. In the final preparation approximately 400 U were obtained from 2 kg of fresh spinach leaves.

Immobilization of enzyme

Nitrite reductase was immobilized according to a modified method reported by Brown et al. [7]. A total of 160 mg of nitrite reductase and 100 mg of bovine serum albumin were dissolved in 4 ml of 0.1 M phosphate buffer, pH 8.0. Four drops of glutaraldehyde were then added to the solution with stirring for 2—3 min. The solution was then frozen by dry ice—acetone coolant and thawed slowly in a refrigerator overnight. The sponge-like copolymer was washed with the same buffer and stored in a refrigerator until use.

Enzyme assays

Nitrite reductase activity was assayed by the method described by Ho and Tamura [6].

Other assays

Protein was determined by the method of Lowry et al. [8]; bovine serum albumin was used as reference. Nitrite was assayed by the method of Snell and Snell [9].

Measurement of nitrite with soluble enzyme

Tris buffer (50 μl of 0.75 M) containing 0.75 μmole of methyl viologen and 3 U of enzyme was pipetted into a sample chamber, followed by 200 μl of a nitrite standard solution. Sodium hydrogencarbonate solution (50 μl of 0.29 M) containing 3.75 mg of sodium dithionite was finally added; the dithionite solution was prepared immediately before use. The sample chamber was put in a water bath (30 °C), incubated for 5 min, and then placed on a magnetic stirrer preset at a desired speed. Sodium hydroxide solution (100 μl of 0.5 *M*) was added and the chamber was immediately covered with the electrode body. The potential change was recorded until a steady state was reached. The pH_e was read directly from the digital pH meter and was plotted versus nitrite concentration to give the standard calibration plot.

Measurement of nitrite with immobilized enzyme

Immobilized enzyme (ca. 4 U) was placed in a 10-ml beaker, followed by 250 μl of nitrite standard solution, 75 μl of 0.75 M tris buffer containing 1.125 μmole of methyl viologen, and 75 μl of the freshly prepared 0.29 M sodium hydrogencarbonate containing 5.125 mg of sodium dithionite. The beaker was then placed in a water bath (30 °C) and incubated for 5 min. The sample was agitated at moderate speed by a stirring bar during incubation. After incubation, the sample was filtered through a Gooch crucible under suction. From the filtrate, 300 μl of reaction mixture were transferred to a clean sample chamber, 100 μl of 0.5 M sodium hydroxide was added, the chamber was imediately closed with the electrode body, and the ammonia was measured as described above. The immobilized enzyme was washed with distilled water and filtered under suction several times, and was then ready for the next assay.

RESULTS AND DISCUSSION

As ammonia is volatilized from solution according to Henry's Law and diffuses to the electrode surface, it reacts with the ammonium ion present at the electrode surface with a resulting decrease in the hydrogen ion concentration. The equilibrium pH of the electrode, pH_e, at constant $[NH_4^+]$ at the electrode surface and at a constant pH of the sample should be proportional to the $\log[NH_4^+]$ of the sample

$$pH_e = \log[NH_4^+]_{sample} + \text{constant}$$

Hansen and Růžička [6] have presented an excellent discussion of the theoretical and practical limits of detection as a function of pH, $[NH_4^+]_s$ and percent conversion of ammonium ion to ammonia. In this study, several steps were adopted in order to obtain better response and higher sensitivity: (1) since the enzyme has a pH optimum between 7.1—7.8, the catalytic reaction was carried out at pH 7.0 to avoid any loss of ammonia during incubation; (2) for better and faster electrode response, the solution was adjusted to pH 10.5 to obtain quantitative conversion of ammonium to ammonia; (3) to extend the detection limit, the sample was diluted with reagents no more than two-fold of its original volume (200 μl to 400 μl); (4) the pH_e observed varied quite drastically with the change of stirring rate, hence the stirrer was preadjusted to a moderate speed so that all samples were stirred under almost identical conditions. Although the thickness of the electrode layer has no essential effect on the final pH_e value, it does affect the response time. This effect became especially apparent at low sample concentration ranges (10^{-4} M—$5 \cdot 10^{-4}$ M) where poor response time was obtained (> 7 min) if the layer was too thick. The temperature was found to have no essential effect on the response time and detection range, as reported by Hsiung [10]. Therefore, all measurements were conducted at room temperature.

Typical response curves are shown in Fig. 1, and standard curves plotted

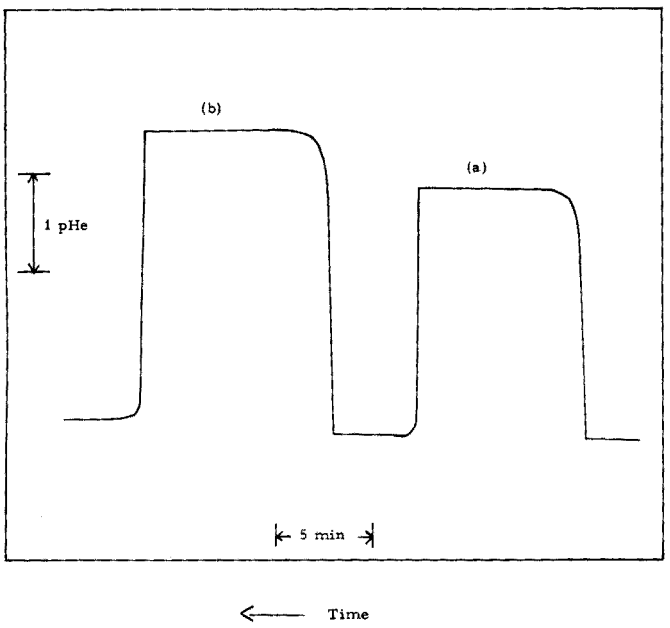

Fig. 1. Typical response curves of air-gap electrode to nitrite ion: (a) $1 \cdot 10^{-3}$ M; (b) $5 \cdot 10^{-3}$ M.

as pH_e vs. $\log[NO_2^-]$ for the soluble and immobilized enzyme methods are indicated in Fig. 2.(a, b). Each point plotted was the average of three measurements. A linear relationship between 10^{-4} M and $5 \cdot 10^{-2}$ M was obtained from both methods. The slope of the linear section was 1.10 for assays based on the soluble enzyme and 1.04 for the immobilized enzyme, which is close to the theoretical value of 1.00. The curve leveled off at higher concentration, possibly because of oxidation of dithionite by molecular oxygen before the completion of the enzymatic reaction. The levelling off observed at low concentrations was due to the detection limit of the electrode sensor used.

Under the same experimental conditions, the reproducibility during assay depends mainly on the stability of the enzyme. Nitrite reductase is quite stable if stored in solutions of high ionic strength. The lyophilized powder was found to be even more stable. No loss of activity was observed for at least four months. Another advantage of lyophilized enzyme is its quick availability for use without the necessity of thawing, which was found to deactivate the enzyme. The slope of the linear section was found to be quite reproducible. For thirty assays, deviations in the slope were only 1.10 ± 0.02. The excellent stability and reproducibility provided by this method offers an economical, convenient and specific technique for routine assay of nitrite. When 200 μl of 0.01 M nitrite was assayed with the

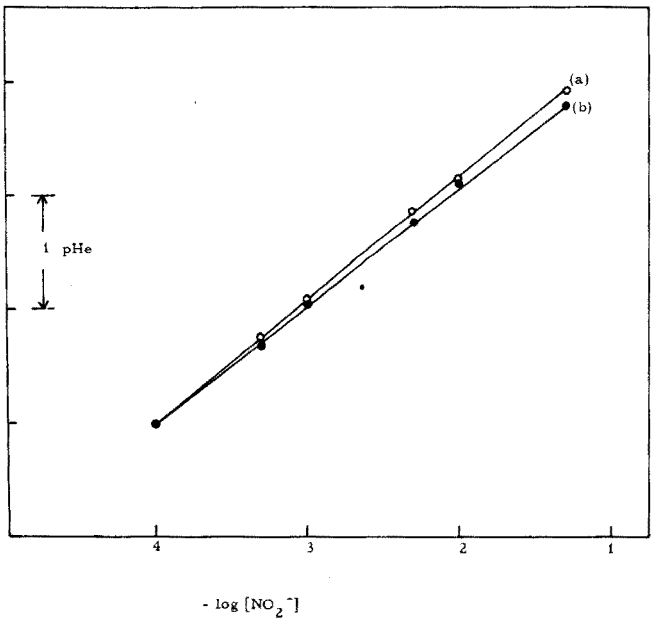

Fig. 2. Calibration curve for nitrite. (a) Soluble enzyme method. (b) Immobilized enzyme method.

immobilized nitrite reductase, consistent pH_e values were always obtained for the first 40 runs; thereafter, the activity of the enzyme decreased gradually to half its initial activity at the end of ca. 100 assays. Thus, a longer incubation time is required for the complete conversion of nitrite at high concentrations.

In order to check the efficiency of ammonia formation from nitrite by enzymatic reduction, the same amount of ammonium chloride was used under the same assay conditions instead of standard nitrite solutions. The results obtained for both nitrite and ammonium ions at different concentrations, from $5 \cdot 10^{-4}$ M to 10^{-2} M were found to be superimposable on each other. It is obvious that more than 99 % conversion of nitrite was achieved.

In an interference study, 100 μl of 0.02 M solutions of sulfite, sulfate, nitrate and perchlorate ions were added to the reaction mixture containing 100 μl of 0.01 M nitrite, and the pH_e was monitored. The results obtained are shown in Table 1. The excellent agreements of all the final pH_e readings indicated that interferences caused by these anions are negligible.

TABLE 1

Interference Test of Anions
(Overall concentrations were 0.0025 M nitrite and 0.005 M diverse anion.)

Anion	—	SO_3^{2-}	SO_4^{2-}	NO_3^-	ClO_4^-
pH_e	8 · 18	8 · 19	8 · 16	8 · 20	8 · 16

The data obtained with nitrite reductase suggest that the use of the air-gap electrode with the immobilized nitrite reductase will be a useful tool for the rapid, selective, and economical analysis of nitrite at micro-levels.

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Analytica Chimica Acta, 80 (1975) 215—221

DETERMINATION OF NITRATE IN PICKLING BATHS WITH A NITRATE-SELECTIVE ELECTRODE AND A STANDARD ADDITION PROCEDURE

JAN OLA BURMAN*
Avesta Jernverks AB, 774 01 Avesta (Sweden)
GILLIS JOHANSSON
Department of Analytical Chemistry, University of Umeå, 901 87 Umeå (Sweden)
(Received 17th March 1975)

SUMMARY

A reverse addition method for the determination of nitrate in pickling baths is described. Calibration solutions of nitric acid were added to the standard solution so that the slope and intercept of the electrode response function could be determined. The standard deviation of the method was better than 1 %. Nitrate in pickling baths containing hydrofluoric and nitric acids was measured and compared to measured hydrogen ion concentrations as a function of running time for the bath. The effects of iron(III) and hydrofluoric acid on the results were studied.

A strict control of the composition of pickling baths would result in considerable savings of acids and in a better quality of the finished product. The amount of pollutant generated can also be reduced by selection of suitable pickling conditions and this reduces the operating cost of subsequent water and air purification. The present study is concerned with the nitrate concentration in stainless steel pickling baths containing nitric and hydrofluoric acids. A nitrate method is necessary in order to decide on the following points: (i) if the hydrogen ion or the nitrate ion is consumed more rapidly; (ii) if the nitrate concentration is of importance for the pickling; (iii) if there is any correlation between the consumption of nitrate and the hydrofluoric acid concentration; and (iv) what is the mechanism of the pickling.

EXPERIMENTAL

A nitrate-selective electrode (Orion 92-07) and a reference electrode (Orion 90-01) filled with 4 M KCl were connected to an Orion 701 digital voltmeter.

The hydrogen ion concentration in the solutions was determined with an

*Present address: Norbottens Järnverk AB, Koksverk 75, 951 01 Luleå, Sweden.

Aveplex electrode [1] and the concentration of hydrofluoric acid was evaluated from the potentials of both the Aveplex electrode and a fluoride-selective electrode (Orion 94-09) as described earlier by Eriksson [2] and Eriksson and Lunner [3]. The total amount of dissolved iron was determined by atomic absorption spectrometry.

A reverse standard addition method (sample addition method) was used. Samples of 0.50 ml were added by a Medical Laboratory Automation Pipette with disposable plastic tip to 50.00 ml of standard solution. The standard solution contained 0.005 M KNO_3, 0.5 M Na_2H_2Y (EDTA) and about 0.25 M ammonia; ammonia solution was added until the pH was 8.3. The standard solutions were dispensed from a 50-ml motor burette (Metrohm Multi-Dosimat, E 415).

In order to test and calibrate the method, calibration solutions of 0.5, 1, 2, 3, 4 and 5 M nitric acid were prepared. These were added with an MLA pipette to the standard solutions in the same way as the samples.

The procedure employed for analysis of pickling baths started with a determination of the slope of the electrode from calibration solutions. The starting potential in a dispensed standard was measured (after 60 s), 0.5 ml of calibration solution was added, and the potential was noted (after 20 s); two more additions were made in the same way. This was repeated with two other calibration solutions containing higher concentrations of nitric acid. Samples from pickling baths were then measured in the same way as described for the calibration solutions. Every fifth sample was a calibration solution. The series was finished with a redetermination of the slope. The result was entered into a computer; the Fortran program JON was used.

Sample addition method

The standard addition method for electrodes has been developed by various workers [4—11]. It is necessary to know the volume of the sample and the standard as well as the slope of the calibration curve for the electrode. This slope has been taken as equal to the theoretical slope [8] measured separately [7] or calculated from a double addition method [8]. In the first and last cases, the accuracy is low. Brand and Rechnitz [9] obtained more accurate results by a multiple addition method. For a liquid-membrane electrode the slope may vary with time; the method was therefore modified so that the calibration could be obtained under identical timing conditions as the measurements on the samples.

In choosing a method, sample addition was preferred as the measurements could be made at almost constant ionic strength. In the present case a more favourable part of the electrode range could also be utilized.

For the calculations, the equation [11] used was

$$C_x = C_s \left[10^{\Delta E/S} \cdot \left(1 + \frac{V_s}{V_x} \right) - \frac{V_s}{V_x} \right] \qquad (1)$$

where C_x is the nitrate concentration of the unknown sample, C_s the nitrate concentration of the standard solution (0.005 M); V_s is the volume of the standard solution (50 ml) and V_x the volume of the sample (0.50 ml); S is the slope of the electrode. If E_0 is the potential measured in the standard solution and E_1 is the potential in the solution after one addition then $\Delta E = E_1 - E_0$. For subsequent additions V_x was multiplied by 2 or 3 and $\Delta E = E_n - E_0$.

A calibration solution of, say, 1 M HNO_3 was added in increments of V_x to the standard solution and ΔE was measured; C_x is known and it is therefore possible to solve for S. In order to obtain a better value the procedure was repeated for two or more standard solutions with higher or lower concentration.

Equation (1) was transformed to

$$E = S \log \left[\left(\frac{C_x}{C_s} + \frac{V_s}{V_x} \right) \Big/ \left(1 + \frac{V_s}{V_x} \right) \right] + \delta \qquad (2)$$

which is of the form $y = S \cdot x + \delta$, where δ is the intercept on the E-axis when a straight line is fitted to the experimental points; δ should be close to zero.

A value S_1 can be calculated from the first measurements in the different calibration solutions by a least-squares procedure. For the second and third additions in the respective solutions, values of S_2 and S_3 can be calculated with two more linear regressions. When a sample is analyzed, the value S_1 will be used for the first sample addition, S_2 for the second, etc. The S-values are inserted into eqn. (2) and C_x is evaluated. If the calibration solutions are selected to cover the expected concentration range for the samples, a better value is obtained for S by this procedure than by repeating the measurements with the same calibration solution. In the latter case, δ must be assumed to be zero.

RESULTS

Electrode evaluation

Table 1 shows the slopes calculated for four series of nitric acid solutions by the calibration procedure described above. It can be seen that the slopes decrease somewhat for the second and third additions compared to the first. This observation forms the basis for the separate slope calculations mentioned. The variations between the series include some temperature changes as well as electrode changes. The slope obtained in this medium may be more than 1 mV/decade lower than a slope calculated from a calibration curve in unbuffered solution; to obtain an accurate result it is essential to determine the slope in the same medium.

In order to determine the precision of the method, solutions of known nitrate concentration were analyzed. The results (Table 2) show that there

TABLE 1

Slope of the nitrate selective electrode (mV/decade)

Series	1st addition	2nd addition	3rd addition
A	56.4	56.4	56.3
B	57.1	56.9	56.7
C	57.0	56.9	56.8
D	57.0	56.8	56.7

TABLE 2

Precision of the standard addition method

Series	Taken	Found					
		1st addn.	*S*	2nd addn.	*S*	3rd addn.	*S*
	M	M	%	M	%	M	%
A	0.500	0.499	0.8	0.499	0.8	0.502	0.8
B	1.000	0.999	0.6	1.001	0.4	1.004	0.6
C	2.000	1.999	0.7	1.995	0.6	1.991	0.7
D	3.000	3.017	0.6	3.030	0.6	3.053	0.6
E	4.000	4.005	0.5	3.996	0.6	3.992	0.8
F	5.000	4.963	0.6	4.923	0.6	4.88	0.7

is no significant difference in precision between the first, second and third additions. The results given in Table 2 were obtained on different occasions; different slopes were calculated and used on each occasion. The relative standard deviation is significantly better than 1 %, which is surprisingly good for a liquid membrane electrode. The potentials E_0 measured in the standard solutions had a good constancy throughout a series. For one of the series the difference between the highest and lowest value was 0.8 mV for 19 values. The total potential change for a standard addition was normally 25—50 mV for the first addition.

Electrode drift was less significant in a buffered standard solution than in an unbuffered solution. The difference between readings made 1 min and 2 min after immersion was 0.5 mV in the buffer and 1.3 mV in the unbuffered solution; in the buffered solutions a reasonably stable reading was obtained after only 10—15 s. As there is no true equilibrium in a liquid ion-exchanger membrane system, drift must be expected; if it remains essentially constant a procedure with a strict time sequence, equal for calibration and standard, should give good results, and this was found to be the case.

A similar evaluation was also made on solutions interspersed between pickling bath samples. The result was the same although the standard deviations were larger, around or slightly below 1 %.

In order to test the accuracy of the measurements in pickling baths, some test solutions containing iron(III) and hydrofluoric acid were prepared. All solutions were made 1 M with respect to nitric acid. The nitrate was determined by the procedure described, three measurements being made on each solution. Table 3 shows the mean of the concentrations found; it can be seen that the standard deviation is less than 1 % for the first nitric acid solution in each series, but is significantly higher on the test solutions and also on the nitric acid solution finishing each series. The added components may affect the equilibrium in the electrode membrane but still the overall result seems to be correct within 5 or 6 %, The effects of iron(III) and hydrofluoric acid seem to cancel each other to some extent.

Measurements on pickling baths

Figure 1 shows measurements of the hydrogen ion and nitrate concentrations in samples from a pickling bath for stainless steel. The concentration is plotted against the running time of the bath. In a fresh bath the hydrogen ion concentration should be equal to the nitrate concentration, as nitric acid almost completely suppresses the dissociation of hydrofluoric acid; this was found to be the case. Hydrogen ions are then consumed at a greater rate (0.08 M h^{-1}) than nitrate ions (0.03 M h^{-1}). The main process in this tank is dissolution of a chromium-deficient layer on the steel. Two additions of more acids to the bath are shown. In another tank in which

TABLE 3

Nitrate determination in some test solutions (Results have been normalized to a nitrate concentration of 1.000 M HNO_3 taken)

Test solution	NO_3^- Found M	σ
1 M HNO_3	0.997	0.4
1 M HNO_3 + 0.2 M $FeCl_3$	0.986	0.5
1 M HNO_3 + 0.5 M $FeCl_3$	0.963	1.2
1 M HNO_3 + 1 M $FeCl_3$	0.974	1.4
1 M HNO_3	1.006	0.9
1 M HNO_3	1.002	0.9
1 M HNO_3 + 0.2 M HF	1.056	1.6
1 M HNO_3 + 0.5 M HF	1.039	1.5
1 M HNO_3 + 1 M HF	1.057	1.7
1 M HNO_3	1.000	1.9
1 M HNO_3	1.003	0.6
1 M HNO_3 + 0.2 M $FeCl_3$ + 0.6 M HF	1.043	1.0
1 M HNO_3 + 0.5 M $FeCl_3$ + 1.5 M HF	1.028	1.3
1 M HNO_3 + 1 M $FeCl_3$ + 3 M HF	1.004	2.0
1 M HNO_3	0.996	1.3

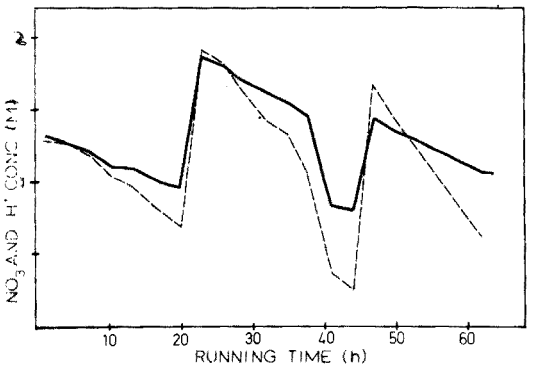

Fig. 1. Nitrate (—) and hydrogen (- - -) ion concentration in a pickling bath for stainless steel. Additions of nitric and hydrofluoric acids were made after 20 and 44 h.

etching of steel was the main process, the rates of consumption were almost equal. The electroneutrality condition is of course balanced by metal ions and complex ions such as FeF^{2+} and FeF_2^+. Nitric oxides also form and are vented to the air. Preliminary investigations show that the method described here can be used for obtaining extra information about the pickling process.

DISCUSSION

The sample addition method described here is capable of good accuracy even when the electrode has a constant drift. The procedure is well suited to routine applications, especially when used with a program for computer evaluation of the result. The change in slope between additions (Table 1) is smaller than the systematic errors which may arise (Table 2). It may be thought that the procedure described by Brand and Rechnitz [9], in which the slope is evaluated from multiple sample additions, would give more accurate results on real samples. This is not the case, however, as the standard deviation also increases. Moreover, it should be noted that the concentrations given in Table 3 are the means from three additions; the first addition produces the most accurate result and the third shows the largest systematic error.

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THE SURFACE MORPHOLOGY OF ION-SELECTIVE MEMBRANE ELECTRODES
PART I. STUDIES ON SILVER IODIDE-BASED SILICONE RUBBER MEMBRANE ELECTRODES

H. MALISSA and M. GRASSERBAUER
Institute for Analytical Chemistry, Technical University, Wien (Austria)
E. PUNGOR, K. TÓTH, M. K. PÁPAY and L. PÓLOS
Institute for General and Analytical Chemistry, Technical University, Budapest (Hungary)
(Received 8th July 1975)

SUMMARY

Electron micrographs, and micrographs of electron absorption and silver and iodine distribution on the surfaces and cross-sections of silver iodide-based silicone-rubber membranes showing Nernstian electrode response are presented. An electron microprobe analyzer was used. The electrochemical effects of concentrated (> 0.6 M) potassium iodide solutions and iron(III) hydroxide or iron(III) ions, are described. The phenomena are interpreted on the basis of the results obtained by electrochemical, atomic absorption and electron microprobe analysis.

The electrochemical behaviour (potential response) of ion-selective membrane electrodes is now interpreted more and more frequently on the basis of reactions taking place at the surface of the electrode membranes. This interpretation seems to be justified by the results of response time measurements and also by the so-called memory effects observed with the electrodes. The potential response of electrodes caused by a steep concentration gradient at the electrode surface is much slower with precipitate-based silicone-rubber electrodes than with homogeneous electrodes prepared from the same precipitate [1]. The inhomogeneity of the electrode surface also affects the response times and memory phenomena of the electrodes. Furthermore, in accordance with the statements made by Sorrentino and Rechnitz [2] about the potential response of silver iodide-based electrodes, we have found that the response of the electrodes ceases completely after the membranes have been soaked in potassium iodide solution of a concentration exceeding $6 \cdot 10^{-1}$ M. In order to interpret the effect of concentrated potassium iodide solutions, and the relatively slow responses and memory effects of silicone-rubber membrane electrodes, investigations on the surface-morphology of electrode membranes have been initiated. In the present paper the results of surface-morphological and structural studies, as well as electrochemical measurements, on silver iodide electrodes are presented.

EXPERIMENTAL

Apparatus and reagents

E.m.f. measurements were made with a Radelkis Type OP-205 precision pH meter.

Morphological studies were done with an International Scientific Instruments Type MSM-2 electron microscope and an Applied Research Laboratories Type EMX-SM electron microprobe analyzer. A Unicam SP-90B atomic absorption spectrophotometer was used for the determination of potassium in solutions.

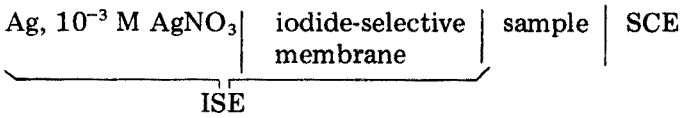
All chemicals used were of analytical grade.

Electrodes

E.m.f. measurements and morphological studies were made on silver iodide-based silicone-rubber membranes. The membranes were prepared by mixing 70 wt. % silver iodide with 30 wt. % dimethylpolysiloxane (Silopren K1000, Farbenfabrik Bayer) and polymerizing at room temperature with cross-linking agent and catalyst [3]. Discs cut from the membrane layer were sealed onto the end of glass or plastic tubes and were used as electrodes, with a suitable internal solution and reference electrode (10^{-3} M silver nitrate and silver wire). In e.m.f. measurements a Metrohm SCE was used as reference electrode.

E.m.f. measurements

All measurements were made at 25 °C in the following cell



Electrochemical investigation of the effect of potassium iodide.

Electrodes exhibiting good electrochemical properties, i.e. giving Nernstian response, were soaked either in 0.1 or 1 M potassium iodide solutions for 24 h. The electrochemical function was tested repeatedly after washing with distilled water, and then the damaged electrodes were selected. Electrodes whose response ceased, were regenerated by soaking in distilled water or in 0.1 M potassium chloride or in 0.1 M lithium chloride.

Electrochemical investigation of the effect of iron(III) hydroxide

Electrochemically sound electrodes were placed in 0.001, 0.01 or 0.1 N iron(III) nitrate solution, all of which were 1 M in nitric acid. Iron(III) nitrate was titrated with potassium hydroxide solution under magnetic

stirring, a glass electrode being used to indicate the pH. The electrodes were taken out at certain pH values and the potential response was measured in standard potassium iodide solutions.

Measurements with the electron microprobe analyzer

Thin sections were prepared from a silver-iodide membrane sheet and sealed on an aluminium sample holder. Gold, or in some cases carbon, was evaporated on to the surface of the samples thus prepared in a Balzers evaporating unit.

RESULTS AND DISCUSSION

Some electron micrographs taken of the surfaces and cross-sections of silver iodide membranes which were found to give Nernstian electrode response, are shown in Fig. 1. As the electron micrographs show, the surface of the membranes is covered by a more or less continuous thin silicone-rubber layer, with some pores with an average diameter of 5—15 μm.

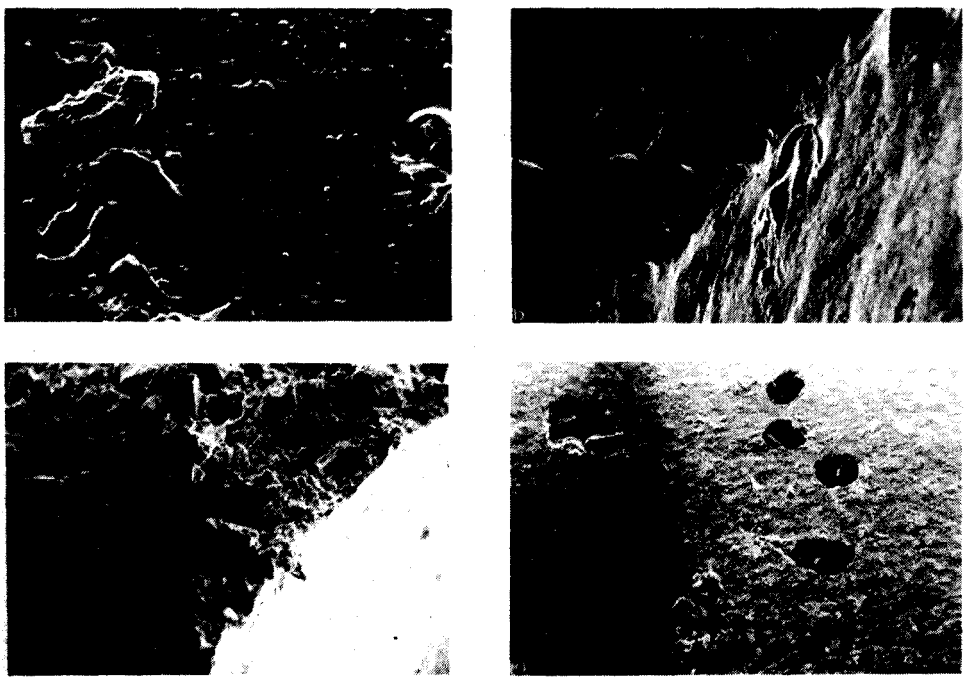


Fig. 1. Electron micrographs of silver iodide-based silicone-rubber membrane electrodes. (a) The surface; magnification 1 : 1000. (b) The surface and cross-section; magnification 1 : 700. (c) The cross-section; magnification 1 : 200. (d) The surface showing the pores; magnification 1 : 100.

The position of precipitate particles in the membrane is shown well in Fig. 2(a). The large precipitate particles do not always contact each other, and small particles are situated between the large ones embedded in the silicone-rubber matrix. The specific distribution of iodide and silver over the cross-section of the membrane is shown in Fig. 2(b, c), which was obtained by means of back-scattered electrons. These photographs, like the electron micrographs, show clearly the heterogeneous particle size distribution of the silver iodide, and the composition of the membrane layer; they also make it possible to calculate the volume fraction of the particles within the membrane. Stereometric evaluation showed that about 70 % of the volume of the membrane comprises silver iodide. Quantitative evaluation of the silver and iodide contents of the silver iodide precipitate in the membrane gave the expected result; namely, that the composition of the precipitate changed during the measurements, in the case of currents of high intensity. The silver iodide crystal decomposes on irradiation with electrons, and a loss of iodine is caused.

Interference from potassium iodide

The potential response of silver iodide membrane electrodes ceases after the membranes have been soaked for a long period of time (minimum 24 h) in potassium iodide solutions with concentrations exceeding $6 \cdot 10^{-1}$ M, because some changes occur on the electrode surface [2]. This phenomenon was also observed with the silver iodide-based silicone-rubber membrane

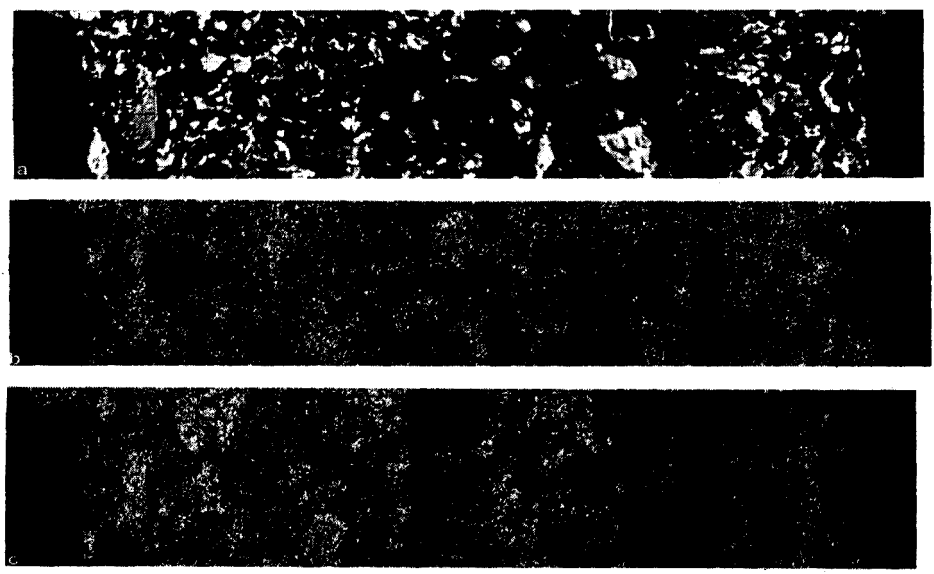


Fig. 2. Micrographs of the total cross-section of the silver iodide-based silicone-rubber membrane electrode. (a) Electron absorption. (b) Iodine distribution. (c) Silver distribution

electrodes. Electrodes soaked in 1 M potassium iodide solution and washed with distilled water do not show Nernstian response, but can be regenerated by soaking in distilled water or 0.1 M potassium chloride or 0.1 M lithium chloride solution for at least 5 h, as shown in Fig. 4. The feasibility of regeneration suggests that the interpretation proposed by Sorrentino and Rechnitz [2], i.e. that potassium iodide interferes because of dissolution of silver iodide in a complex form, is incomplete.

Accordingly, the dissolution process was investigated in 0.1 M and 1 M potassium iodide solutions; it was found that dissolution is really due to the complex formation equilibrium [4]

$$AgI_{(S)} + 2\,I^- \rightleftharpoons AgI_3^{2-};\ K_{S3} = 10^{-2.52}\ mol^{-1}$$

which continues until silver iodide is completely dissolved from the active surface layer of the electrode.

The same silver iodide membrane was used repeatedly to study the dissolution of silver iodide from the surface layer with freshly prepared 1 M potassium iodide solution, as well as the regeneration process. The amount of silver iodide dissolved gradually decreased, as shown by data obtained by atomic absorption spectrometry; thus the active surface layer became poorer and poorer in silver iodide. Membrane electrodes submitted to repeated treatment could not be regenerated, because the surface layer no longer contained any silver iodide precipitate. The average amount of silver iodide dissolved from one electrode membrane with a diameter of 7 mm, was found to correspond to 1200 μg of silver. The area of the electrode surface was 0.2 cm^2, and it can be assumed that the active surface at which the precipitate-exchange reaction could take place was one third of the total surface, hence the thickness of this active surface layer of the electrode could be calculated as 60 μm. This corresponds to the thickness of the surface precipitate layer determined from the average particle size.

The amount of silver dissolved from the silver iodide membrane by 0.1 M potassium iodide solution was much smaller. Consequently, the amount of silver dissolved in 2—3 repeated experiments remained almost constant, i.e. the surface layer did not become significantly poorer in silver.

In addition to the dissolution of silver iodide, a chemisorption process also occurred, potassium and iodide being bound on the surface of the electrode. After the electrode had been washed, the potassium and iodide sorbed on the surface were dissolved, and the potassium and iodide were measured in the resulting solution by flame spectrometry and by electrochemical methods, respectively. The amounts of potassium and iodide sorbed from 1 M solution were found to be greater than those from 0.1 M solution. Potassium and iodide were chemisorbed on the surface inhomogeneously, blocking the active points of the surface (Fig. 3). However, it is supposed that the interference cannot be attributed solely to dissolution and adsorption, but also to the simultaneous formation of an electrochemically inactive — chemisorbed or recrystallized — new layer on the electrode

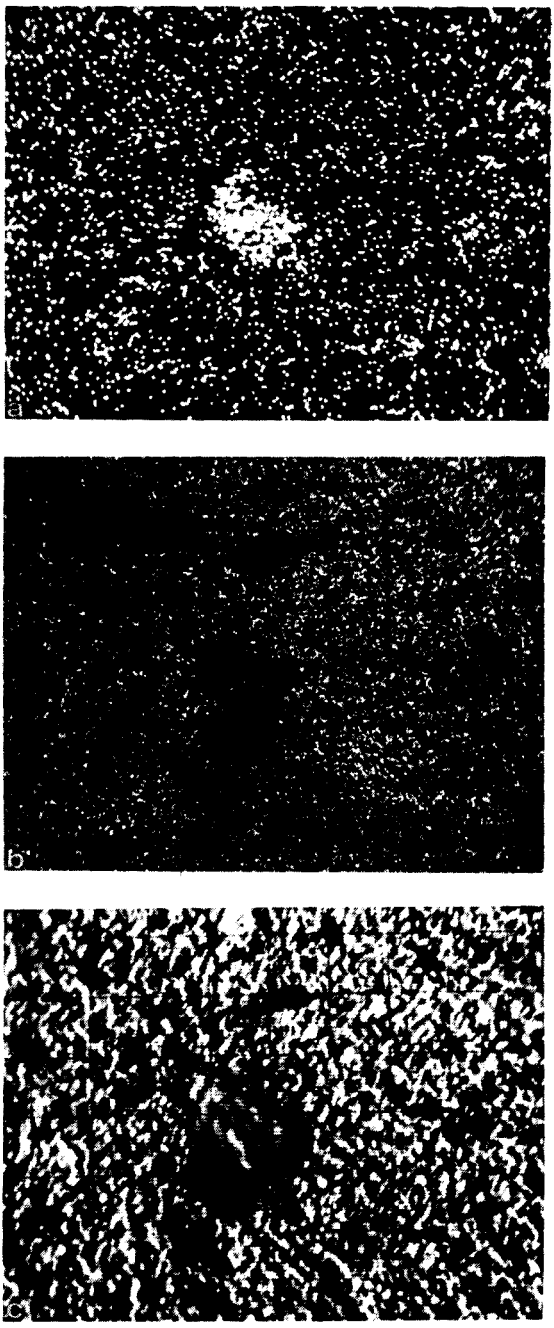


Fig. 3. Micrographs of the surface of a silver iodide-based silicone-rubber membrane electrode damaged in 1 M potassium iodide solution. (a) Potassium distribution. (b) Silver distribution. (c) Iodine distribution.

surface which causes the electrode response to cease. This results in the phenomenon that the electrode potential is constant, irrespective of great variations in the activity of iodide in the sample solution. The constant potential exhibited by a damaged electrode is more negative than the value measured in 0.1 M potassium iodide solution, which indicates that an iodide layer with a constant and extensive coverage has been formed on the electrode surface (Fig. 4). Investigations of the nature, the crystalline form and the chemisorption of the barrier layer on the electrode surface are in progress.

Investigation of the interference by iron(III) hydroxide

The electrochemical function of silver iodide-based membrane electrodes changes, mainly at low iodide levels, in solutions where iron(III) hydroxide may be formed (Fig. 5). No interference is observed, however, if the electrodes are immersed in acidic solutions containing iron(III) ions or iron(III) hydroxide. Thus the potential response of the electrodes deteriorates only if iron(III) hydroxide is precipitated when the electrodes are in the solution. Some electrodes damaged by iron(III) hydroxide were submitted to electron microprobe analysis, to study the iodine and iron(III) distributions on the electrode surface which had contacted the sample solu-

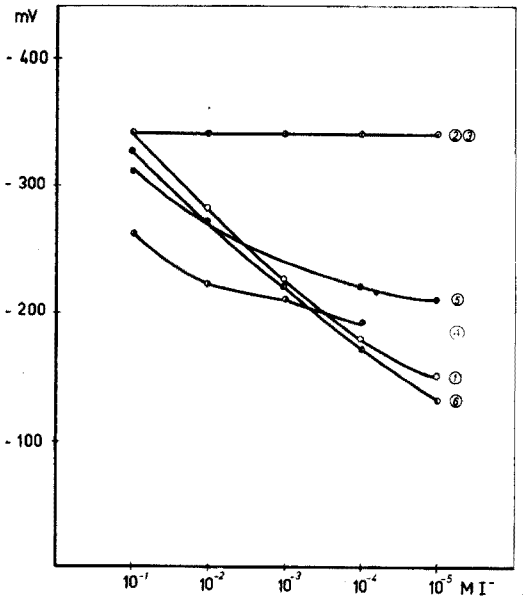


Fig. 4. Changes in the potential response of a silver iodide-based silicone-rubber membrane electrode after soaking in 1 M KI and during regeneration in 0.1 M KCl solution. (1) Untreated electrode. (2) Immediately after soaking in 1 M KI solution. (3)—(6). After soaking in 0.1 M KCl for 1 h, 2 h, 3 h, and 5 h, respectively.

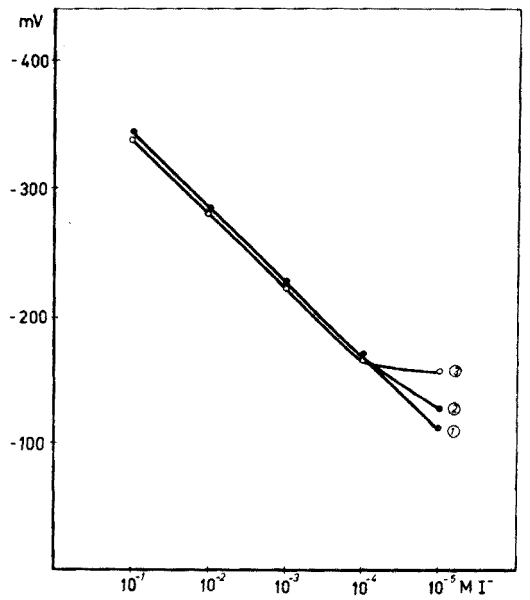
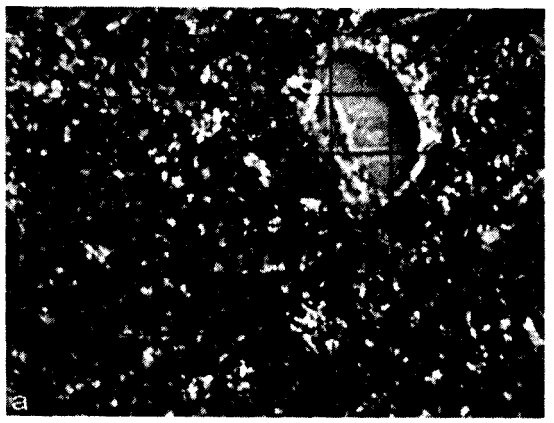
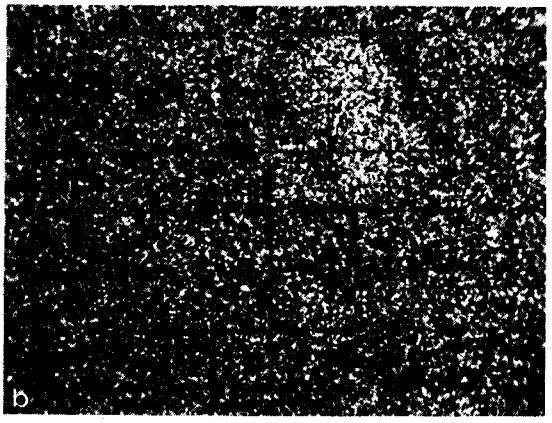


Fig. 5. Potential response of a silver iodide-based silicone-rubber membrane electrode. (1) Theoretical response. (2) Response of untreated electrode. (3) Response of an electrode which had been present during the precipitation of iron(III) hydroxide.

tion (Fig. 6). The photographs show clearly that iron is concentrated on the areas where the surface was found to be poor in silver and iodine. This suggests that the interference from iron(III) is quite different in nature from that of concentrated (> 0.6 M) potassium iodide, because iron(III) hydroxide covers mainly the inactive areas of the electrode surface and not the electrochemically active sites.





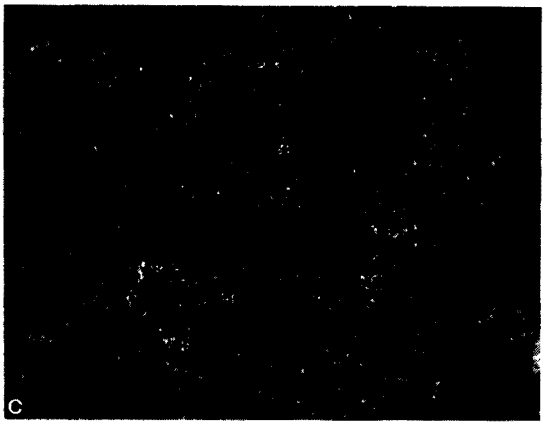


Fig. 6. Micrographs of the surface of an electrode which had been present in the solution during the precipitation of iron(III) hydroxide. (a) Electron absorption. (b) Iodine distribution. (c) Iron distribution.

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ACID-BASE EQUILIBRIA OF SOME 6-MEMBERED N-HETEROCYCLIC COMPOUNDS

W. FRANKLIN SMYTH, T. JENKINS, J. SIEKIERA and A. BAYDAR
Chemistry Department, Chelsea College, Manresa Road, London S.W.3 (England)
(Received 10th March 1975)

SUMMARY

The acid—base equilibria existing in aqueous solutions of barbiturates, thiobarbiturates, Persedon, Sedulon, Noludar, Doriden and methaqualone were investigated by u.v. spectrophotometry and polarography. All the compounds give pK_a values in the region 7—11.5 except Noludar and Sedulon, which exist as neutral molecules over the pH range 0—13. Methods for the analysis of mixtures of trace amounts of these 6-membered heterocyclic compounds are suggested based on selective solvent extraction and/or differential pulse polarography after ascertainment of the pH regions of existence of the neutral molecules.

N-Heterocyclic compounds containing amide linkages are widely used in medicine, principally as hypnotic drugs. Barbiturates, thalidomide and methaqualone, which produce depressive effects on the central nervous system, are structurally similar in that they contain 6-membered rings with amide linkage(s) that can undergo enolization to varying degrees. The earlier literature, which was particularly concerned with barbiturates and thiobarbiturates, abounds with debate on the state of ionization of these molecules at physiological pH and on the importance of this factor in determining the action of these drugs. The only references to acid—base equilibria of later members of this "barbiturate-like" series are concerned with tabulation of some pK_a values with no discussion on the structures of the species participating in the equilibria or on their extractability into organic solvents. Such information is of fundamental importance in studies of the absorption and excretion of these drugs and their interaction with various membranes, and in the improvement of analytical methodology for their extraction from body fluids.

This paper presents a systematic study of the acid—base equilibria in aqueous solutions of barbiturates, thiobarbiturates, pyrithyldione (Persedon), piperidinedione (Sedulon), methyprylone (Noludar), glutethimide (Doriden) and quinazolines (e.g. methaqualone). Where necessary, model compounds which are of no therapeutic value are included. These equilibria are proposed after studies of the variation with pH of u.v. absorption bands, polarographic reduction waves (where appropriate) and extractability. Some analytical possibilities are then suggested.

EXPERIMENTAL

Apparatus

Ultraviolet spectra were recorded with a Perkin-Elmer model 137 spectrophotometer, for matched 10-mm silica cells thermostatted at 20 °C; the instrument was flushed with dry nitrogen to eliminate stray radiation effects. Spectral bands below 220 nm were recorded with a Cary spectrophotometer.

A P.A.R. Model 174 Polarographic Analyser was operated in the "Tast" mode in conjunction with a micro Kalousek vessel with a saturated calomel electrode. The dropping mercury electrode used had the following characteristics: outflow velocity $m = 1.73$ mg s^{-1} drop time $t = 4$ s at the potential of the saturated calomel electrode and a mercury pressure $h = 80$ cm in 1 M potassium chloride. The controlled drop time was 0.5s with a modulation amplitude of 100 mV.

Reagents

Samples of heterocyclic compounds were obtained from various sources (see Acknowledgements). Stock solutions (about 10^{-3} M), prepared in Analar methanol, were kept in the dark under refrigeration to minimize decomposition.

A stock Britton—Robinson buffer solution (pH ca. 2.0), containing boric acid, phosphoric acid and acetic acid, (all 0.04 M), was prepared from Analar reagents. This solution was adjusted to varying pH values with 0.1 M sodium hydroxide (pH meter).

Techniques

Spectrophotometry

Solutions were prepared by diluting an appropriate amount of stock solution with the appropriate buffer to give a $5 \cdot 10^{-5}$ M solution. In addition to the buffered solutions, M HCl, 0.1 M HCl, 0.1 M NaOH and M NaOH were used. The pH range was scanned for each compound in increments of 1 pH unit to determine the approximate position of each pK_a value by observation of spectral changes; the region around each pK_a value was then studied in more detail at intervals of ca. 0.3 pH units. From the spectra obtained, pK_a values were evaluated from the Henderson equation. Spectra were scanned from 220 to 390 nm, at a slow scan speed (8 min for the range) with a buffer solution containing the same amount of methanol as the samples as reference.

Polarography

A 2-ml portion of a $5 \cdot 10^{-5}$ M solution of the sample in the appropriate supporting electrolyte was deaerated with nitrogen for 3 min and the i—E curve was recorded at a rate of 10 mV s^{-1} (modulation amplitude 100 mV).

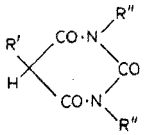
Solvent extraction

A 5-ml portion of a $5 \cdot 10^{-5}$ M sample solution, adjusted to the appropriate pH with buffer, sodium hydroxide or hydrochloric acid was used as the aqueous phase. The u.v. spectral bands of the aqueous phase were recorded before and after shaking for 10 min with 5 ml of the solvent. If the compound was completely extracted, the organic layer was separated, the solvent evaporated off and the residue made up to 5 ml in the appropriate buffer solution before the u.v. spectrum was recorded.

RESULTS AND DISCUSSION

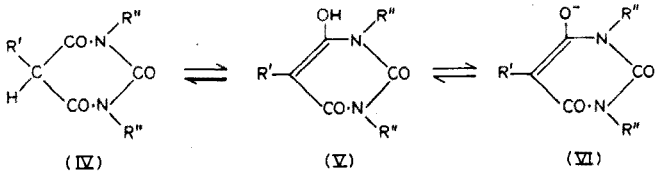
2,4,6-Trioxopyrimidines with at least one hydrogen atom in position 5

The u.v. bands and pK_a values observed for barbituric acid I, 5-ethylbarbituric acid II, and 1,3-dimethylbarbituric acid III are given in Table 1.



(I) R′ = R″ = H
(II) R′ = $-C_2H_5$, R″ = H
(III) R′ = H, R″ = $-CH_3$

All these compounds are relatively strong acids with pK_a values of about 4. In passing through the pK_a value, the major band intensifies but does not change wavelength. This can be understood in terms of 5,6-enolization.



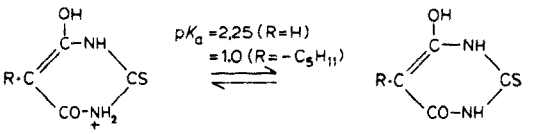
(IV) (V) (VI)

TABLE 1

U.v. bands and pK_a values for some barbituric acids

Compound	HA		A^-		pK_a
	λ max	ϵ ($\cdot 10^4$)	λ max	ϵ ($\cdot 10^4$)	
I	257	0.1	230S 257	4.0 30.0	4.1
II	268	0.1	240S 268	0.57 1.05	4.0
III	227 260	0.7 0.04	230S 260	0.35 2.0	4.4

In acidic solution (pH < pK_a), IV predominates and only a weak carbonyl n—π^* absorption would be expected over 200 nm ($\epsilon \sim 20$) [1]. The enol form, which is present to a small degree, is responsible for the observed π—π^* absorption. At pH > pK_a, the equilibrium shifts to the right and enol V is deprotonated to give VI, the absorptivity being enhanced. The π—π^* absorption wavelengths for the 5,6-enols can be calculated empirically by the Woodward—Hoffman rule. These enols constitute an enone system in which the double bond and carbonyl chromophores are capable of conjugation. Calculations of the λ_{max} values for barbituric acid and 5-ethyl-barbituric acid give the values 257 and 267 nm, respectively, whereas the observed values are 257 and 268 nm. The pK_a value of about 4 corresponding to deprotonation of I, II and III can therefore be assigned to the 5,6-ehol centre. It is useful to compare these results with those obtained previously [2] on the corresponding 2-thio compounds, for which the equilibrium proposed was:

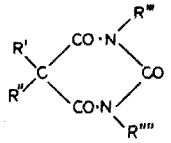


(VII) (VIII)

The same 5,6-enol structure is proposed but the pK_1 value is assigned to deprotonation on the 3-N atom, rather than the 6-OH group, giving rise to the neutral VIII. Since the pK_1 value for the 2-thio compounds is much more affected by 5-alkyl substitution, it is reasonable to assign the deprotonation to a centre other than the 6-OH group. Thus it seems more reasonable to consider VII protonated on the 1-N atom so that on deprotonation, the lone pair on this N-atom will allow for conjugation from the R group to the S atom. This would result in a lower pK_a value for 2-thiobarbituric acid compared with barbituric acid, as is observed.

5,5'-Disubstituted, 1,5,5'-trisubstituted and 1,3,5,5'-tetrasubstituted barbituric acids

Substitutions of both hydrogen atoms in position 5 weakens the acid strength of 2,4,6-trioxopyrimidines; the pK_a value shifts from 4 to 7—8, depending on the substituents. Further substitution on the 1-N atom moves it a further 0.5 pK_a units. Complete substitution of all hydrogen atoms results in a weak acid, p$K_a \triangleq 10.5$. Table 2 lists the observed u.v. spectral bands together with the observed pK_a values for 1,3 di-n-butyl-5,5'-dimethyl-barbituric acid (IX), 1-methyl-5'-ethyl-5-phenylbarbituric acid (phenitone) (X) and 5,5'-diethyl-barbituric acid(barbitone) (XI).



(IX) R' = R'' = $-CH_3$, R''' = R'''' = $n-C_4H_9$

(X) R' = $-C_2H_5$, R'' = $-C_6H_5$, R''' = $-CH_3$, R'''' = H

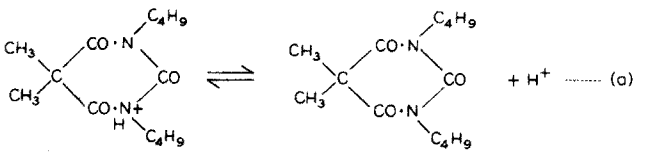
(XI) R' = R'' = $-C_2H_5$, R''' = R'''' = H

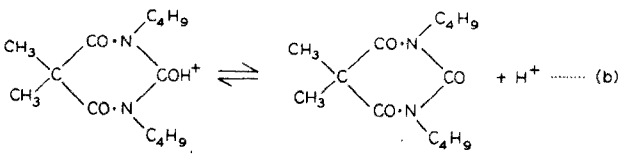
TABLE 2

U.v. bands and pK_a values for some substituted barbituric acids

Compound	H_2A^+		HA		A^-		pK_a
	λ max	ϵ ($\cdot 10^4$)	λ max	ϵ ($\cdot 10^4$)	λ max	ϵ ($\cdot 10^4$)	
IX	200 230	2.4 0.66	230	0.47			10.7
X			185	3.6	245	0.86	7.8
XI			192 210	2.8 1.3	240	1.10	7.5

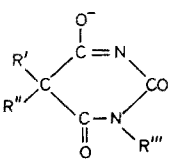
The completely substituted barbiturate (IX) cannot lose any hydrogen ions, therefore the pK_a value at 10.7 must refer to either process (a) or (b)



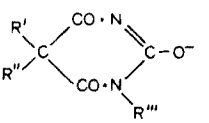


Equilibrium (a) is favoured since the corresponding tetrasubstituted 2-thiobarbituric acid has a similar pK_a value (10.5) [3].

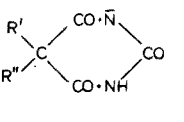
Compounds X and XI show similar u.v. spectral behaviour, which differs from that of IX, particularly on the acid side of the pK_a value; this suggests the existence of different acid—base equilibria. The absorptions of the HA species are clearly due to pure π—π^* electronic transitions within the carbonyl centre (including the benzene absorption in the case of X). The absorptions of the A^- species are much more difficult to assign. The proposed possibilities are



(XII)



(XIII)



(XIV)

Both XII and XIII constitute enone systems and would be expected to show π—π^* absorptions at about the observed wavelengths, even on the acid side of the pK_a value; but this was not observed and these barbiturates are

not polarographically active in the pH range 1—13, which suggests that the process is not tautomeric. Species XIV offers the possibility of proton abstraction from the amide nitrogen, and this process has been shown to require the least energy by a molecular orbital computer method [4]. Deprotonation of a neutral species leading to anions such as XIV is further confirmed by the solvent extraction results shown in Fig. 1. The uncharged 5,5′- and 1,5,5′-trisubstituted barbiturates (HA) are readily extracted into chloroform and ether and to a lesser extent into petroleum spirit, whereas the anion A^- is not. Extractions of therapeutically used barbiturates from body fluids are done at acid pH values [5].

The production of an anion from a neutral molecule with no tautomerization has been suggested by Zuman [6] who has used the fact that 5,5′-disubstituted barbiturates can form a dianion above ≙ pH 12 with λ max ≙ 270 nm, to differentiate a mixture of 5,5′- and 1,5,5′-substituted barbiturates.

The acid—base equilibria in aqueous solutions of the corresponding 2-thio-5,5′-disubstituted and 1,5,5′-trisubstituted barbiturates are quite different because of the tendency of the thio group to enolize. These equilibria have been studied [3] in detail with the following conclusions.

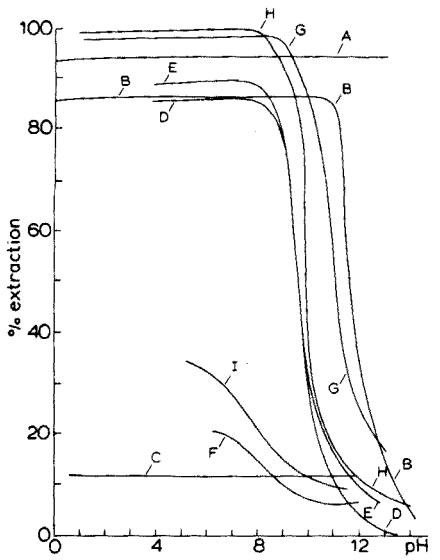
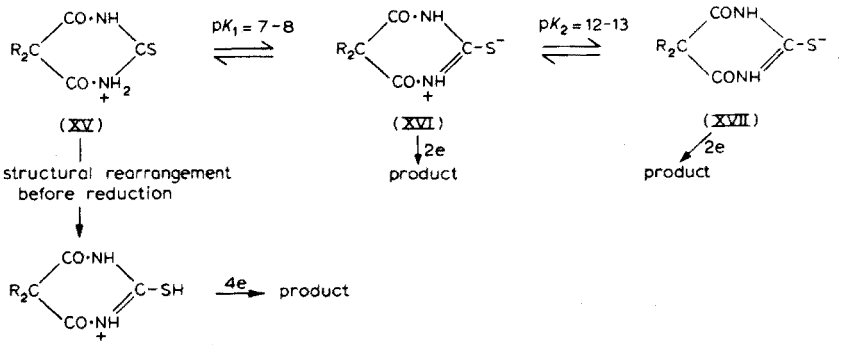


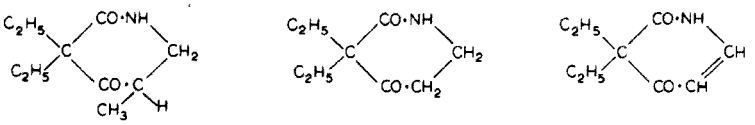
Fig. 1. Solvent extraction profiles of some 6-membered N-heterocycles. A, Noludar and sedulon with chloroform. B, Persedon with chloroform. C, Persedon with petroleum ether (40—60°). D, Pentabarbitone with chloroform [a]. E, Pentabarbitone with ether[a]. F, Pentabarbitone with petroleum ether (40—60°)[a]. G, Phenitone with ether[b]. H, 5-Ethyl-5′-butyl-2-thiobarbituric acid with chloroform[c]. I, 5-Ethyl-5′-butyl-2-thiobarbituric acid with petroleum spirit[c]. % Extraction calculated at, [a]λ = 240 nm, [b]λ = 245 nm and [c]λ = 288 nm.



The distribution characteristics of 5-ethyl-5′-butyl-2-thiobarbituric acid (Fig. 1) show that this compound and similarly substituted barbiturates behave analogously; in fact, thiobarbiturates are usually extracted from body fluids from acid solutions [5]. This suggests that XV should be represented as a neutral molecule, and XVI and XVII as the inextractible monoanion and dianion, respectively. This is rather like the barbiturates except that the 2-thio compounds exist in thioenol forms giving rise to reduction waves which are not shown by the barbiturates[3].

Noludar, Sedulon, Persedon and Doriden

Data on the spectral behaviour and pK_a values of the three 3,3′-diethyl-2,4-dioxo N-containing heterocycles, Noludar (XVIII), Sedulon (XIX) and Persedon (XX) are given in Table 3.



(XVIII) (XIX) (XX)

TABLE 3

U.v. bands and pK_a values for Noludar, Sedulon, Persedon and Doriden

Compound	HA		A^-		pK_a value
	λ max	ε	λ max	ε	
(XVIII) Noludar	290	40	—		> 13
(XIX) Sedulon	295	30	—		> 13
(XX) Persedon	307	7700	307 366	2000 10000	11.5
(XXI) Doriden	260	410	260 233	410 104	10.5

Both XVIII and XIX give low molar absorptivities of 30—40 l mol^{-1} cm^{-1} at 290—295 nm; this probably corresponds to transitions involving the carbonyl group with no 1,6- or 3,4-enolization taking place. The molecules exist as uncharged species in the pH range 0—13 as shown in the extraction profile (Fig. 1), and deprotonate only above pH 13 if at all.

Persedon (XX) exhibits a pK_a value of 11.5 as shown on Fig. 2. On the acid side of the pK_a value, a band appears at 307 nm, and at pH > pK_a, this band decreases considerably in intensity with the appearance of a new band at 366 nm (Table 3). Like Noludar and Sedulon, Persedon is readily extracted into chloroform below pH 11 and negligibly into petroleum spirit. Above pH 11, the percentage extraction of Persedon into chloroform falls off whereas Noludar and Sedulon continue to be extracted (Fig. 1).

An analogy can be made between this "dienone" structure and similarly conjugated cyclohexadienones [7], which exhibit a K band at 290—340 nm ($\epsilon = 10^3$—10^4 l mol^{-1} cm^{-1}) which should be compared with the 307 nm band of Persedon. At pH < pK_a, tautomeric processes, similar to those in 2- and 4-hydroxypyridines, occur and it is impossible to describe a deprotonation site.

Noludar, Sedulon and Persedon all give rise to anodic waves because of complex formation with mercury, as has been observed for barbiturates [8]

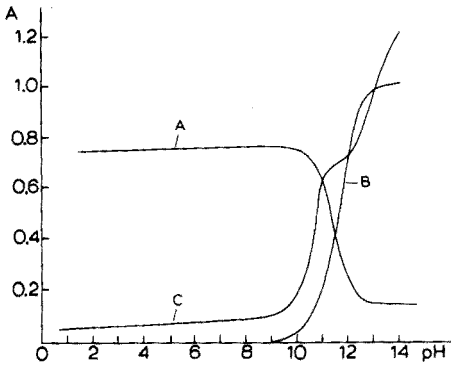


Fig. 2. The variation of u.v. absorbance with pH for Persedon (A, measured at 307 nm: B, measured at 366 nm) and Doriden (C, measured at 233 nm).

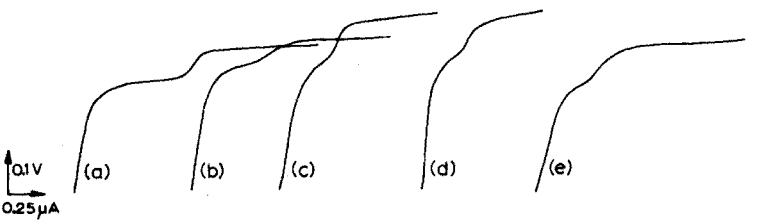
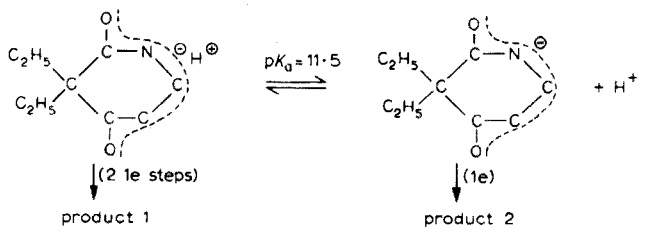
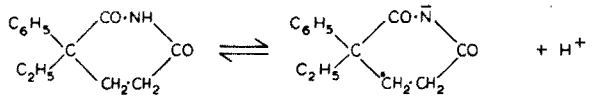


Fig. 3. D.c. polarographic curves of anodic waves of 10^{-4} M (a) barbiton, (b) Sedulon, (c) Noludar, (d) Persedon and (e) glutethimide, in borax buffer, pH 9.2. Starting voltage, —0.3 V. Sampled d.c. mode. Drop time 1 s.

and thiobarbiturates [3] (Fig. 3). The anodic wave of barbitone under similar conditions is included for comparison purposes and it can be seen that the anodic waves of Noludar, Sedulon, Persedon and Doriden are smaller and less well defined than that of barbitone. Differential pulse polarography of these anodic waves results in ill-defined peaks, all at ca. OV; at levels lower than 10^{-5} M, the waves merge into the supporting electrolyte decay waves. Persedon is the only one of the three to undergo polarographic reduction in aqueous buffers over a wide pH range [9]. This compound is reduced in two 1e steps at pH 0—10, above which the second wave begins to decay, leaving a 1e step by pH 12.0. The $E_{\frac{1}{2}}$—pH plot shows a break at pH 11.0. A possible scheme for the acid—base equilibria in aqueous solutions of Persedon and for its polarographic reduction is



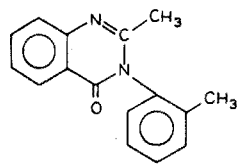
Glutethimide (Doriden) (XXI) exhibits a pK_a value of 10.5 (Fig. 2). A further change in spectrum is observed above pH 12 corresponding to ring opening. [10] At pH < pK_a a band at 260 nm is observed and at pH > pK_a, two bands appear at 233 nm and 260 nm (Table 3). This behaviour is similar to that of 5,5′-disubstituted barbiturates which have only weak absorption at pH < pK_a and stong absorption at pH > pK_a in the region of 240 nm. The weak absorption at 260 nm is presumably due to the phenyl group in the case of Doriden. The pK_a value of 10.5 corresponds to deprotonation to yield XXII:



(XXI) XXII

Quinazolines

Methaqualone (XXIII) is the active constituent of Mandrax and is the most widely known hypnotic of this class of compound. In contrast to the



(XXIII)

other drugs discussed, there are no "free" hydrogen atoms to be involved in enolization processes or abstraction of hydrogen ions in alkaline media. Application of the Henderson equation to the u.v. spectrum, measured at 288 nm, yields a pK_a value of 2.5 (Fig. 4) corresponding to protonation on the azomethine nitrogen. This compound can be extracted from biological fluids at pH > pK_a [11] and is not extracted at pH < pK_a. The inextractability of compounds containing the protonated azomethine group has already been observed for 1,4-benzodiazepines [12]. Pflegel and Wagner [13] have thoroughly investigated the polarographic reduction of the azomethine group in Methaqualone and from the inflexion in their $E_{\frac{1}{2}}$—pH plot, a pK_a value of ca. 2 was obtained, which agrees well with the spectral result.

It is of interest to compare the present spectral and polarographic results with results obtained on compounds such as 1-diethylaminoethyl-3-(4-methoxybenzyl)-chinoxalone [14] and chlordiazepoxide [15].

Analytical applications

Only thiobarbiturates, Persedon and methaqualone can be detected by differential pulse polarography at trace levels; after solvent extraction of the neutral species from aqueous solutions. If BR buffer (e.g. pH 8.0) is used the reduction wave of methaqualone merges into the supporting electrolyte, but the reduction peaks of a thiobarbiturate and Persedon are well defined

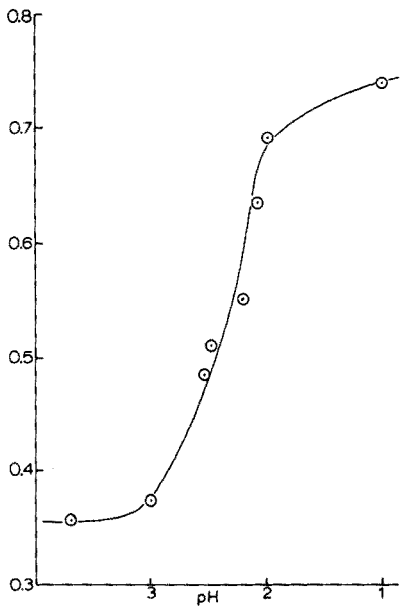


Fig. 4. Absorbance vs. pH for a 10^{-4} M solution of methaqualone measured at 288 nm.

and resolved, and can be measured down to 10^{-7} M. If 0.01 M tetraethylammonium perchlorate is used as supporting electrolyte, all three peaks can be observed, but the peaks for a thiobarbiturate and methaqualone are too close for complete resolution at the trace level. Table 4 gives the differential pulse polarographic peak potentials of these species in the two supporting electrolytes.

TABLE 4

D.p.p. peaks in different buffers

	pH 8 BR buffer	0.01 M Et_4NClO_4
5-Ethyl-5-(1-methylpropyl) 2-thiobarbiturate	−1.29	−1.32
Methaqualone	—	−1.36
Persedon	−1.20	−1.10

Other mixtures of such 6-membered N-heterocycles can be resolved by gas—liquid chromatography or by selective solvent extraction (as can be seen from Fig. 1); eg., Noludar, Sedulon (and methaqualone) can be separated from the others by extraction into chloroform at pH 14, a pH at which they all exist as neutral molecules.

The authors thank Mr. E. H. P. Young of I.C.I. Ltd., Pharmaceuticals Division, Macclesfield, for the provision of 1,3,5,5′-tetrasubstituted barbiturates; Roche Products Ltd., Welwyn Garden City, for samples of Noludar, Sedulon and Persedon; Ciba Laboratories Ltd., Horsham, Sussex, for a sample of Doriden, and Mr. L. E. Spencer for the preparation of 5-ethylbarbituric acid [16]. In addition, Dr. J. Barrett is thanked for valuable discussions.

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AN INVESTIGATION OF POROUS MEMBRANES SATURATED WITH HYDROPHOBIC SOLVENTS AS ION-SELECTIVE ELECTRODES

OVE ÅSTRÖM

Department of Analytical Chemistry, University of Umeå, S-901 87 Umeå (Sweden)

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SUMMARY

The ion-selective characteristics of Orion nitrate, chloride, calcium electrode membranes and Millipore filters saturated with organic solvents (*o*-dichlorobenzene, 1,2-dichloroethane, 2,2-dichlorodiethyl ether and 1,1,2,2-tetrachloroethane) have been studied. The saturated membranes were not inert, but showed ion-selective qualities. Almost Nernstian behaviour was obtained for several anions. The addition of an ion exchanger scarcely affected the potentiometric behaviour for most ions, except when a selective interaction occurred.

In order to understand the behaviour of liquid membrane ion-selective electrodes it is necessary to study the effects of solvent, membrane support and ion exchanger separately. It has often been supposed that the membrane support is electrochemically inert, but several observations reported in the literature suggest that this might not be the case.

Tendeloo and Krips [1] made membranes from paraffin wax and cotton gauze which showed a potentiometric response of 15—20 mV/decade change in molarity for calcium. The results were confirmed by Shatkay [2], who found that the gauze — far from being inert — took part in the perm-selectivity of the membrane.

Ilani [3—5] studied the discrimination between Na^+ and K^+ by porous filters saturated with organic solvents; the filters were ordinary Millipore membrane filters of various types. It was assumed that there was an interaction between the filter material and the solvent and that the filter material imparted fixed negative charges to the membrane.

Kedem, et al. [6] stated that the support material for a potassium liquid—membrane electrode should be slightly charged, so that the solvent and support together have a negative charge of 10^{-6}—10^{-3} meq g^{-1} of solvent. Potassium could be measured only when this charge condition was fulfilled. Orion [7] have reported that diphenyl ether, without valinomycin, used in an Orion potassium electrode responds to changes in potassium ion activity over the range 10^{-3}—10^{-1} M with a nearly theoretical slope and shows about a 40:1 preference for potassium over sodium. Fiedler and Růžička [8] have reported similar results obtained with the Philips potassium electrode. These

authors also reported that membranes made from pure polyurethane and dioctyladipate, without valinomycin, gave a slope of 30.0 mV/decade for potassium and pK_{KNa} = 2.50, and that a silicone rubber membrane with dioctyladipate gave a slope of 38.7 mV/decade and pK_{KNa} = 3.64. Růžička et al. [9] investigating the calcium(II) Selectrode, found that a membrane made of only PVC and dioctylphenylphosphonate showed a calcium response over several decades with nearly theoretical slope, but this was ascribed to impurities in the solvent.

The present work was undertaken to study the role of the support materials in more detail. Membranes already in use in commercial ion-selective electrodes were included, as their electrode properties when an ion exchanger is added, are well established.

When liquid membrane electrodes are used, the solvent must be water-immiscible and should be chemically inert, so that extensive investigations can be done with an ion exchanger in the solvent; moreover, the solvent must have a high boiling point and moderate viscosity. The solvents used here were *o*-dichlorobenzene, 1,2-dichloroethane, 1,1,2,2-tetrachloroethane and 2,2-dichlorodiethylether.

EXPERIMENTAL

Membrane and solutions

Orion series 92 membranes for nitrate, chloride and calcium electrodes were used. Millipore Solvinert and Teflon filters were also investigated. 1,1,2,2-Tetrachloroethane (Merck) was washed with 18 M sulphuric acid and distilled. 2,2-Dichlorodiethyl ether (Fluka) was washed with 12 M hydrochloric acid and distilled. *o*-Dichlorobenzene (BDH) and 1,2-dichloroethane (Fisher) were used without further purification. Sodium tetrafluoroborate was recrystallized from an ethanol—water mixture; all other salts were of analytical grade and were used without further purification.

Electrodes

The body of an Orion series 92 liquid-membrane electrode was used with the Orion series 92 membranes or with membranes (3 mm diam.) punched from Millipore filters. The electrode was assembled according to the manufacturer's instructions. The inner reference system in Fig. 1 was an aqueous solution of sodium chloride and sodium perchlorate, each 10^{-3} M. For all the remaining cases the concentrations were 0.05 M and 10^{-3} M, respectively. The outer reference electrode was an Orion 92-02 double liquid junction electrode, with the outer chamber filled with aqueous 10 % ammonium chloride.

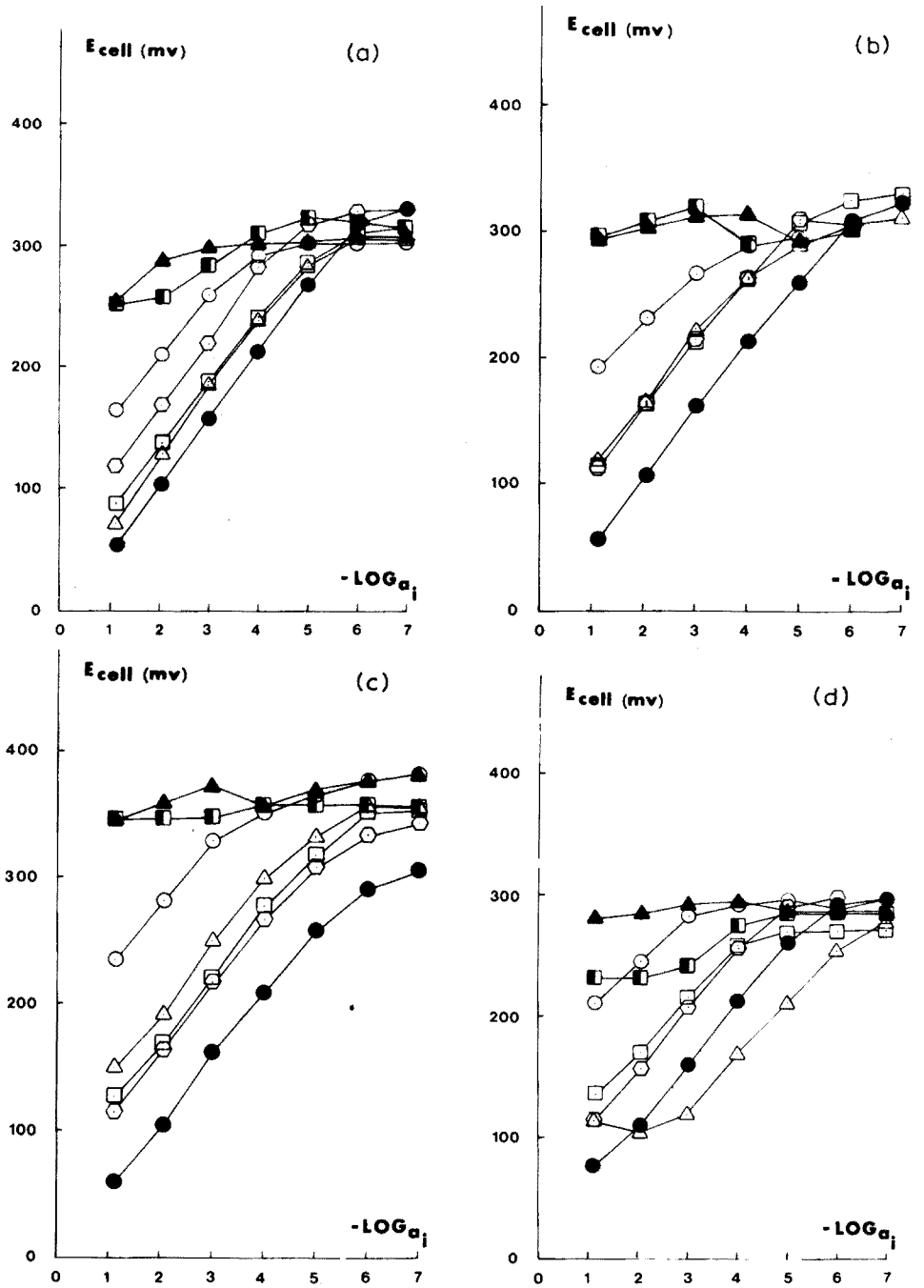

Fig. 1. Response of Orion nitrate membrane saturated with different solvents for different anions. (●) ClO_4^-, (⬭) BF_4^-, (▫) SCN^-, (△) I^-, (○) NO_3^-, (▲) Cl^-, (◧) OAc^-. (a) 1,1,2,2-Tetrachloroethane. (b) 1,2-Dichloroethane. (c) *o*-Dichlorobenzene. (d) 2,2-Dichlorodiethyl ether.

Measurement techniques

The potential was measured in the following cell:

—Ref electrode|sample aq.|Membrane|NaCl, $NaClO_4$, aq.|AgCl|Ag +

at room temperature (21 °C) with an Orion 801 digital pH meter. The internal reference solution of the Orion 92 liquid-membrane electrode was always pre-equilibrated with the organic solvent used. Serial dilutions of unbuffered solutions were made and in general sodium salts of the appropriate anions were used. Measurements were made with the electrode immersed to a constant depth in the sample solution and with the solution stirred by a magnetic bar. Readings were taken after 5 min, but for most cases stable values were obtained earlier. Single-ion activities were calculated from the extended Debye—Hückel relation with the ion size parameters of Kielland [10]. The organic solvents were equilibrated with water before use.

Selectivity coefficients were evaluated from the equation: $\log K_{\text{ref/interf.}} = (E_{\text{ref}} - E_{\text{interf}})/59.8$, at 10^{-3} M concentrations.

RESULTS

Figure 1(a) shows the potentiometric response of the membrane delivered by Orion with their nitrate-selective electrode impregnated with 1,1,2,2-tetrachloroethane. No ion exchanger is present, but the electrode still shows an almost Nernstian anion response for several ions. The slope is 56 mV/decade for perchlorate which indicates a high degree of cation exclusion from the membrane—solvent phase. This effect could be due to the membrane, the solvent or to a combination of both. Figure 1 (b—d) shows the responses of the membrane impregnated with various solvents. All the measurements shown were made about 15 min after the electrodes had been assembled; reasonably consistent results were obtained when the measurements were repeated about 24 h later. In all cases there is an anion response which for some ions is almost Nernstian over several decades. All solvents are inert, the dielectric constants being 8.2, 10.4, 9.9 and 21.2 for the solvents in Fig. 1 (a—d), respectively. There are some changes in selectivities between them, e.g. the dichlorodiethyl ether membrane (Fig. 1d) is most selective for iodide until the response breaks down at high concentrations, presumably because of loss of cation exclusion. The other membrane—solvent combinations are most selective towards perchlorate but show some changes in selectivity order for the other anions tested, and none has selectivity for chloride. The selectivity orders have many features in common with the Orion nitrate electrode based on the phenanthroline ion exchanger, but there are differences in the selectivity coefficients. $K_{ClO_4^-, NO_3^-}$ is 0.001 for the Orion electrode [11] with ion exchanger, and 0.018, 0.015, 0.0014 and 0.008 for the combinations in Fig. 1 (a—d) respectively.

To investigate the importance of the membrane material alone,

tetrachloroethane was chosen as a solvent, and the Orion chloride and calcium electrode membranes were tested. The potentiometric response with the "chloride" membrane was tested for the same anions as for the "nitrate" membrane and the results were practically identical with those shown in Fig. 1 for the same solvent. For the "calcium" membrane with tetrachloroethane, there is only slight response to perchlorate but no consistent selectivity pattern (Fig. 2). The results indicate that there are few fixed positive charges on the membrane. Millipore Solvinert UGWP and UGHP filters (pore size, 0.25 μm) tested with tetrachloroethane gave a slight anion response with practically no selectivity, but in some cases even a cation response was noted. In contrast to the other membranes studied, the properties changed somewhat with time, in the direction of a loss of cation response. Millipore Teflon filters type FHLP and LSWP, which differ in thickness and porosity, showed initially an anion response only at the highest concentrations and had poor selectivity, but when these membranes were stored overnight in pure solvent, the anion response improved substantially. The electrode behaviour was then similar to that shown in Fig. 1 but the linear range was shorter and the response levelled off at around ten times higher concentration, i.e. ca. 10^{-5} M. The Teflon membranes obviously acquired more fixed positive charge with time.

Table 1 summarizes the behaviour of the various membranes tested and it can be seen that the membrane material contributes to the electrode function. The selectivity order for the various anions remained the same on all the membrane materials which showed a consistent anion response. Thus the membrane material as well as the solvent is important. It should be

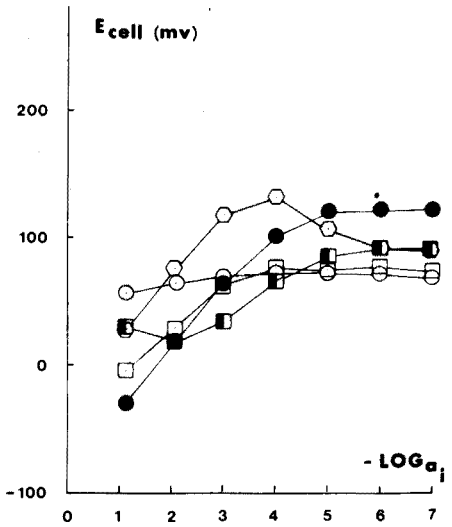

Fig. 2. Response of Orion calcium membrane saturated with 1,1,2,2-tetrachloroethane for different anions. For the symbols used, see Fig. 1.

TABLE 1

Specification of various membranes and 1,1,2,2-tetrachloroethane

Membrane type		Lower linear range for perchlorate	Apparent Selectivity coefficient $K_{ClO_4^-/NO_3^-}$	Slope mV/decade
Orion	nitrate	$5 \cdot 10^{-6}$	0.018	56
	chloride	$5 \cdot 10^{-6}$	0.014	56
	calcium	10^{-3}	0.8	48
Millipore	UGHP	—		
	UHWP	—		
Solvinert[a]	UGWP	—		
Millipore Teflon	FHLP	10^{-3}[b]	0.07	46
		10^{-5}[c]	0.02	54
	LSWP	10^{-3}[b]	0.15	48
		$5 \cdot 10^{-5}$[c]	0.02	53

[a]These membranes showed a slight cation response which diminished with time.
[b]Freshly prepared electrode.
[c]After storage in pure solvent overnight.

emphasized that the differences between the various Orion membranes are real. When different batches of the same type of membrane were tested, the same pattern of results was obtained, although the linear ranges could change somewhat.

With regard to the effect of cations, Fig. 3 shows the potentiometric response in solutions of different perchlorate salts; obviously, the cation effect is negligible. Figure 4 shows the response of the filter alone; there is only a slight change with concentration and no selectivity. Similar results were obtained with the chloride membrane.

In most modern liquid ion-selective electrodes, an ion exchanger is present. In order to study the effect of adding an ion exchanger, a compound was selected which has been studied in the solid state; the ion radical salt N-ethyl-benzothiazole-2,2′-azaviolene perchlorate (abbreviated NEBA · ClO_4), first prepared by Hünig et al. [12] and later used in an ion-selective electrode by Sharp [13], was chosen. When the Orion nitrate membrane was used with tetrachloroethane as solvent and NEBA · ClO_4 as ion exchanger, the results were very similar to those shown in Fig. 1(a) except for iodide, although discrepancies occurred at the lowest concentrations. For iodide a selectivity coefficient of the order of +25 was obtained, indicating selectivity for iodide rather than perchlorate; this effect has been noted for the solid-state electrode [13], where a selectivity coefficient of 75 was reported. The potential stability was improved compared to a membrane-solvent without ion-exchanger. Similar results were obtained with the "chloride" membrane—

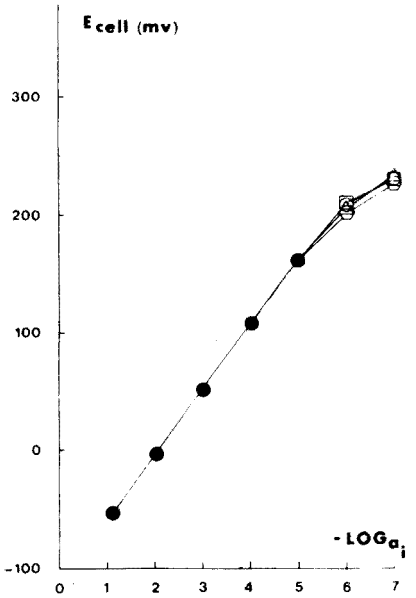

Fig. 3. Response of Orion chloride membrane saturated with 1,1,2,2-tetrachloroethane for the following perchlorate salts: (⊂) $NaClO_4$, (△) $LiClO_4$, (□) $KClO_4$, (○) $Ba(ClO_4)_2$.

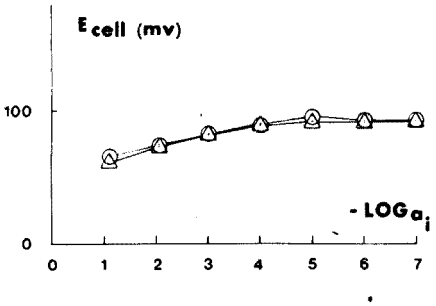

Fig. 4. Response of Orion nitrate membrane without organic solvent for different anions. For significance of symbols used, see Fig. 1.

tetrachloroethane—NEBA · ClO_4 combination; the ion exchanger did not affect the response, except for iodide, where a selectivity coefficient of 8 was obtained compared to 0.2 for the membrane—solvent only. As mentioned by Sharp, iodine reacts with the radical salt, so that iodide produces anomalous results. It must therefore be concluded that the ion exchanger has no effect on the potentiometric response but improves the stability somewhat.

As mentioned above, Millipore Solvinert and Teflon filters initially showed a slight cation response, which changed towards anion response after storage in the solvent. The experiments were repeated with NEBA · ClO_4 present; it was evident that storage in the solvent rather than the ion exchanger affected

the response. Figure 5 shows the perchlorate response for a UGWP-filter system measured at various times after preparation. The same results were obtained when the filter was stored in solvent only and then filled with ion exchanger just before the measurement. The first change of the membrane—solvent phase thus occurs independently of the presence of the ion exchanger; once this change has occurred the ion exchanger is necessary for a good anion response. The response of the filter—solvent system alone, comes close to curve (a) in Fig. 5, independently of the storage time.

DISCUSSION

Ilani studied Millipore, cellulose acetate and nitrate filters, which are not of the same type as those studied here, and found that most membrane—solvent combinations resulted in cation selectivity independently of the anions used; Nernstian slopes were obtained in several cases. Ilani [4, 5] found that different filters had different ion-exchange capacities: VF-filters had typically a capacity of 10^{-7} moles for a 3-cm^2 filter area. There was selectivity for potassium over sodium and lithium in filling the fixed sites. Later Ilani and Tzivoni [14] showed that the sites had properties similar to strong acid exchangers; the sulfur content of the filter corresponded to the measured capacity, if sulfonic acid groups were assumed to be present. Various other measurements made by Ilani [5] and by Ilani and Tzivoni [14] on these membranes have indicated that the fixed negative charges in the membrane dominate the electrochemical behaviour of the membrane,

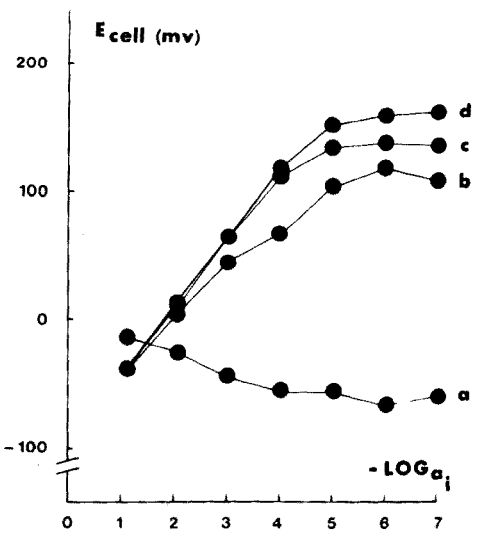

Fig. 5. Response of Millipore UGWP filter saturated with NEBA · ClO_4 in 1,1,2,2-tetrachloroethane with time. (a) freshly prepared, (b) 1 day, (c) 5 days, (d) 6 days. For significance of symbols used, see Fig. 1.

despite their low concentration in comparison with common ion-exchange resins.

The results presented above show that the membrane materials used in commercial ion-selective electrodes as well as other filter materials, contain enough fixed positive charges to convey anion selectivity. The potentiometric behaviour of the filters investigated is similar to that reported earlier [5, 6, 14] for other filters, except that these materials contain positive charges. The solvent influences the selectivity order to some degree. The presence of an ion exchanger has little effect unless there is a selective interaction between the ion exchanger and one or more of the ions. In other cases the mobile charges of the ion exchanger seem to have the same properties as the fixed charges on the membrane.

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COMPUTERIZED ELECTROANALYSIS. PART III. MULTIPLE SCANNING ANODIC STRIPPING AND ITS APPLICATION TO SEA WATER*

DANIEL JAGNER** and LARS KRYGER

Department of Inorganic Chemistry, University of Aarhus, Langelandsgade, DK-8000 Aarhus C (Denmark)

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SUMMARY

The determination of copper, lead, zinc, cadmium and bismuth in Standard Sea Water samples by multiple scanning anodic stripping has been investigated. The influence of plating potential and sample pH has been studied.

Since it is a highly sensitive multi-elemental technique requiring no sample pretreatment, anodic stripping voltammetry has been applied to the determination of toxic metals in sea water by several authors [1—8]. The method has, however, certain draw-backs. Because of the low concentrations of the metals in sea water, very long plating times are usually necessary. Moreover, as the method must be calibrated by means of standard additions, several plating periods have to be included in a single analysis, making the total time for analysis well over an hour. This time can be reduced by using an in situ mercury-plated glassy carbon electrode as the sensor. In thin film electrodes [9—15], however, copper and zinc form an intermetallic compound and these elements will consequently interfere mutually in the analyses.

By means of the multiple scanning technique described previously [16, 17], it ought to be possible to overcome these draw-backs. The increased sensitivity of this technique facilitates short plating times. Multiple stripping should allow the complete oxidation of the copper—zinc intermetallic compound, making it possible to correct for the interference of copper in the determination of zinc and vice versa. Moreover, because of the high degree of automation, the multiple scanning technique makes it possible to study the effects of varying the plating potentials and acidifying the samples. Although of considerable importance in the characterization of reducible metal species in natural waters these aspects have scarcely been dealt with by previous authors.

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**On leave from Department of Analytical Chemistry, University of Göteborg, Sweden.

EXPERIMENTAL

Instrumentation

In the electroanalytical instrument described previously all experimental parameters are under computer control [16]. Standard additions were thus made automatically by means of a motor syringe buret.

Chemicals

All reagents were of analytical grade. The water used for preparing standard solutions had been passed through a Millipore set-up, consisting of a prefilter, an organic adsorber, an ion-exchanger and a filter. Ultra-pure hydrochloric acid (Merck Suprapure) was used to acidify the samples. Analysis of the mercury solution used for plating the electrodes showed only insignificant amounts of other metals. Standard Sea Water (lot No. P_{67}, August 1974) was obtained from Charlottenlund Slot, Copenhagen. Samples of this water were used without filtering. Argon purified with active charcoal was used as inert gas for deaerating samples and reagents.

Electrodes

Polished glassy carbon "Ringsdorff glassartiger Kohle" was used as sensor electrode material [17]. The electrode was preplated daily with mercury in a solution containing 20 p.p.m. mercury. In order to achieve reproducibility, the plating potential was held under computer control. The plating sequence started at 0 V, and the potential was then continually decreased to −1.2 V in steps of 0.06 V, the plating time at each potential being 20 s. At the end of the plating cycle, the potential was returned to 0 V. The reference electrode was a Radiometer K401 saturated calomel electrode.

Procedure

Mercury(II) was added to 60-ml portions of the Standard Sea Water samples to yield a total concentration of 20 p.p.m. The samples were deaerated for 10 min before the start of the analysis. The multiple scanning analysis was performed according to the general features described previously [17].

STANDARD ADDITION ANALYSIS

A Standard Sea Water sample containing 20 p.p.m. of mercury(II) was plated for 3 min at −1.25 V. Ten analytical scans between −1.25 and −0.15 V were then recorded, the scan time being 1 s. The background was registered with ten similar scans after the electrode had been cleaned at 0 V. The difference between the sum of the currents from the analytical scans and the

sum of those from the background scans is shown in Fig. 1. The curves registered after three standard additions, each consisting of 6.25 p.p.b. zinc, 1.25 p.p.b. copper and lead, and 0.5 p.p.b. of cadmium, are also indicated. The total time for the analysis, including evaluation of the results, was less than 20 min. The results, which are summarized in Table 1, were evaluated as shown for lead in Fig. 2. Although the cadmium peak is scarcely discernible in Fig. 1, its height and area could be evaluated by the computer program. The results shown in Table 1 are in agreement with results previously obtained in sea-water analysis [1—15], but since the samples had been stored in sealed glass vessels for several months, too much significance should not be attributed to these results. The precision of the multiple scanning technique when applied to sea water was found, on consecutive analysis of the same sample, to be better than 3 %. This is illustrated in Fig. 3. The same experimental parameters as in Fig. 1 were used except that the plating time was 1 min only and the sample was analyzed three times without any addition of standard.

Determination of copper

It is well known that amalgamated copper and zinc form an intermetallic compound which is oxidized at a potential very close to the oxidation potential for copper. The peak denoted as copper in Fig. 1 can in fact be

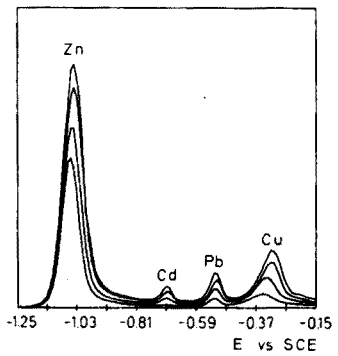


Fig. 1. Standard addition analysis of Standard Sea Water. Increments of 6.25 p.p.b. zinc, 1.25 p.p.b. copper and lead, and 0.5 p.p.b. cadmium were added. Plating time, 3 min; scan time, 1 s.

TABLE 1

Concentrations of metal ions in Standard Sea Water.
(The zinc value has been corrected for copper interference.)

Element	Cu	Zn	Pb	Cd	Bi
Concn. found (p.p.b.)	1.0	24	1.2	0.17	<0.01

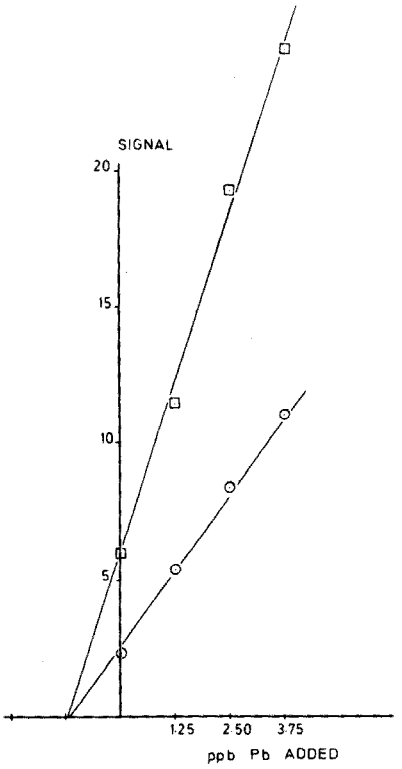


Fig. 2. Peak area (⊙) and peak height (⊡) plotted against p.p.b. lead added in the determination of lead in a Standard Sea Water sample.

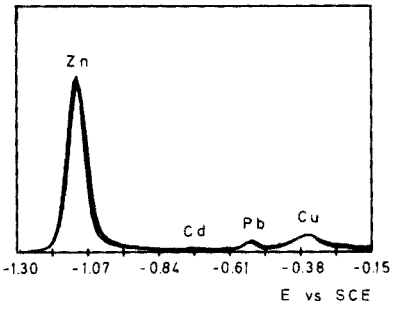


Fig. 3. Reproducibility of three plating and scanning sequences in the same Standard Sea Water sample. Plating time, 1 min; scan time, 1 s.

attributed to the copper—zinc compound.

The zinc concentration in sea water is normally considerably greater than the copper concentration. If the formation constant for the copper—zinc compound is sufficiently high, copper will always be bound quantitatively to

zinc at a constant ratio in the mercury phase. This will apply both to the sample and to the sample plus standard additions of copper and zinc. Consequently, the standard addition technique will yield accurate results for copper when the area or peak height of the copper—zinc peak is measured, provided that the multiple stripping technique allows the complete stripping of this peak.

The accuracy of the standard addition technique for the determination of copper was confirmed by analysis at two different plating potentials, namely −1.25 and −0.90 V. At −1.25 V zinc is reduced at the mercury electrode, while this is not possible at −0.90 V. Both analyses, by the multiple scanning technique, gave the same results, within the estimated experimental errors, demonstrating the accuracy of this technique for copper determinations.

Determination of zinc

Formation of the copper—zinc compound does, of course, decrease the height of the zinc peak. However, it has been shown that copper forms this compound quantitatively, so that it should be possible to correct the zinc value for copper interference, provided that the copper to zinc ratio in the compound is known. In order to determine this ratio, a Standard Sea Water sample was electrolyzed at −1.30 V for 2 min. Twenty analytical and ten background scans were then registered between −1.30 and −0.85 V, the scan time being 1 s. Three standard increments of 5 p.p.b. copper were then added. The result is shown in Fig. 4. A plot of the zinc peak height versus p.p.b. copper added (Fig. 5) showed that the copper to zinc ratio in the compound is 1 : 1. On extrapolation to zero, a zinc concentration of 25 p.p.b. is obtained. This value is close to the 24 p.p.b. obtained previously (cf. Table 1). Figure 5 shows that zinc can in fact be titrated with copper.

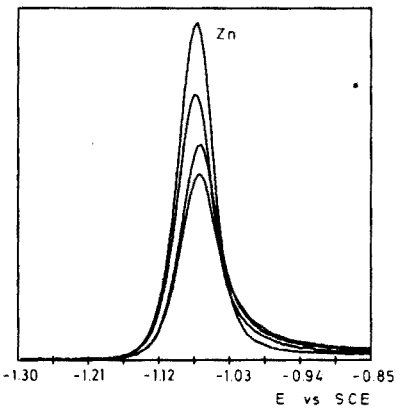


Fig. 4. Copper interference in the determination of zinc. Three aliquots of 5 p.p.b. copper were added to a Standard Sea Water sample. Plating time, 2 min; scan time, 1 s.

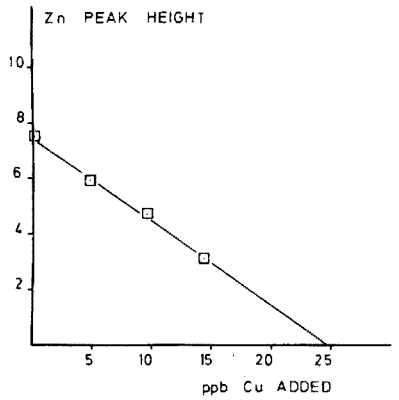


Fig. 5. Zinc peak height plotted against p.p.b. copper added.

From the results shown in Figs. 4 and 5, it can be concluded that zinc can be determined in the presence of copper by means of the multiple scanning technique. The value obtained for copper is simply added to that obtained from the zinc peak (or area) measurements. This correction is, however, only applicable if zinc is in excess of copper.

Addition of gallium

It has recently been shown that the interference of copper in the determination of zinc can be eliminated by the addition of an excess of gallium, the gallium—copper intermetallic compound having a higher formation constant than the corresponding zinc—copper compound [18]. Addition of gallium was attempted in the analysis of sea water but was found to have little effect on the copper—zinc interference. This was attributed to the extensive hydrolysis of gallium in neutral solutions and it was concluded that addition of gallium eliminates the copper interference only in very acidic solutions.

Determination of bismuth

In acidic solutions bismuth can be amalgamated at a mercury electrode. In sea water the metal is stripped off at a potential of approximately −0.10 V [14, 15]. Since this is in a potential range with large capacitance background currents and close to the commencement of stripping mercury itself, the multiple scanning technique should be particularly suitable for bismuth analysis, since it involves background correction.

The possibility of determining bismuth was investigated by adding 0.1 p.p.b. bismuth to a Standard Sea Water sample acidified to pH 0.8 with hydrochloric acid. The sample was plated for 30 min at −0.5 V, and thirty

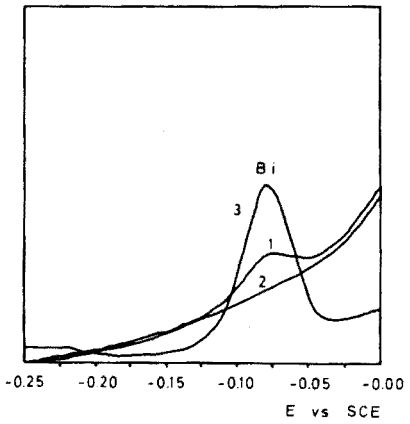


Fig. 6. The sum of thirty analytical scans (curve 1), thirty background scans (curve 2) and the difference between these sums (curve 3) for the analysis of a Standard Sea Water sample spiked with 0.1 p.p.b. bismuth at pH 0.8.

analytical and background scans were registered between —0.25 and —0.02 V, the scan time being 0.2 s. Figure 6 shows the sum of the analytical scans (curve 1), the sum of the background scans (curve 2) and the difference between these sums (curve 3). The experiment was repeated for a sample to which no bismuth was added; no signal was registered. It was therefore concluded that the concentration of reducible bismuth in the Standard Sea Water sample was less than 0.01 p.p.b., this value being the detection limit estimated from Fig. 6.

ACIDIFIED SAMPLES

Hitherto, anodic stripping has been used only to determine total reducible (often denoted soluble) metal concentrations in natural waters. Consequently, concentrated acid has been added to the samples before analysis in order to liberate metal ions from any chelate complexes present in the sample. This addition has normally been made in connection with sampling.

In order to study the effect of adding acid, a Standard Sea Water sample was electrolyzed for 3 min at —0.90 V. Thirty analytical and fifteen background scans were then recorded over the lead peak between —0.65 and —0.40 V and with a total scan time of 0.3 s. Concentrated hydrochloric acid was then added until a pH value of 0.8 was obtained, and the experiment was repeated. As can be seen from Fig. 7, the addition of acid increased the lead peak by a factor of almost two. This does not, however, necessarily mean that a standard addition analysis on an acidified sample would yield a lead concentration twice that obtained in a neutral sample under the same experimental conditions. If, for example, the lead in the neutral solution is bound to a complexing agent with a total concentration considerably greater

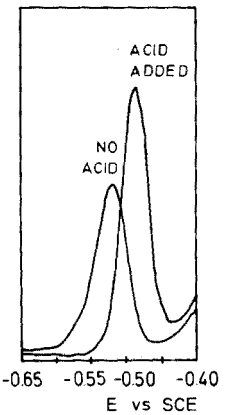
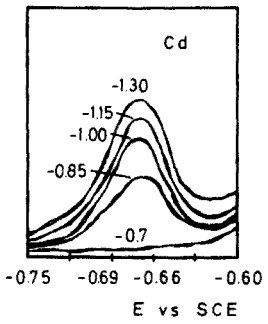


Fig. 7. Determination of lead in the same Standard Sea Water sample at pH 8 and pH 0.8. Plating time, 3 min; scan time, 0.3 s.

than the lead concentration, then lead from the standard aliquots will be complexed to the same degree as the lead in the sample. In order to find out whether this was the case for the particular sample under consideration, standard addition analysis, with the same experimental parameters as in Fig. 7, was done both on a neutral and on an acidified sample, the latter giving almost twice the lead concentration. There is no self-evident explanation for this. Hydrolysis [19] would, indeed, explain the factor of two, but only provided that the dominant hydrolytic species, $PbOH^+$, is formed slowly and cannot be reduced at the mercury electrode. However, as the lead concentration is only of the order of magnitude of 10^{-9} M, it is not unlikely that the difference could be explained by the presence of organic complexing agents. Furthermore, metal species either adsorbed on or contained in solid particles could be made reducible at the mercury film by means of acidification. It can, however, be concluded that when analytical results obtained by anodic stripping analysis are reported, the experimental conditions with regard to filtering, acidification and storage ought to be specified in detail.

PLATING POTENTIALS

When anodic stripping is used to determine the total amount of reducible metal species, it is natural to select a plating potential as low as possible. In a neutral sea-water sample, this value is approximately −1.35 V, the minimum value increasing, of course, by 0.06 V when the pH is decreased by one unit. If, however, the metal species are reducible at different potentials then plating at varying potentials would yield information concerning the relative abundance of the different species. This was studied for cadmium in Standard Sea Water samples by plating for 6 min at five different potentials, namely −0.7, −0.85, −1.00, −1.15 and −1.30 V. Thirty analytical and thirty background scans over the cadmium peak between −0.75 and −0.60 V



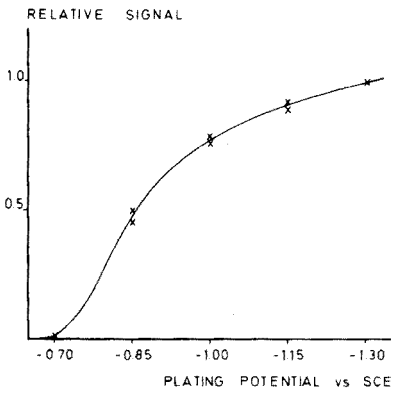


Fig. 8. Signals obtained in the determination of cadmium in the same Standard Sea Water sample when plating at −0.7, −0.85, −1.00, −1.15 and −1.30 V. Duplicate runs at each potential.

Fig. 9. Relative signals obtained in the determination of cadmium in the same Standard Sea Water sample plotted against plating potential. The signal obtained at −1.30 V is set equal to one.

were then registered, the total scan time being 0.4. Duplicate plating and scannings were performed at each potential. The results (Fig. 8) show that a decrease in plating potential, increases the signal. Duplicate analyses at the same plating potential give reproducible results. In Fig. 9 the relative signals have been plotted against the plating potential, the signal obtained at −1.30 V being set equal to one. The experiment shown in Fig. 8 was repeated five times, all experiments yielding distribution curves very similar to that given in Fig. 9.

The effect of varying the plating potential was also studied for the copper signal at four different potentials, namely −0.50, −0.60, −0.75 and −0.95 V. At potentials lower than −0.95 V formation of the zinc–copper intermetallic compound seriously affects the copper signal; this might also be the case to a certain extent at −0.95 V. The result is shown in Fig. 10. Obviously, the copper signal is also greatly affected by decreasing the plating potential, indicating that copper may exist in the form of several complexes reducible at different potentials. It must, however, be emphasized that in a complete investigation, standard addition calibrations must also be performed at each plating potential. This compensates for changes in plating current with plating potential occurring at high stirring rates where the plating current is not entirely diffusion-controlled.

α-coefficients

Analogously to the α-coefficient concept [20] for complexing agents, an α-coefficient, α_E, based on the plating potential, E, can be defined by

$$\log \alpha_E = (E_{1/2} - E)nF(RT\ln 10)^{-1} \quad (1)$$

where R, T, n and F have their usual meanings and $E_{1/2}$ is the half-wave potential for the metal-metal ion pair under consideration.

In Fig. 11 the relative cadmium and copper signals from the experiments shown in Figs. 8 and 10, respectively, have been plotted against $\log \alpha_E$.

For analytical reagents based on, for example, chelate formation or ion-exchanger separation, an α-coefficient can be calculated for each metal to be determined, provided that the relevant stability constants are available [20]. For example, at a specified free concentration of dithizone and at a specified

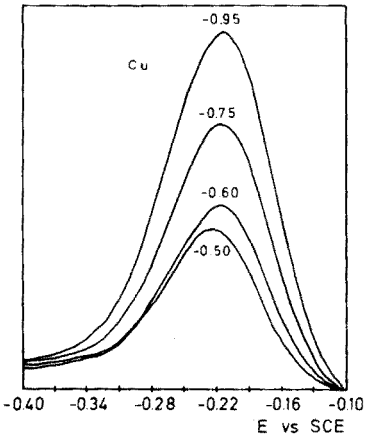


Fig. 10. Signals obtained in the determination of copper in the same Standard Sea Water sample when plating at —0.50, —0.60, —0.75 and —0.95 V.

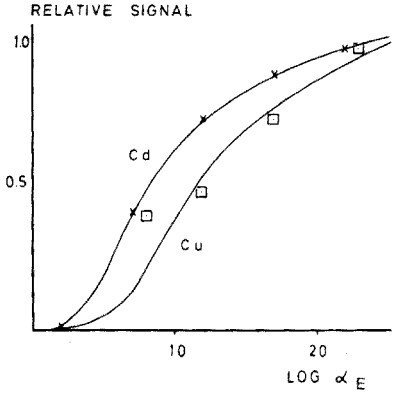


Fig. 11. Relative signals obtained in the determination of copper and cadmium in Standard Sea Water at different plating potentials plotted against α_E. For copper the signal obtained at —0.95 V has been set equal to one, and for cadmium the signal obtained at —1.30 V has been set equal to one.

pH value, the α-coefficient for the extraction of cadmium into chloroform can be calculated. Provided that the system is in equilibrium, the value of the α-coefficient is an indication of the completeness of the analytical reaction. Even though α-coefficients obtained from chelating reagents and those obtained from plating potentials are not directly comparable, those obtained from plating potentials being relevant for reducible species only, the results shown in Fig. 11 could well explain the discrepancies often found between results obtained by means of anodic stripping analysis and those obtained by means of solvent extraction—atomic absorption analysis. Figure 11 can also explain why the anodic stripping results are sometimes higher and sometimes lower than those obtained by atomic absorption analysis.

CONCLUSIONS

Multiple scanning anodic stripping with background subtraction has been shown to be suitable for the determination of reducible species of zinc, copper, lead and cadmium in sea water. The technique yields accurate values, since the zinc interference with the copper determination is eliminated and the copper interference with the zinc determination is corrected for. Because of the sensitivity of the technique, a standard addition multi-element analysis can be performed automatically in 20 min, after deaeration has been completed.

It has been shown that the plating potential and the pH of the sample are vital parameters in the anodic stripping analyses. It is also apparent that anodic stripping may yield information concerning the nature of metal species in samples of natural water. In this respect, the method is complementary to methods such as atomic absorption analysis with a graphite furnace, or x-ray fluorescence analysis, which yield total concentrations only. With automatic instrumentation, the nature of species occurring in a particular sea-water sample could be investigated by analyzing at, e.g., six different pH values and at, e.g., five different plating potentials for each pH value. Such analyses could be of interest in the investigation of toxic metals in coastal or polluted areas.

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VOLTAMMETRY AND ELECTROCHEMICAL STRIPPING ANALYSIS FOR ANTIMONY IN AQUEOUS AND NONAQUEOUS MEDIA AFTER EXTRACTION

T. V. NGHI and F. VYDRA

The J. Heyrovský Institute of Physical Chemistry and Electrochemistry, Czechoslovak Academy of Sciences, Prague 1, Jilská 16 (Czechoslovakia)

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SUMMARY

Cyclic voltammetry of antimony was studied in aqueous media (HCl—LiCl) and in nonaqueous media after extraction with 20 % tri-n-butylphosphate in toluene, with a rotating glassy carbon disc electrode. Reduction of antimony to the element in aqueous media is nearly reversible, but irreversible in nonaqueous media. Anodic stripping voltammetric and chronopotentiometric determinations were also studied in nonaqueous media; methanol and LiCl, NH_4SCN or NH_4NO_3 were used as base electrolytes. In nonaqueous media, antimony can be determined down to concentrations of 10^{-8} M by stripping voltammetry, and 10^{-7} M by stripping chronopotentiometry. Electrochemical stripping determinations of 10^{-6} M antimony(III) were not affected by Co^{2+}, Ni^{2+}, Cd^{2+}, Zn^{2+} or As^{3+} ($5 \cdot 10^{-3}$ M), Ag^{+} ($4 \cdot 10^{-4}$ M in stripping voltammetry or 10^{-3} M in stripping chronopotentiometry), Hg^{2+} ($5 \cdot 10^{-4}$ M), Pb^{2+} ($3 \cdot 10^{-4}$ M), Cu^{2+} ($1.5 \cdot 10^{-4}$ M) Sn^{2+} and Sn^{4+} ($7 \cdot 10^{-4}$ M), Fe^{3+} ($4 \cdot 10^{-4}$ M), Au^{3+} ($5 \cdot 10^{-5}$ M) and Bi^{3+} ($1.5 \cdot 10^{-5}$ M). The stripping chronopotentiometric determination showed better selectivity.

Antimony is one of the elements that can be favorably determined by electrochemical stripping methods on solid graphite and various carbon electrodes [1, 2], with voltammetric measurement. However, elements such as Sn^{4+}, Cu^{2+}, Bi^{3+}, etc., interfere with its determination in aqueous solutions [2], so that separation is needed for the analysis of complex materials. The advantages of separation of antimony by extraction and its direct determination in nonaqueous media have been shown recently [3, 4].

Antimony can be quantitatively and selectively extracted as an ion associate with a 20 % solution of tri-n-butyl phosphate (TBP) in toluene from 2 M HCl—1 M LiCl media [5—7]. In this paper is presented a study of the voltammetry of antimony in aqueous and nonaqueous media, and of an electrochemical stripping determination of antimony after extraction.

EXPERIMENTAL

Apparatus and reagents

The cyclic voltammetric and stripping voltammetric polarization curves were recorded with a Radelkis OH-102 three-electrode polarograph. The rotating disc electrode was made from glassy carbon (Tokay Electrode Mfg. Co., Japan) with a diameter of 5 mm, fixed in a teflon tube 15 mm in diameter. The rotor apparatus was designed in this Institute. An electrolytic vessel with a teflon lid, a saturated calomel reference electrode and a platinum auxiliary electrode were employed. Before each measurement the glassy carbon electrode surface was polished with metallographic sand-paper (SIA No 6).

For the chronopotentiometric curves, the OH-102 polarograph was used for the electrolysis at constant potential and a Polarecord E 261 (Metrohm, Switzerland) was used as the recording potentiometer. Constant current was supplied from a 100-V battery with a suitable resistance. The rotating disc electrode and the other equipment were the same as for the voltammetric measurements. E-τ curves were recorded under the following conditions: chart speed, 2.4 cm s^{-1}; potential scan rate, 80 or 40 mV s^{-1}. All potentials were measured against the saturated calomel electrode.

Reagent-grade TBP (BDH Chemicals Ltd, England) and toluene (Lachema, Brno) were used. All the other chemicals used were of reagent grade, except for Suprapure hydrochloric acid and methanol (Merck, West Germany). Twice-distilled water from quartz apparatus was used in all measurement. To increase the conductivity of the nonaqueous media, a 0.5 M solution of lithium chloride or ammonium thiocyanate, or a 0.35 M solution of ammonium nitrate was used.

Extraction of antimony and preparation of solution for electrolysis

Antimony was extracted from 2 M HCl—1 M LiCl solutions with an equal volume of 20 % TBP in toluene. Because the distribution ratio of antimony is high [5], only one extraction was carried out; the extraction efficiency was verified by analysis of the aqueous phase. The solution for electrolysis comprised 30 ml of extract and 20 ml of electrolyte solution in methanol. During the deposition and stripping processes, nitrogen was allowed to flow over the solution to prevent contact with air; the nitrogen used was passed previously through a solution identical in composition with the analyzed solution

Voltammetric measurements

Cyclic voltammetry

The voltammetric curves were recorded during rotation of the electrode at 2200 r.p.m.; the potential was shifted from + 0.5 V to the more negative potential selected and the polarity was immediately reversed.

Stripping voltammetry

Electrolysis of aqueous and nonaqueous solutions of antimony was realized during rotation of the electrode at a constant potential. After completion of the electrolysis, the electrode was polarized to a more positive potential and the stripping peak of antimony was recorded.

Stripping chronopotentiometry

After electrolysis at constant potential on the OH-102 polarograph (as in the case of stripping voltammetry), the deposit was dissolved by application of a suitable small constant current, and the E—τ curve was recorded (Polarecord E 261).

RESULTS AND DISCUSSION

Cyclic voltammetry of antimony in aqueous media

Figure 1 shows the cyclic voltammogram of antimony in 0.4 M HCl—0.2 M LiCl medium in the potential range +0.5 to —0.5 V; a cathodic wave with the half-wave potential $(E_{1/2})_{cat}$ = —0.29 V and an anodic peak at about 0 V can be seen. The existence and the size of the peak depends on the potential at which cathodic polarization was stopped (or the starting potential of the "anodic" polarization), i.e. the peak depends on the range of the polarization potential. Figure 2 shows cyclic voltammograms of antimony in various polarization ranges; for the range +0.5 V to —0.25 V (—0.25 V is the potential corresponding to the bottom of the antimony wave), there is no peak (curve a), but in the range +0.5 V to —0.3 V (—0.3 V is the half-wave potential of antimony) the peak starts to form (curve b), and if the cathodic polarization potential is more negative, the peak is bigger (curve c and Fig. 1). These results show that the oxidation peak of antimony forms only at anodic polarization potentials in the range of the wave corresponding to reduction of antimony(III) to the metal.

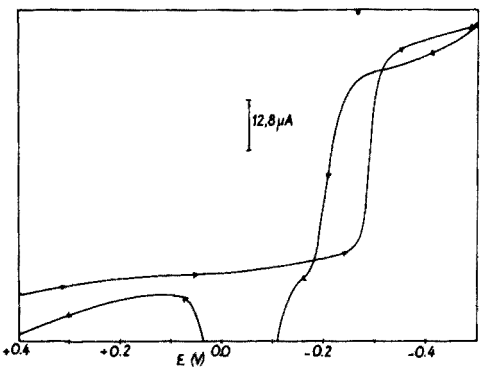

Fig. 1. Cyclic voltammogram of antimony in aqueous medium. 10^{-4} M Sb^{3+}; 0.4 M HCl—0.2 M LiCl; $\omega \doteq 2200$ r.p.m.; v = 4.2 mV s^{-1}; $d_{electrode}$ = 5 mm.

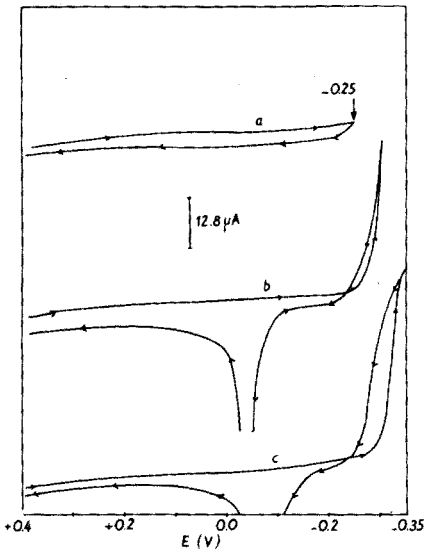

Fig. 2. Cyclic voltammograms of antimony in aqueous medium in various polarization ranges. Conditions as in Fig. 1.

To establish reversibility, and to calculate the number of electrons involved in the electrode process, logarithmic analysis was carried out. According to Delahay [8], for reversible reduction of metal ions to the metal at a rotating electrode or in a stirred solution the equation relating the current and potential has the form

$$E = \text{const.} + \frac{0.058}{n} \log(i_d - i) \text{ (at 20 °C)} \quad (1)$$

This equation was deduced on the assumption that the activity of the solid deposit is unity, i.e. the surface of the electrode is completely covered by the deposit. Figure 3 shows the $\log(i_d - i)$ vs. E plot for the reduction wave of antimony(III) in the potential range −0.265 to −0.33 V; in the range −0.265 V to −0.29 V the relationship is not linear (part AB on the curve), so that eqn. (1) is not valid, possibly because the electrode surface is not completely covered. In the range −0.29 V to −0.31 V, the relationship is linear, the slope being 1 : 24 (part BC of curve), which gives a value of 2.5 electrons, so that the reduction of antimony(III) in this range is not quite reversible. Above −0.31 V, eqn. (1) is valid for the reduction of antimony(III): part CD of the curve has a slope of 1 : 20, which corresponds to 3 electrons. At these potentials the surface of the electrode is completely covered by the deposit under the given experimental conditions.

The reduction wave of antimony(III) is diffusion-controlled; its height is directly proportional to the concentration of antimony in the solution in the range $8 \cdot 10^{-5}$ to $2 \cdot 10^{-4}$ M, and is directly proportional to the square root of the angular velocity of the rotating disc electrode.

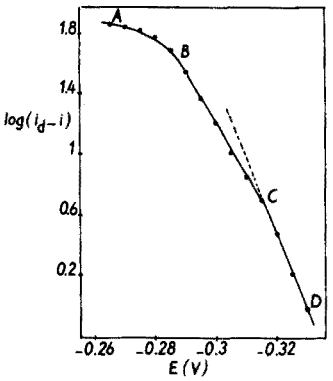

Fig. 3. Logarithmic analysis of the antimony wave in aqueous medium. Conditions as in Fig. 1.

Anodic stripping voltammetry of antimony in aqueous media

Figure 4 shows typical anodic stripping curves of antimony in 0.1 M HCl—0.2 M LiCl media. Under optimal conditions antimony could be determined down to 10^{-8} M concentrations (curve 2 of Fig. 4). The maximum current (peak height) depends on the deposition potential, as shown in Fig. 5. At potentials more negative than —0.25 V, electrolysis starts, in agreement with the results of the cyclic voltammetric studies; i_p shows a plateau in the potential range —0.6 V to —0.75 V, but at potentials more negative than —0.85 V peak heights decrease rapidly, probably because at these very negative potentials antimony anions are formed in the electrode double layer [4].

The anodic stripping peak of antimony is well developed in acidic media

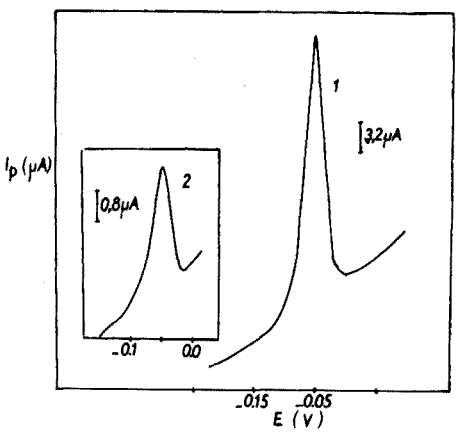

Fig. 4. Anodic stripping voltammetric curves of antimony in aqueous medium. 0.1 M HCl—0.2 M LiCl; ω = 2200 r.p.m., v = 25 mV s^{-1}; $d_{electrode}$ = 5 mm. (1) 10^{-6} M Sb^{3+}, t_{el} = 2 min, E_{el} = —0.7 V (2) $2 \cdot 10^{-8}$ M Sb^{3+}, t_{el} = 18 min, E_{el} = —0.7 V.

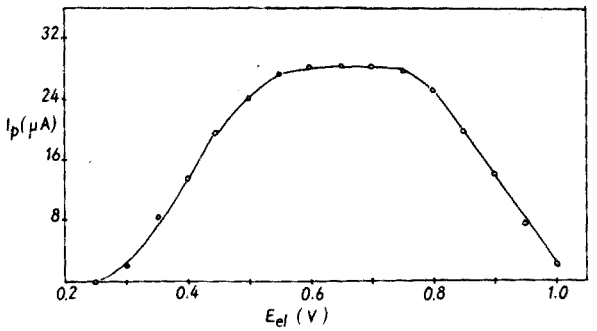

Fig. 5. Dependence of maximum current, i_p, on the deposition potential, E_{el}, in aqueous medium. 10^{-6} M Sb^{3+}; 01. M HCl—0.2 M LiCl; ω = 2200 r.p.m.; v = 25 mV s^{-1}; t_{el} = 2 min; $d_{electrode}$ = 5 mm.

containing various electrolytes (Table 1). The optimal deposition potential for the determination of antimony in these media is —0.7 V. For various concentration ranges, the optimal time of electrolysis (under the conditions given in the experimental part) is as follows:

10^{-8}—$5 \cdot 10^{-8}$ M, 20—18 min; $5 \cdot 10^{-8}$—10^{-7} M, 15—13 min; 10^{-7}—$5 \cdot 10^{-7}$ M, 10—8 min; $5 \cdot 10^{-7}$—10^{-6} M, 5—3 min.

Anodic stripping chronopotentiometry of antimony in aqueous media

Figure 6 shows typical stripping chronopotentiometric curves for antimony in 0.2 M HCl—0.2 M LiCl medium, in which, under the optimal conditions, antimony can be determined down to $5 \cdot 10^{-8}$ M (curve 2 of Fig. 6). The dissolution time depends on the deposition potential and this dependence is the same as for stripping voltammetry. The best deposition potentials are again in the potential range —0.6 to —0.75 V. The dissolution time depends on the current; this dependence is not linear, as in the case of other metals.

TABLE 1

Anodic stripping voltammetry and chronopotentiometry of antimony in aqueous media (10^{-6} M Sb^{3+}, ω = 2200 r.p.m., E_{el} = —0.7 V, $d_{electrode}$ = 5 mm)

Electrolyte	Voltammetry[a]		Chronopotentiometry[b]	
	E_p	i_p	E_{dis}	τ_{dis}
	(V, SCE)	(μA)	(V, SCE)	(s)
0.2M LiCl + 0.1M HCl	—0.05	27.4	—0.06	10.0
0.2M NH_4SCN + 0.1M HCl	—0.04	26.5	—0.03	9.8
0.2M NH_4NO_3 + 0.1M HCl	—0.02	26.6	—0.02	9.7

[a] t_{el} = 2 min; v = 25 mVs^{-1}.
[b] t_{el} = 5 min; i_{dis} = 34 μA.

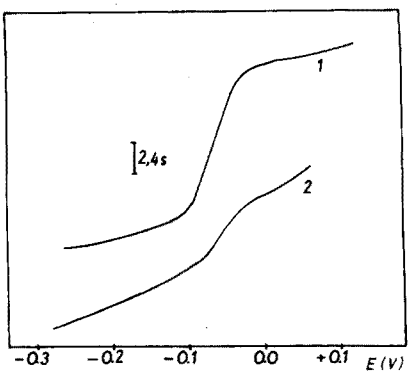

Fig. 6. Anodic stripping chronopotentiometric curves of antimony in aqueous medium. 0.1 M HCl—0.2 M LiCl; ω = 2200 r.p.m.; E_{el} = —0.7 V; $d_{electrode}$ = 5 mm. (1) $2 \cdot 10^{-6}$ M Sb^{3+}; t_{el} = 4 min; i_{dis} = 34 μA. (2) $5 \cdot 10^{-6}$ M Sb^{3+}; t_{el} = 20 min; i_{dis} = 24 μA.

The dissolution potential (E_{dis}) depends on the value of i_{dis} and the dependence E_{dis} — log i_{dis} is linear. Under the conditions given in the experimental part, the optimal parameters for the determination of antimony are as follows:

$[Sb^{3+}]$ (M)	t_{el} (min)	i_{dis} (μA)
$5 \cdot 10^{-8}$—10^{-7}	20	20—25
10^{-7}—10^{-6}	10	25—30
10^{-6}—$5 \cdot 10^{-6}$	4—6	30—35

As for stripping voltammetry, antimony can be successfully determined in acidic media containing various electrolytes (Table 1).

Cyclic voltammetry of antimony in nonaqueous media after extraction

Figure 7 shows the cyclic voltammogram of antimony in TBP—toluene—methanol (3:12:10) medium containing 0.2 M LiCl in the potential range +0.5 V to —0.6 V. As in aqueous solutions, a wave and a peak were observed; for the wave, $E_{1/2}$ = —0.52 V, and the peak potential is 0 to —0.1 V, while the peak size again depends on the polarization range. This dependence is shown in Fig. 8; —0.45 V is before the antimony wave (curve a), whereas —0.5 V and —0.54 V are near the half-wave potential of antimony (curves b and c), —0.6 V corresponding to the limiting current of the antimony wave (Fig. 7). At the concentrations used above in aqueous media, the reduction of antimony(III) is irreversible in nonaqueous media; logarithmic analysis showed that eqn. (1) was not valid at all. However, the reduction wave of antimony has some diffusion-controlled characteristics; the height of the wave is directly proportional to the antimony concentration and to the square root of the angular velocity of the rotating disc electrode.

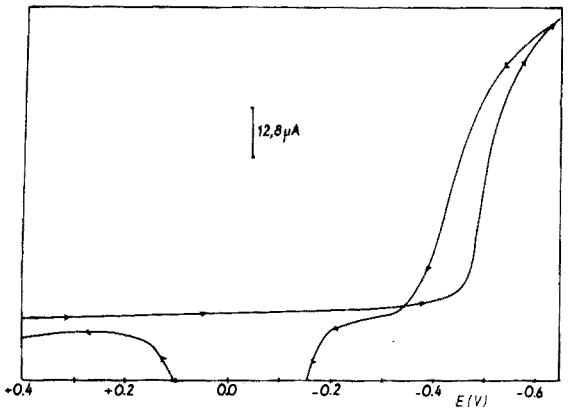

Fig. 7. Cyclic voltammogram of antimony in nonaqueous medium after extraction. $2 \cdot 10^{-4}$ M Sb^{3+}; 30 ml of TBP—toluene + 20 ml of CH_3OH, 0.2 M LiCl; ω = 2200 r.p.m.; v = 4.2 mV s^{-1}, $d_{electrode}$ = 5 mm.

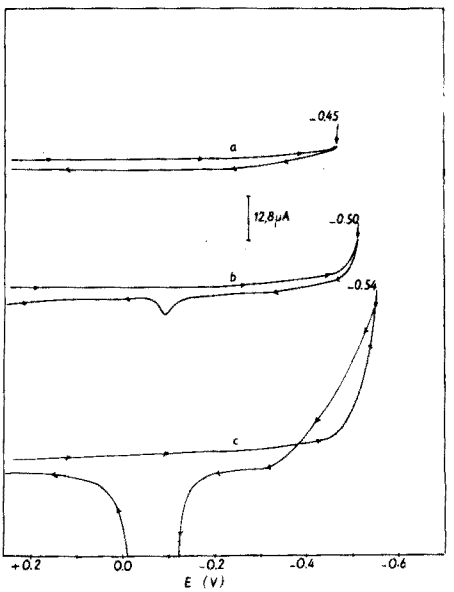

Fig. 8. Cyclic voltammograms of antimony in nonaqueous medium in various polarization ranges. Conditions as in Fig. 7.

The electrochemical behaviour of antimony in the nonaqueous and aqueous media is therefore quite similar, except that in nonaqueous media the reduction is irreversible and the $E_{\frac{1}{2}}$ value is more negative so that the anodic peak is formed at more negative cathodic polarization potentials.

Anodic stripping voltammetric determination of antimony in nonaqueous media after extraction

The anodic stripping curves of antimony in nonaqueous media are similar to those in aqueous media. Antimony can be determined down to $2 \cdot 10^{-8}$ M, which is almost the same concentration limit as in aqueous media, and the dissolution peak is obtained in the presence of various electrolytes (LiCl, NH_4SCN and NH_4NO_3), as shown in Table 2.

TABLE 2

Anodic stripping voltammetry and chronopotentiometry of antimony in nonaqueous media
(30 ml TBP + 20 ml CH_3OH, ω = 2200 r.p.m., $d_{electrode}$ = 5 mm)

Electrolyte	Voltammetry[a]		Chronopotentiometry[b]	
	E_p	i_p	E_{dis}	τ_{dis}
	(V, SCE)	(μA)	(V, SCE)	(s)
0.2 M LiCl	−0.06	10.2	−0.065	7.5
0.2 M NH_4SCN	−0.05	9.6	−0.04	7.2
0.15 M NH_4NO_3	+0.02	9.6	−0.02	7.2

[a] $5 \cdot 10^{-7}$ M Sb^{3+}, E_{el} = 0.8 V, t_{el} = 5 min, v = 25 m Vs^{-1}.
[b] $2 \cdot 10^{-6}$ M Sb^{3+}, E_{el} = −0.78 V, t_{el} = 6 min, i_{dis} = 25 μA.

The maximum current i_p depends on the deposition potential, E_e. In the presence of lithium chloride, i_p is maximal for E_e values of −0.75 to −0.8 V, and in the presence of ammonium thiocyanate or nitrate for E_e values of −0.78 to −0.85 V; at more negative potentials, i_p decreases rapidly. The optimal times of electrolysis for different antimony concentrations are as follows: 10^{-8}—$5 \cdot 10^{-8}$ M, 20 min; $5 \cdot 10^{-8}$—10^{-7} M, 15 min; 10^{-7}—$5 \cdot 10^{-7}$ M, 10 min; $5 \cdot 10^{-7}$—10^{-6} M, 5—3 min; 10^{-6}—$5 \cdot 10^{-6}$ M, 2 min.

The peak height is directly proportional to the antimony concentration under the optimal conditions.

Anodic stripping chronopotentiometric determination of antimony in nonaqueous media after extraction

Antimony can be determined down to 10^{-7} M by anodic stripping chronopotentiometry, the curves being similar to those in aqueous solutions. The dissolution time (τ_{dis}) depends on the deposition potential, and this dependence is similar to that found in stripping voltammetry; the optimal deposition potentials are in the range −0.75 to −0.8 V. The values of τ_{dis} and E_{dis} in nonaqueous media in the presence of various electrolytes (Table 2) depend on the dissolution current i_{dis}; as in aqueous media the $\tau_{dis} - i_{dis}$ relationship is not linear, and the $E_{dis} - \log i_{dis}$ dependence is linear

(Fig. 9). The optimal conditions for the determination of antimony in different concentration ranges are as follows:

$[Sb^{3+}]$ (M)	t_{el} (min)	i_{dis} (μA)
$8 \cdot 10^{-8}$—10^{-7}	20	20
10^{-7}—$5 \cdot 10^{-7}$	15	25—30
$5 \cdot 10^{-7}$—10^{-6}	12	25—30
10^{-6}—$5 \cdot 10^{-6}$	6—4	30—35

Reproducibility of measurements

Under the recommended conditions, the stripping voltammetric and chronopotentiometric determinations of antimony show good reproducibility. For anodic stripping voltammetry of $4 \cdot 10^{-7}$ M Sb^{3+} in thiocyanate medium under the conditions shown in Table 2, at a sensitivity of $1.2 \cdot 10^{-7}$ A mm^{-1}, a mean peak height of 72.2 mm was found, with a range of 71.5 to 73.0 mm (5 results). For stripping chronopotentiometry in ammonium nitrate medium for 10^{-6} M Sb^{3+}, the mean τ_{dis} value was 7.74 s with a range of 7.68—7.805 s (5 results). When 10^{-6} M antimony(III) in 2 M HCl—1 M LiCl solution was extracted under the recommended conditions, and anodic stripping chronopotentiometry was applied under the conditions outlined in Table 2 for 0.2 M LiCl medium, the τ_{dis} value found was 5.28 s with a range of 5.16—5.40 s (4 results).

ANALYTICAL APPLICATIONS

The efficiency of the extraction of antimony depends on the concentration of hydrochloric acid and lithium chloride in the aqueous phase. Antimony is quantitatively extracted from a 2 M HCl + 1 M LiCl solution (% E = 100,

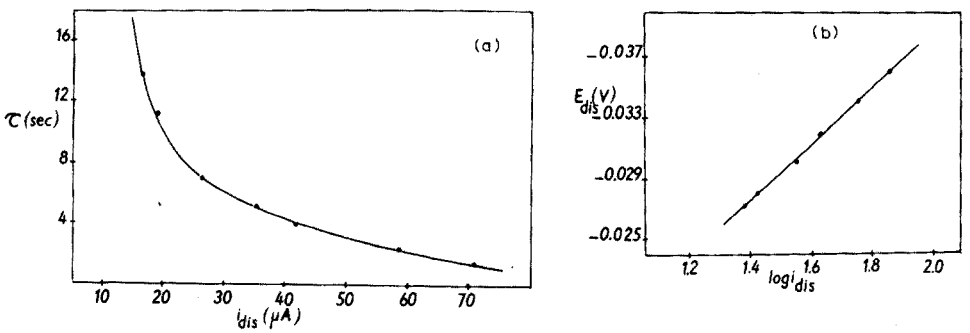

Fig. 9. The τ_{dis}—i_{dis} (a) and E_{dis}—log i_{dis} (b) relationships in nonaqueous medium. 10^{-6} M Sb^{3+}; 30 ml of TBP in toluene + 20 ml of CH_3OH; 0.15 M NH_4NO_3: ω = 2200 r.p.m.; E_{el} = —0.8 V; t_{el} = 7 min; $d_{electrode}$ = 5 mm.

D $\sim \infty$)[6]. From this medium Co^{2+}, Ni^{2+}, Cd^{2+}, Zn^{2+}, As^{3+} are practically not extracted. In the presence of these metal ions at concentrations of $5 \cdot 10^{-3}$ M, 10^{-6} M antimony(III) could be determined with a standard deviation of 3 %. Ag^{+}, Hg^{2+}, Pb^{2+}, Cu^{2+}, Fe^{3+}, Bi^{3+}, Au^{3+}, Sn^{2+} and Sn^{4+} were extracted to some extent. The influence of these elements on the determination of antimony was therefore studied in detail.

When the concentration of silver(I) in the aqueous phase exceeded $4 \cdot 10^{-4}$ M, it was partially extracted and gave a peak in stripping voltammetry (E_p = +0.13 V in 0.15 M NH_4NO_3) and a dissolution "wave" in stripping chronopotentiometry (E_{dis} = +0.1 V in 0.15 M NH_4NO_3); the voltammetric peak of antimony was then deformed and the determination of antimony was inaccurate. However, antimony could still be successfully determined by stripping chronopotentiometry; 10^{-6} M Sb^{3+} was determined ($s_r \approx 5$ %) in the presence of 10^{-3} M Ag^{+} (Fig. 10). Tin(II) and tin(IV) interfered when their concentrations exceeded $7 \cdot 10^{-4}$ M; in the nonaqueous solutions, the tin ions gave a peak with E_p = −0.13 V (in 0.1 M NH_4NO_3) but 10^{-6} M antimony(III) could be determined (s_r = 4 %) in the presence of $7 \cdot 10^{-4}$ M tin in the initial aqueous media. In the presence of $3 \cdot 10^{-4}$ M lead(II) in the initial aqueous phase, antimony could be determined with an error of ±4 % after its extraction.

Copper and bismuth in nonaqueous media gave peaks at the same potential as antimony, but 10^{-6} M antimony could be determined ($s_r \approx 4$ %) in the presence of $1.5 \cdot 10^{-4}$ M Cu^{2+} and $1.5 \cdot 10^{-5}$ M Bi^{3+}. Iron(III) decreased the height of the antimony peak at concentrations above $4 \cdot 10^{-4}$ M. Gold at concentrations of $5 \cdot 10^{-5}$ M did not affect the determination of antimony.

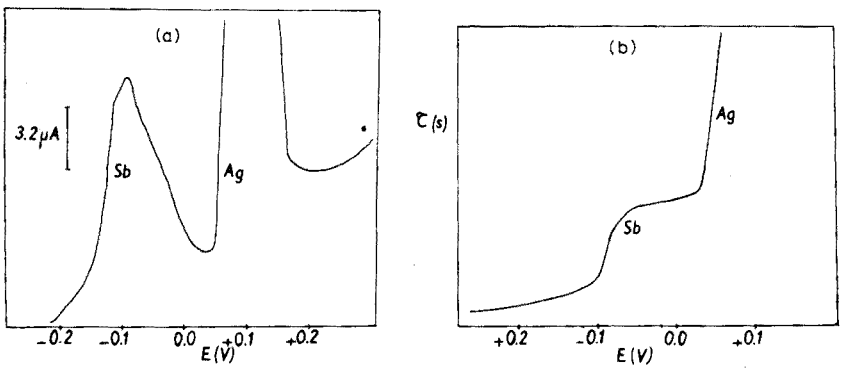

Fig. 10. (a) Anodic stripping curves of antimony and silver after extraction. 10^{-6} Sb^{3+} and 10^{-3} M Ag^{+} in the initial aqueous medium; final medium as in Fig. 9; t_{el} = 3 min, v = 25 mV s^{-1}. (b) Anodic stripping chronopotentiometric curves of antimony and silver after extraction; conditions as for (a), but t_{el} = 6 min, i_{dis} = 25 μA.

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THE DETERMINATION OF MANGANESE IN SMALL SAMPLES OF BIOLOGICAL TISSUE BY FLAMELESS ATOMIC ABSORPTION SPECTROMETRY

G. B. BELLING and G. B. JONES

C.S.I.R.O., Division of Human Nutrition, Kintore Avenue, Adelaide, South Australia 5000 (Australia)

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SUMMARY

A method for the determination of manganese in small samples of biological tissues is described. Interferences are removed by extracting iron with cupferron-MIBK below pH1; the pH is raised to 7.5 and the manganese extracted with cupferron-MIBK to separate it from sodium, potassium, calcium, and magnesium which are shown to interfere. After evaporation of the MIBK, the manganese cupferrate is dissolved in 70 % nitric acid and determined by flameless atomic absorption spectrometry.

To determine the manganese content of small samples of muscle and liver taken at biopsy, very small amounts, often less than $3 \cdot 10^{-8}$ g in the entire sample, must be measured. Neutron activation is suitable [1] but is not widely available. In the most sensitive spectrophotometric methods [2—6], manganese catalyses the oxidation of some organic compounds to form intensely colored compounds, but most of these methods are not sensitive enough for small biopsy tissue samples and some lack reliability [7, 8]. Although flame atomic absorption spectrometry is not sensitive enough for these low levels, Ebdon et al. [9] have obtained a detection limit of $5 \cdot 10^{-11}$ g for manganese with a carbon filament atomizer and have shown that other elements may interfere.

This paper presents a novel scheme for the separation of manganese from interfering elements before its determination by atomic absorption spectrometry with a carbon furnace atomizer. An examination of the effect of other elements, in the quantities likely to be present in biological samples, showed that sodium, potassium, calcium, magnesium and iron interfere seriously. Van Ormer and Purdy [10] extracted manganese cupferrate quantitatively into methyl isobutyl ketone (MIBK) at pH 7.1; the present work has shown that manganese is not extracted by cupferron-MIBK below pH 3, although extraction is complete at pH 5—9. Since iron cupferrate is extracted completely into organic solvents over the pH range 0—12 [11], manganese has been separated from interfering elements by extracting with cupferron-MIBK below pH 1, then raising the pH to 7.5 and extracting the manganese

with cupferron-MIBK, leaving the other interfering elements in the aqueous phase. With this separation procedure, and with an atomic absorption spectrometer with a carbon furnace attachment, which gave a detection limit of $5 \cdot 10^{-13}$ g, a method has been developed for the determination of very small quantities of manganese in biological tissues.

EXPERIMENTAL

Apparatus

A Varian Techtron model 1200 atomic absorption spectrometer with a model 63 carbon rod atomizer was used. The atomizer consists of a cylindrical graphite tube (3 mm id., 9 mm long) with the optical path aligned with the tube length. Instrument settings were: lamp current, 5 mA; wavelength, 279.5 nm; slit width, 0.2 nm; drying voltage, setting 5 for 60 s; ashing voltage, setting 5 for 30 s; atomizing voltage, setting 7½ for 3.5 s; nitrogen flow 4 l min^{-1}.

Solutions in test tubes were mixed with Townson Tru-Mix vortex mixers. Pyrex test tubes (200 × 24 mm) were used for digestion and extraction. Glassware was cleaned with hot 16 M nitric acid and rinsed well with glass-redistilled water.

Reagents

All chemicals were analytical-reagent grade except the tris(hydroxymethyl)-aminomethane (TRIS), which was Sigma 7—9 buffer grade. Nitric acid, sulphuric acid, and MIBK were redistilled before use. Water redistilled from glass was used throughout.

Recommended procedure

Digest muscle (0.3 g of wet tissue or 0.1 g of dried tissue) or liver (0.03 g of wet tissue or 0.01 g of dried tissue) by gently boiling with 2 ml of nitric acid and 20 drops of sulphuric acid. Add a few more drops of nitric acid if necessary, and when digestion is complete, heat to fumes of sulphur trioxide. When the digest has cooled, add 10 ml of water and again heat until fumes appear. Cool, add 10 ml of water, and cool to ice water temperature. Add 1 ml of aqueous 5 % (w/v) cupferron solution and extract with 2 × 5-ml portions of MIBK, using the vortex mixer. Draw off the MIBK layer by pasteur pipette and a suction pump with a liquid trap in the suction line. Cool the remaining aqueous phase to ice water temperature, and add 20 drops of concentrated ammonia solution (28—30 % w/w) slowly with vigorous mixing. Add 1 ml of M TRIS buffer and adjust to pH 7.5 with ammonia solution; use 1 drop of cresol red (0.1 % in 20 % ethanol) as internal indicator (orange—brown color). Add 1 ml of cupferron solution and extract

with 3 × 5 ml portions of MIBK. Draw off the MIBK layer with a pasteur pipette, and collect the combined extracts in a 30-ml beaker. Evaporate the MIBK to less than 0.5 ml on a hot plate at low heat, and take to dryness by directing a stream of dry nitrogen on to the surface of the solvent. Take up the residue in 1.00 ml of 70 % (v/v) nitric acid and determine the manganese content by injecting 5-μl aliquots into the carbon rod atomizer.

Prepare standards from manganese sulphate dissolved in 70 % nitric acid for the calibration graph. The plot shows only slight curvature for the range $0—8 \cdot 10^{-8}$ g ml^{-1}.

RESULTS AND DISCUSSION

Interferences

The effect of foreign ions on the absorbance of a manganese solution containing $5 \cdot 10^{-8}$ g ml^{-1} was investigated by adding these ions separately, in the amounts likely to be found in samples of tissue, and determining the atomic absorption with the carbon rod atomizer. The results shown in Table 1 indicate that manganese must be separated from sodium, potassium, calcium, magnesium and iron. The interference is increased if these metals are present as chlorides.

Efficiency of extraction procedure

The efficiency for manganese was tested by adding known amounts of manganese(II) to 20 drops of concentrated sulphuric acid and determining

TABLE 1

Effect of foreign ions on the absorbance of a solution of manganese containing $5 \cdot 10^{-8}$ g ml^{-1}

Ion	Concentration of ion, μg ml^{-1}	Compound used	% Change in signal
Na^+	1000	$NaNO_3$	−13
Na^+	1000	NaCl	−86
K^+	1500	KNO_3	−7
K^+	1500	KCl	−69
Ca^{2+}	1000	$Ca(NO_3)_2$	0
Ca^{2+}	1000	$CaCl_2$	−97
Mg^{2+}	500	$MgSO_4$	−34
Mg^{2+}	500	$MgCl_2$	−97
Fe^{2+}	500	$FeSO_4$	−17
Cu^{2+}	10	$CuSO_4$	0
Zn^{2+}	10	$ZnSO_4$	0
PO_4^{3-}	200	$NH_4H_2PO_4$	0
Cl^-	2000	HCl	0
NO_3^-	1000	HNO_3	0
SO_4^{2-}	1000	H_2SO_4	0

manganese by the recommended procedure, without the digestion step. The results (Table 2) show that manganese is extracted completely over the concentration range likely to be found in tissues.

To test the effectiveness of the separation, solutions containing 1000 μg Na ml^{-1}, 1500 μg K ml^{-1}, 1000 μg Ca ml^{-1}, 500 μg Mg ml^{-1} (all added as the chlorides) and 500 μg Fe ml^{-1} (as iron(II) sulphate), and either 0.03 or 0.06 μg Mn ml^{-1}, in 5 *M* sulphuric acid were analysed; ten 1-ml aliquots of each solution were tested. The results obtained were 0.029 μg Mn ml^{-1} (s = 0.0015) and 0.059 μg Mn ml^{-1} (s = 0.0019), which indicates that the combined interferences from these elements are eliminated by the extraction procedure.

Percentage extraction of manganese(II) vs. pH

The percentage extraction of manganese(II) as a function of pH was determined by adding 0.02 μg of manganese (II) to 10 ml of water and 20 drops of concentrated sulphuric acid, the pH being adjusted with concentrated ammonia solution and TRIS or acetate buffer; 1 ml of the cupferron solution was added and the resulting solution was extracted with 3 × 5 ml of MIBK. After evaporation of the extract and dissolution in nitric acid as in the recommended procedure, manganese was determined with the carbon rod atomizer. The results showed that manganese is extracted completely over the pH range 5—9 but is not extracted below pH 3.

Comparison of results with a flame a.a.s. method

In order to compare the results obtained by the recommended procedure with those obtained by flame atomic absorption spectrometry, large samples of freeze-dried muscle and liver obtained at autopsy were pulverized with a glass pestle and mortar. For determinations by the flame mode, samples of muscle (3 g dry weight) and liver (1 g dry weight) were ashed in a muffle furnace at 800 °C. The ash was dissolved in 1 ml of 6 M hydrochloric acid and diluted to a suitable volume with water. The manganese concentrations measured were compared with standards in 6 M hydrochloric acid. The results obtained by the recommended procedure with the carbon rod agree well with those obtained by the larger scale flame procedure (Table 3).

TABLE 2

Recovery of manganese by the recommended extraction procedure

Mn added, μg	0.02	0.04	0.06	0.08	0.10
% Recovery[a]	98.4	99.3	100.0	100.4	100.3
s	±3.98	±2.49	±1.04	±2.47	±1.63

[a]Mean of 6 determinations.

TABLE 3

Results obtained by the recommended flameless method and by a flame a.a.s. method

Sample	Recommended method		Flame method
	Mn content[a] ($\mu g\ g^{-1}$)	s	Mn content ($\mu g\ g^{-1}$)
Muscle A	0.27	0.016	0.29
Muscle B	0.32	0.015	0.31
Liver A	5.00	0.150	5.15
Liver B	4.90	0.140	4.85
Adductor muscle	0.31	0.014	—
Biceps femoris muscle	0.34	0.010	—
Heart	0.56	0.014	—

[a]Mean of 6 determinations.

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THE ATOMIC ABSORPTION DETERMINATION OF ANTIMONY, ARSENIC, BISMUTH, CADMIUM, LEAD, AND TIN IN IRON, COPPER, AND ZINC ALLOYS WITH THE GRAPHITE FURNACE

WILLIAM B. BARNETT and EDWARD A. McLAUGHLIN, JR.*
The Perkin-Elmer Corporation, Norwalk, Connecticut 06856 (U.S.A.)
(Received 23rd April 1975)

SUMMARY

Antimony, arsenic, bismuth, cadmium, lead, and tin can be determined in metallurgical samples by flame atomic absorption spectrometry at levels of 0.005 wt%, but lower concentrations frequently necessitate preconcentration. The graphite furnace allows determination of these elements at concentrations 1—2 orders of magnitude lower than is possible with flame techniques. All six elements have detection limits at or below 1 $\mu g\ g^{-1}$ in a variety of alloys. Calibration for antimony and lead was done with standards containing the principal component of the alloy as a synthetic matrix. Bismuth, cadmium, and tin could be determined accurately only by the standard addition method. Arsenic could be determined in iron alloys with synthetic standards, but standard additions were required for copper alloys.

The determination of trace elements in metallurgical samples is of considerable interest, because these elements frequently affect alloy properties. Among the elements of interest are antimony, arsenic, bismuth, cadmium, lead, and tin. As a group, these elements are frequently cited as being among the more difficult to determine by atomic absorption. Their most sensitive absorption lines occur at short wave-lengths where background absorption interferences are most severe; and because these elements are relatively volatile, it is difficult to make good hollow-cathode lamps for several of them.

The commercial development of electrodeless discharge lamps [1], which are now available for all these elements, provides an improvement in detectability particularly for arsenic. Better instrumental background corrector accessories such as more stable power supplies and double-beam operation, have allowed further improvements. The flame detection limits for these elements in aqueous solutions and in brass and steel samples vary [2] between 0.2 and 17 $\mu g\ g^{-1}$, with a 2-fold improvement possible for some alloys, such as brass, where a solution containing 2 g of alloy per 100 ml can be aspirated

*Present address: Department of Toxicology, Food and Drug Administration, Washington, D.C. 20204.

instead of 1 g/100 ml. These detection limits are not adequate for some applications. It was decided to extend the previous study [2] on the determination of the "difficult" elements in metallurgical samples by using the HGA-2100 Graphite Furnace. Several papers [3—5] have dealt with similar analyses with an earlier version of the Graphite Furnace. Two of these [3, 4] describe the determination of lead in steel and in copper, while a third [5] describes the determination of lead, bismuth, selenium, tellurium, and thallium in nickel alloys. The determination of antimony in steel with a furnace similar to the HGA-2000 has also been discussed recently [6].

In the present work, several sets of standard samples (Table 1) with concentrations of the elements ranging from 3 to 260 $\mu g\ g^{-1}$, were selected. These concentrations were somewhat higher than the expected detection limits, but the assays are reliable. It is extremely difficult to obtain well-characterized samples with concentrations much lower than flame detection limits. The factors affecting detection limits are discussed below, and data are presented to show that detection limits can be decreased by one or two orders of magnitude compared to those of the accepted flame methods.

EXPERIMENTAL

Equipment

All analyses were done with a Perkin-Elmer Model HGA-2100 Graphite Furnace, with either a Perkin-Elmer Model 503 or a Model 305B atomic absorption spectrophotometer. Both instruments were equipped with simultaneous Deuterium Arc Background Correctors which were used for all determinations. All data were obtained by reading peak heights, either on a recorder or with the peak reader of the Model 503.

The purge gas for all analyses was nitrogen. Eppendorf micropipets were used to introduce 20 μl of the solutions (samples or standards) into the

TABLE 1

Metallurgical samples analyzed as part of this study

Matrix	Source	Elements determined
Mild steel	NBS British chemical standards	As, Pb, Sb
Copper base alloys	British non-ferrous metals research association[a] NBS	As, Pb, Sn, Sb, Bi
Zinc alloys	Zinc et Alliages[a] NBS	Pb, Sn, Cd

[a]Obtained through Brammer Standard Co.

graphite furnace. Perkin-Elmer electrodeless discharge lamps were used for antimony, arsenic, cadmium, and tin determinations. An Intensitron hollow-cathode lamp was used for bismuth. Lead was determined in steel with an electrodeless discharge lamp, while an Intensitron lead hollow-cathode lamp was used for the zinc and copper alloys.

Sample preparation

All alloys (0.5-g samples) were dissolved by adding 10 ml of (1 + 1) nitric acid (Ultrex grade, J. T. Baker Co.), and heating if necessary. The samples were diluted to 50 ml. These stock solutions were then diluted further with water as required to bring the concentrations of the elements of interest within the optimal analytical range. Undissolved residues from samples containing carbon or silicon were allowed to settle before an aliquot was taken for further dilution.

Standard preparation

Standards were prepared by dilution of commercial atomic absorption standards. To each standard was added the principal alloy component as a synthetic matrix. For example, when brass samples were analyzed, all standards contained an amount of pure copper equivalent (on a weight basis) to the concentration of brass in the samples.

RESULTS AND DISCUSSION

Choice of experimental conditions

Nitric acid was chosen for sample dissolution for several reasons. In the case of arsenic, for example, no loss occurs at charring temperatures up to 1000 °C when 5% (w/v) nitric acid is present. Other workers [3, 7] have shown that hydrochloric acid causes serious element losses, even at fairly low charring temperatures. Tables of compound properties [8] indicate low melting and boiling temperatures for the chlorides of all the elements determined in this study. A recent study [9] on the thermodynamics of reactions occurring within the furnace postulated that most oxy-anion salts of the metals (such as nitrate) decompose to the oxide on heating; these oxides then react with carbon to form free atoms during the atomization step. Chloride salts give a much higher degree of molecular volatilization which decreases the atom population in the furnace.

The only problem expected as a result of this choice was for tin; tin oxide is listed as insoluble [8]. As will be discussed later, tin was successfully determined in one alloy series in the presence of nitric acid, indicating some solubility, but a second alloy type could not be analyzed, and hydrochloric acid did not improve matters.

Preliminary experiments showed that standards containing only nitric acid could not be used. The presence of iron, zinc or copper enhanced the absorption signal by varying amounts. For example, the lead signal from a solution containing 0.1% (w/v) zinc was enhanced 10% over the lead signal from a solution containing only nitric acid. When copper was substituted, the enhancement was 35%. This meant that the standards had to be matched to the samples, at least with respect to major constituents. Careful matching is not considered acceptable, because samples vary in composition, frequently in an unknown way. Therefore only the primary matrix component was matched, e.g. in brass analysis, pure copper was used in standards preparation and the zinc was ignored, and in steel analysis, only iron was added to the standards. When this approach proved inadequate in obtaining accurate results, the method of standard additions was employed.

Table 2 shows the graphite furnace parameters used. Drying temperatures were 100 or 125 °C, but in later work 125 °C was used in all cases, because better precision was sometimes achieved. Welcher et al. [5] noted a similar effect when determining selenium in nickel alloys. In every case drying time was 30 s, charring time was 30 s, and atomization time was 8 s. Charring temperatures were usually chosen to be well below the point where element loss begins.

The usual charring temperatures for copper, iron and zinc — the three principal alloy components — are 900, 1100 and 500 °C, respectively. Thus it is not possible to remove either copper or iron from the furnace before atomization, but the detection limits for arsenic, bismuth, antimony, and tin in a zinc matrix can be improved by proper choice of charring temperature. Atomization temperatures were selected to provide maximum sensitivity for the element of interest. Tube life is prolonged at lower temperatures, which should be used whenever possible.

Arsenic

Arsenic was present in the iron and copper alloys. The data obtained are summarized in Table 3. The samples were diluted to give matrix concentrations of 0.02 or 0.05%; this kept all arsenic absorbance values below 0.3 where the calibration curve was linear. The steel data have been separated

TABLE 2

HGA-2100 operating parameters

	As	Bi	Cd	Pb	Sb	Sn
Wavelength (nm)	193.7	223.1	228.8	283.3	217.6	286.3
Drying temperature (°C)	125	125	100	125	100	100
Charring temperature (°C)	500	500	250	500	500	1000
Atomization temperature (°C)	2700	2500	2100	2500	2700	2700
Nitrogen purge flow (units)	30	30	50	30	30	interrupt

TABLE 3

The determination of arsenic in iron and copper alloys

Sample no.	Arsenic concentration (wt%)		
	Synthetic standard[b]	Standard addition	Certificate value
Iron alloys			
NBS 10d[a]	0.0056		0.005
8d[a]	0.0038	0.0043	0.007
4d[a]	0.0077	0.0080	0.008
9c[a]	0.0062		0.009
34a[a]	0.0091		0.009
13c[a]	0.0099		0.010
16b[a]	0.0091		0.011
19b[a]	0.0098		0.012
30c[a]	0.0157		0.016
10e	0.0044		0.004
16c	0.0087		0.007
12d	0.0087		0.008
21d	0.0100		0.010
4i	0.0157		0.018
464	0.0173[c]		0.018
1147	0.023[c]		0.022
462	0.046[c]		0.046
Copper alloys			
C38-15	0.0049	0.0039	0.004
NBS 1118	0.0091	0.0071	0.007
C38-14	0.0095	0.0078	0.009
C38-16	0.0156	0.0136	0.015

[a]These standard samples issued before 1930.
[b]0.05% matrix concentration, except where specified.
[c]0.02% matrix concentration.

into two groups: those samples issued before and after 1930. For over half the samples issued before 1930, agreement with the certificate values is good, but variations up to a factor of two are noted for the remainder. However, many of these early samples were certified on the basis of only one analysis. Agreement is good for samples issued after 1930 which were certified on the basis of multiple determinations. Two of the pre-1930 samples were analyzed by the standard addition method (see Table 3); the values obtained by this method and with synthetic standards agreed well. The correlation coefficient (all samples included) is 0.991. The regression coefficient is 1.01.

Table 3 also contains data on the determination of arsenic in copper alloys. For these, synthetic standards gave results about 20% higher than those obtained by the standard addition method.

Antimony

Data obtained for the determination of antimony in iron and copper alloys are shown in Table 4. The presence in solution of either iron (0.5%) or copper (0.1%) enhanced the absorbance of antimony by 15% and 25%, respectively, over the absorbances observed when nitric acid solutions were analyzed.

TABLE 4

The determination of antimony in iron and copper alloys

Sample no.	Antimony concentration (wt%)		
	Synthetic standard	Standard addition	Certificate value
Iron alloys			
BCS 326	0.0047		0.005
BCS 330	0.0176		0.018
BCS 328	0.026		0.026
BCS 327	0.034		0.033
Copper alloys			
C38-15	0.0064	0.0059	0.008
NBS 1118	—	0.0099	0.010
C38-16	0.0142	0.0149	0.011
C38-14	0.0117	0.0116	0.012

The analytical data for the iron samples correlated well with the certificate values. Those for the copper samples were less satisfactory, but the results obtained with synthetic standards agreed well with those by the standard addition method.

Lead

The determination of lead in iron [3] and copper [4] alloys has already been reported; the present data (Table 5) extend the method to the HGA-2100 furnace and to zinc alloys. As with the other elements, the metallurgical matrix enhanced the sensitivity for lead: 15% for iron (0.2%), 10% for zinc (0.1%), and 35% for copper (0.02%). In all cases analysis by comparison with synthetic standards produced data which correlated well with the certificate values.

Cadmium

Cadmium was certified only in the zinc alloys. The values were quite high for analysis with the graphite furnace, so that large dilutions were required. The matrix concentration varied between 0.02 and 0.005% (w/v). Table 6

TABLE 5

The determination of lead in iron, copper, and zinc alloys

Sample no.	Lead concentration (wt%)	
	This study	Certificate value
Iron alloys		
BCS 330	0.0032	0.003
BCS 327	0.0110	0.010
BCS 326	0.0149	0.014
BCS 328	0.0159	0.015
Copper alloys		
NBS 52c (cast bronze)	0.0114	0.011
C38-15 (brass)	0.0121	0.012
C38-14 (brass)	0.021	0.020
C38-16 (brass)	0.024	0.023
NBS 1118 (aluminum brass)	0.024	0.025
NBS 157a (Cu—Ni—Zn alloy)	0.034	0.034
Zinc alloys		
1	0.00065	0.0010
5	0.00135	0.0011
2	0.0030	0.0032
3	0.0051	0.0052
4	0.0101	0.010

TABLE 6

The determination of cadmium in zinc alloys

Zinc alloys	Cadmium concentration (wt%)		
	Synthetic standard	Standard addition	Certificate value
1	—	0.00020	0.0004
5	0.0015	0.00107	0.0011
2	0.0043	0.0035	0.0032
3	0.0065	0.0052	0.0050
4	—	0.0094	0.0098

summarizes the data obtained. Analysis with synthetic standards produced data about 30% high. The method of standard additions gave data which correlated well with the certificate values except for sample 1; poor correlation for lead in this sample was also observed. In both cases, the values were well above the HGA detection limit, and perhaps the concentrations of lead and cadmium in this sample were near the limits of detection of the reference methods.

Bismuth

Bismuth was certified in the copper alloys. Preliminary experiments showed that use of synthetic standards would not be adequate so the method of standard additions was adopted. The data obtained (Table 7) showed good correlation with the certificate values.

TABLE 7

The determination of bismuth in brass

Sample no.	Bismuth concentration (wt%)	
	Standard addition	Certificate value
C38-14	0.0056	0.005
C38-16	0.0078	0.007
C38-15	0.0097	0.010

Tin

Tin was certified in the zinc and copper alloys. Agreement with certificate values was good for the zinc alloys, but not for the copper alloys (Table 8). As was noted earlier, tin should not be soluble in solutions containing nitric acid, hence hydrochloric acid was used first to dissolve the zinc alloys. However, the results were high by approximately two-fold. Substituting

TABLE 8

The determination of tin in zinc and copper alloys

Sample no.	Tin concentration (wt%)			
	Synthetic standards		Standard addition	Certificate value
	In HCl	In HNO_3		
Zinc alloys				
1	—	0.00025	0.00076	0.0003
5	0.0032	0.0024	0.00146	0.0014
2	0.0051	0.0040	0.0027	0.0027
3	0.0099	0.0083	0.0049	0.0049
NBS 94b	—	—	0.0055	0.006
4	—	0.0153	0.0092	0.0097
Copper alloys				
C38-15		0.0140	0.0120	0.010
C38-14		0.028	0.030	0.019
NBS 157a		0.032	0.028	0.021
C38-16		0.034	0.039	0.024

nitric acid improved the results, but they were still high by 50—70%. Adding aluminum to the standards in an attempt to match the sample matrix more closely made the results even worse. The method of standard additions, with the zinc samples dissolved in nitric acid, was ultimately successful. Good correlation was obtained between the present results and the certificate values except again, for sample 1.

Table 8 also summarizes attempts to determine tin in brass, which were not completely successful, with either synthetic standards or standard additions. However, the signals obtained were proportional to the tin concentration in the alloy so that a well-characterized alloy could probably be used as a standard to obtain meaningful data.

These analyses illustrate the necessity of having well-characterized samples for the development of flameless atomic absorption methods; without such samples, it is impossible to evaluate correctly the results obtained. These experiments also cast doubt on the validity of interference studies, since the aluminum in the alloys appears to have had a different effect on the tin absorption signal than aluminum added to the standards.

Detection limits

It is difficult in some cases to measure the detection limits directly, because the so-called pure iron, zinc, and copper contain traces of some of these elements. Accordingly, selected elements were measured directly in the presence of a matrix and those values were compared with aqueous detection limits. The detection limits measured in acidic solutions deteriorated by an average of three-fold when the matrix was present. For example, arsenic had a detection limit of 10 pg in aqueous solutions, but when the various matrices were added, the absolute detection limit varied between 23 and 38 pg. In all cases the detection limit was defined as the concentration giving a signal twice the fluctuations in the baseline.

The poorer detection limits are caused by the presence of background absorption from the matrix. Figure 1 shows background signals measured for the three matrices considered here. These plots were constructed by pipeting 20 μl of the solutions shown and measuring the absorbance from a deuterium arc used instead of the hollow-cathode lamp. The peak around 250 nm in the iron curve is not background, but is due to several strong iron lines in that wavelength region. Copper has a weak line at 202.4 nm which probably accounts for that peak.

The factor which restricts detection limits is the amount of background generated by the matrix. The simultaneous background corrector has been shown [10] to correct up to one absorbance unit of background in some cases. However, when a 10-fold scale expansion, which is typical for measurements near detection limits, is used, one absorbance unit of background is too much. Experience indicates that the background from 0.1—0.2% iron, 0.2—0.5% copper, and 1% or more zinc is a reasonable limit, though it may

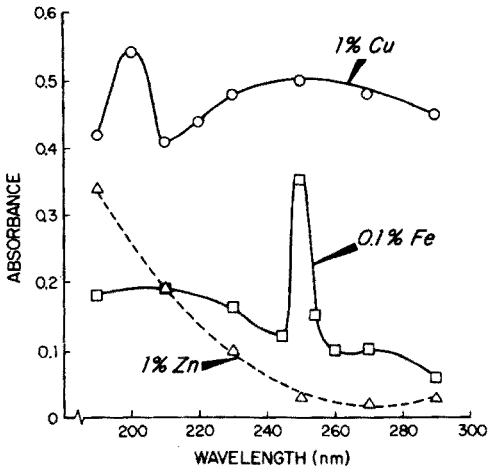

Fig. 1. Background absorption in the HGA-2100.

be possible to use higher concentrations in some cases.

In the case of the zinc matrix, its relative volatility improves the detection limits. The zinc matrix can be removed during the charring step without losing arsenic, bismuth, antimony, or tin. At the lead 283-nm wavelength the background is already low, and removal is not necessary.

The estimated detection limits for these elements in the presence of a metallurgical matrix are presented in Table 9 along with flame detection limits for similar matrices. The HGA values assume that the aqueous detection limits are degraded by 3-fold and that 20 μl of a 1% solution of the metal is pipeted. These values are reasonable for the zinc matrix, perhaps conservative, but are further degraded in the case of the copper or iron samples

TABLE 9

Estimated detection limits in metallurgical samples

Element	Detection limits		
	HGA (pg) aqueous	HGA ($\mu g\ g^{-1}$)[a] metal matrix	Flame ($\mu g\ g^{-1}$)[b,c] metal matrix
As	10	0.2	17
Bi	10	0.2	16
Cd	0.3	0.005	0.2
Pb	5	0.08	4
Sb	20	0.3	9
Sn	10	0.2	7

[a]Assumes 20 μl of a 1% (w/v) solution of the metal matrix.
[b]Assumes a 1% (w/v) solution of the metal matrix.
[c]1 $\mu g\ g^{-1}$ = 0.0001 wt%.

because of the higher background. For comparison, the measured detection limits for arsenic were 0.23 $\mu g\ g^{-1}$ in a zinc matrix and 0.9 $\mu g\ g^{-1}$ in a copper matrix. For antimony, actual measurements gave 0.13 $\mu g\ g^{-1}$ in a zinc matrix and 1.0 $\mu g\ g^{-1}$ in a copper matrix. Thus, the estimated values are reasonable if properly adjusted for each metal matrix. Figure 2 shows some representative tracings obtained near the detection limits for arsenic and antimony.

Conclusions

The HGA-2100 Graphite Furnace provides detection limits which are about 6- to 85-fold better than those obtainable with flames, depending on element and matrix. The determinations of lead and antimony are straightforward and can be done with synthetic standards containing an appropriate amount of the principal component of the alloy. Tin, bismuth, and cadmium are slightly more difficult, because they require the method of standard additions. Arsenic requires the method of standard additions for the copper alloys investigated, while synthetic standards are adequate for the iron alloys.

The HGA-2100 is a reasonable alternative to the flame when better detection limits are required or when the amount of sample is small. The flame, however, remains the method of choice when one has adequate sample, and the concentrations are high enough.

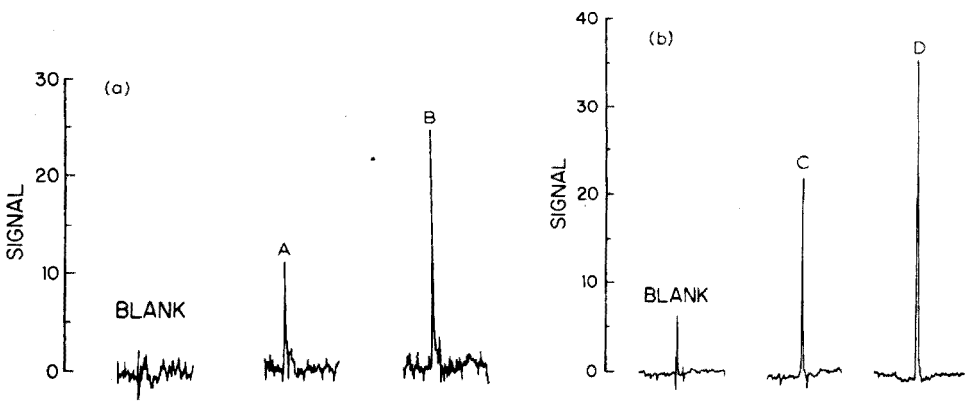

Fig. 2. Typical tracings for the measurement of detection limits. (a) Arsenic in the presence of copper. Each run represents 20 μl of a solution containing 0.2% copper (w/v) and (A) 0.005 μg As ml^{-1}, (B) 0.01 μg As ml^{-1}. (b) Antimony in the presence of zinc. Each run represents 40 μl of a solution containing 0.5% zinc (w/v) and (C) 0.005 μg Sb ml^{-1}, (D) 0.01 μg Sb ml^{-1}.

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ATOMIC ABSORPTION SPECTROMETRIC DETERMINATION OF MANGANESE, SILVER AND ZINC IN DENTAL MATERIAL BY ATOMIZATION DIRECTLY FROM THE SOLID STATE

F. J. LANGMYHR and T. LIND

Department of Chemistry, University of Oslo, Oslo 3 (Norway)

J. JONSEN

Department of Microbiology, University of Oslo, Oslo 3 (Norway)

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SUMMARY

The direct-atomization technique of atomic absorption spectrometry was applied to the determination of manganese, silver and zinc in 14 whole erupted or unerupted human teeth. Pulverized sample—graphite mixtures of 5—10 mg, to which known volumes of metal standard solution had been added, were dried, ashed and atomized in a graphite furnace, as in the standard addition technique. The technique was also used for determining silver and zinc in an international reference sample of calcined animal bone.

In a previous paper [1] the atomic absorption spectrometric determination of cadmium and lead in dental material was described; the analyses were based on direct atomization of the two elements from the solid samples. The feasibility of this technique was demonstrated by analyzing 14 whole erupted or unerupted human teeth. The present paper describes the application of this technique to the determination of manganese, silver and zinc in the same dental material.

According to Goldschmidt [2] manganese may replace calcium in apatite, and substitution is also possible to a certain extent with zinc. Silver is rarely found in calcium minerals.

EXPERIMENTAL

Apparatus

The Perkin-Elmer 303 atomic absorption spectrophotometer used was equipped with an arc-source deuterium lamp for background correction.

The construction of the graphite furnace (heated by a high-frequency induction generator) has been described elsewhere [3]. The signals from the photometric detector were registered with a 3-channel recorder: one of the

channels plotted the peak (in percent absorption), a second channel transformed the detector signal to absorbance and registered the integrated peak area, and the third channel was connected to the energy meter of the spectrophotometer, registering the capability of the background corrector to compensate for non-specific absorption of radiation.

The equipment and procedure for crushing, pulverizing, sieving and weighing the samples has been described elsewhere [1].

The measurements were made at the following wavelengths: manganese 279.5 nm, silver 328.1 nm, and zinc 307.6 nm.

Reagents and standard solutions

Metal standard solutions were prepared from high-purity manganese and zinc metal, and from reagent-grade silver nitrate. The nitric acid was of "Suprapur" quality (E. Merck). Graphite of the quality used in the furnace was finely pulverized in an agate mortar and employed as a dispersing agent.

The argon used for purging the furnace was of 99.9 % purity (by volume).

In some instances, hydroxyapatite for column chromatography (Bio-Gel HTP, Bio-Rad Labs., U.S.A.) was used as the solid standard. Before use the reagent was finely pulverized in an agate mortar.

Manganese and zinc standard solutions (1000 p.p.m.) were prepared by dissolving the proper amounts of the metals in a small excess of (1 + 1) nitric acid. A 500 p.p.m. silver standard solution was prepared by dissolving 0.787 g of silver nitrate in water and diluting to 1l. In all diluted metal standard solutions, a concentration of 3 % nitric acid was maintained. Highly diluted solutions were prepared immediately before use.

All solutions were stored in plastic bottles.

Sample preparation

The teeth were cleaned carefully by scraping (with plastic equipment) and brushing. The samples were then pulverized in an agate mortar to pass a 100-mesh (149 μm) sieve; from these portions smaller amounts were ground to pass a 270-mesh (53 μm) sieve. Before analysis 50 mg of the latter samples and 50 mg of pulverized graphite were mixed intimately by grinding in an agate mortar.

Preliminary investigations

Preliminary measurements were made to ensure linear relationships between the integrated values and different amounts of the three metals (added as varying volumes of separate standard solutions), and between integrated values and different masses of a tooth or of hydroxyapatite.

Analysis of the standard samples

Hydroxyapatite, tooth No. 16(r) and 28 were employed as solid standards for the determination of manganese, silver and zinc, respectively.

Determination of manganese

The content of manganese in hydroxyapatite was determined as follows. A solution of hydroxyapatite was prepared by dissolving 1 g in 3.0 ml of nitric acid (1 + 1) and diluting the solution to 25 ml with water. From this solution constant volumes were taken and different known volumes of manganese standard solution were added for the plotting of two standard addition curves. The two series of solutions were atomized in the graphite furnace, and gave the results 0.83 and 0.91 p.p.m. manganese in the solid sample.

The concentration of manganese in the hydroxyapatite was also established by the following variation of the standard addition technique. From a series of preliminary measurements a certain ideal mass w_i of the solid sample (or the sample—graphite mixture), and a series of ideal volumes (v_1, v_2 and v_3) for addition are chosen. In the actual analysis four portions of mass $w_{1,x}$, $w_{2,x}$, etc. are weighed out. The actual volumes $v_{1,x}$, $v_{2,x}$, etc. to be added to the samples $w_{1,x}$, $w_{2,x}$, etc. are found from the formulae

$$v_{1,x} = v_1 w_{1,x}/w_i; \quad v_{2,x} = v_2 w_{2,x}/w_i; \quad \text{etc.}$$

The samples $w_{1,x}$, $w_{2,x}$, etc. with the added volumes $v_{1,x}$, $v_{2,x}$, etc. of standard solution are atomized and give the integrals $I_{1,x}$, $I_{2,x}$ etc. From the following formula these experimental integrals are recalculated to integrals I_1, I_2, etc. produced by the ideal mass w_i with the added ideal volumes v_1, v_2 and v_3: $I_1 = I_{1,x}\, w_i/w_{1,x}$; $I_2 = I_{2,x}\, w_i/w_{2,x}$; etc.

In the determination of manganese in hydroxyapatite, a mass of 5 mg of the sample—graphite mixture and volumes of 5, 10 and 15 μl of a 0.25 p.p.m. manganese solution were chosen as the ideal amounts.

The method of least squares was employed to establish the position of the standard addition curves. A computer performed the necessary calculations, including the averages and the standard deviations. Four standard addition curves were plotted, and the averages ($\bar{x}$ in p.p.m.) and relative standard deviations (s in %) were found to be: $\bar{x}_1 = 0.86$, $s_1 = 8.5$; $\bar{x}_2 = 0.96$, $s_2 = 4.7$; $\bar{x}_3 = 0.83$, $s_3 = 5.6$; $\bar{x}_4 = 0.93$, $s_4 = 5.6$. From these four averages the overall average of 0.90 p.p.m. was employed as the standard value for manganese in hydroxyapatite.

Determination of silver

By direct atomization of solid samples of hydroxyapatite and by using tooth No. 16(r) as the solid standard for silver, the content of this metal in hydroxyapatite was found to be 32 p.p.b. The concentration of silver in tooth No. 16(r) was determined by the standard addition technique described above,

the ideal mass being 10 mg of the sample—graphite mixture and the ideal volumes 4, 8 and 12 μl of a 25 p.p.b. silver solution. From the four standard addition curves the following averages ($\bar{x}$ in p.p.b.) and relative standard deviations (s in %) were calculated: $\bar{x}_1 = 44$, $s_1 = 15$; $\bar{x}_2 = 54$, $s_2 = 15$; $\bar{x}_3 = 38$, $s_3 = 13$; $\bar{x}_4 = 50$, $s_4 = 27$. The overall average of 47 p.p.b. was used as the standard value.

Determination of zinc

The content of zinc in tooth No. 28 was also established by the above standard addition technique; the ideal mass chosen for the sample—graphite mixture was 10 mg, and the ideal volumes, 5, 10 and 15 μl of a 100 p.p.m. solution. The averages ($\bar{x}$ in p.p.m.) and relative standard deviations (s in %) from five standard addition curves were: $\bar{x}_1 = 162$, $s_1 = 5.4$; $\bar{x}_2 = 148$, $s_2 = 5.0$; $\bar{x}_3 = 157$, $s_3 = 11$; $\bar{x}_4 = 149$, $s_4 = 6.1$; $\bar{x}_5 = 172$, $s_5 = 9.6$. The overall average 158 p.p.m. zinc was used as the standard value.

Procedure

Before the start of the measurements, the hollow-cathode and deuterium lamps were heated for about 1 h. The flow of argon was adjusted to 6 ml s^{-1}, and 5—10 mg of the sample—graphite or standard—graphite mixture were weighed in a small tantalum scoop and placed in the middle of the pre-heated graphite tube by means of a specially constructed adjustable inserting device. The scoop was reweighed and the furnace was moved to its pre-adjusted position.

Samples and standards were atomized in the following order: standard, duplicate portions of the first sample, standard, duplicate portions of the second sample, etc.

For manganese and silver ashing was done at 850 °C, atomization at 1600 °C, and cleaning of the tube at 1950 °C; each operation lasted for 60 s.

For zinc, ashing was done at 700 °C for 60 s, and atomization and cleaning were as for manganese and silver.

RESULTS

The analytical results from determinations of manganese, silver and zinc in 14 whole erupted or unerupted human teeth are listed in Table 1; the water contents of these samples have been given elsewhere [1].

The concentrations of manganese and silver in tooth No. 7 were also determined by neutron activation analysis [4], and were found to be 0.78 p.p.m. and 59 p.p.b., respectively.

The proposed technique was also used in the analysis of an international reference sample of calcined animal bone. The results from two determinations of zinc with tooth No. 28 as the solid standard are listed in Table 1. The present data for zinc compare favourably with the value — 183 p.p.m. —

TABLE 1

Analytical results for manganese, silver and zinc in whole human teeth, and for silver and zinc in calcined animal bone

Sample designation	Manganese (p.p.m.)	Silver (p.p.b.)	Zinc (p.p.m.)
2	0.51	17	191
6	0.75	34	137
7	0.87[a]	86[b]	226
9b(r)[c]	0.47	16	194
12	0.85	66	168
16(r)	0.64	47	157
17(r)	0.81	18	227
19	0.46	22	186
20	0.46	11	185
23(r)	0.43	23	111
28	0.52	9	158
29	0.45	12	153
37(r)	0.51	15	155
41(r)	0.55	69	192
Bone[d]	n.d.[e]	17, 19	188, 180

[a]6 determinations.
[b]8 determinations.
[c](r), unerupted.
[d]International Atomic Energy Agency (Vienna, Austria), reference material A-3/1, calcined animal bone.
[e]Not determined.

recommended by the issuing organization. As can be seen from Table 1, the content of silver in the sample of bone was found to be 18 p.p.b., but a recommended value for this element has not been given; in these measurements tooth No. 16 was employed as the solid standard. Because of the high content of manganese in the bone, compared to the concentrations in the teeth, this element was not determined.

The precision of the proposed technique was calculated from 6 and 8 determinations of manganese and silver, respectively, in tooth No. 7. The relative standard deviation for manganese was 3.4 %, and for silver 36 %. The less satisfactory precision obtained for silver may result from an inhomogeneous distribution of the element. From five determinations of zinc in tooth No. 7, the relative standard deviation was calculated to be 9.5 %.

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HIGH-PRESSURE LIQUID CHROMATOGRAPHY OF POLYNUCLEAR AROMATIC HYDROCARBONS OF CIGARETTE SMOKE CONDENSATE*

A. F. HAEBERER, M. E. SNOOK and O. T. CHORTYK

Tobacco Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Athens, Georgia 30604 (U.S.A.)

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SUMMARY

Reverse-phase, high-pressure liquid chromatography (h.p.l.c.) on Permaphase ODS and ETH was applied to the fractions of cigarette smoke condensate containing polynuclear aromatic hydrocarbons (PAH). A PAH-enriched fraction was first separated by gel filtration, and selected fractions were subjected to h.p.l.c. Although standard PAH could be easily separated on Permaphase ODS, results with smoke condensate were unsatisfactory. Better results were obtained with Permaphase ETH, which gave sufficient resolution for identification. PAH identification was aided by means of a stop-flow technique and measurement of the u.v. spectra of eluting peaks. Benzo(a)pyrene in the smoke condensate could be determined. However, for resolution of the PAH in smoke condensates, the total overall performance of h.p.l.c. was judged inferior to that of gas chromatography.

Polynuclear aromatic hydrocarbons (PAH) from numerous sources have been investigated rather extensively by liquid chromatography [1—3]. Reverse-phase liquid chromatography was first described [4] in 1950; the column-packing materials consisted of celite or silicic acid coated with a low-molecular-weight hydrocarbon stationary phase, but these columns were easily stripped of the stationary phase and thereby rendered useless. More recently, the stationary phases for reverse-phase liquid chromatography have been bonded to the solid supports. Two such materials are Permaphase ODS and Permaphase ETH [5]. The performances of these materials in the high-pressure liquid chromatography of PAH-containing fractions of cigarette smoke condensate are described below.

EXPERIMENTAL

Cigarette smoke condensate was prepared in 1.0-kg quantities as described previously [6]. The details of the total fractionation procedure have been described elsewhere [7, 8], and the isolation procedure for the PAH from

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the neutral compounds in the condensate is shown in Fig. 1. The neutral portion of the condensate was eluted from silicic acid by 25% benzene in petroleum ether (B—PE) solvent. The DMSO solubles of this fraction were subjected to gel filtration (GF) on Bio-Beads SX-2 (Bio-Rad Laboratories, exclusion limit of 2700, acetone solvent) to yield the PAH-enriched fraction (F-55). This fraction was then further subjected to analytical gel filtration on Bio-Beads SX-12 (molecular exclusion 400, benzene solvent) [9]. Briefly, portions of F-55 (100 mg) were placed onto the first of three coupled 43 × 0.5 in. columns with a 1-ml loop injection valve. The column eluate was monitored at 280 nm (Chromatronix Model 230 u.v. detector, equipped with a 2-μl flow cell), and collected in 8-ml portions. The gel filtration fractions 28—42 (PAH isolate) were analyzed individually by high-pressure liquid chromatography (h.p.l.c.), after solvent removal and dissolution in 50 μl of acetonitrile.

H.p.l.c. was performed on a DuPont Model 830 liquid chromatograph equipped with a gradient elution accessory and a 254-nm u.v. photometric detector. The detector was connected directly to a u.v.—visible spectrophotometer, equipped with 8-μl flow cells (Aerograph Variscan, Model 635, Varian Instruments) [10]. Permaphase ODS and ETH columns (stainless steel, 2.1 mm i.d. × 100 cm) were packed by the manufacturer (DuPont Instruments). The column temperature was 60 °C, and the flow rate was 1 ml min^{-1}. The best mobile phase for the ODS column was deionized water modified with nanograde methanol [5]. The mobile phase for the ETH column was water modified with isopropanol. A linear elution gradient increase of 3% min^{-1} from 25 to 40% (v/v) isopropanol—water was employed.

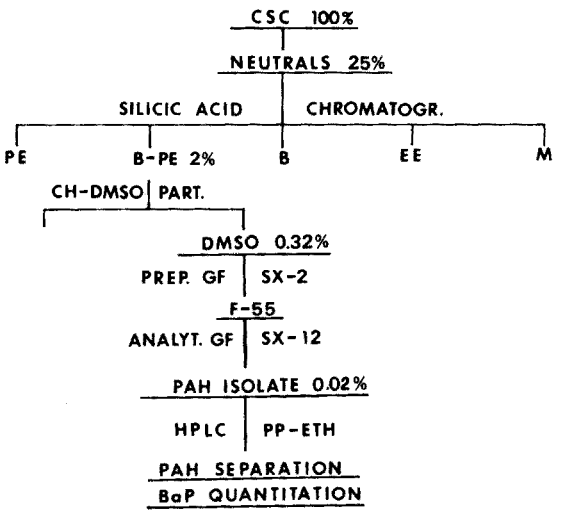

Fig. 1. PAH separation scheme for cigarette smoke condensate. See text.

RESULTS AND DISCUSSION

Since h.p.l.c. separates PAH more rapidly than conventional gas chromatographic methods, the possibility of separating and identifying the PAH components of cigarette smoke condensate by h.p.l.c. was investigated. A considerable volume of material has been published recently on the reverse-phase h.p.l.c. of PAH standards on Permaphase ODS (PP-ODS) [1, 3, 5]. Relatively little material has been published on PAH analysis on Permaphase ETH (PP-ETH). Consequently, both substrates were investigated. For this purpose, the PAH-enriched fraction, F-55 (Fig. 1), subjected to analytical gel filtration, provided relatively clean PAH material for the h.p.l.c. experiments. U.v. absorbance curves for gel filtration elutions of F-55 and several PAH standards are shown in Fig. 2. Gel filtration removed 60% of the F-55 material and concentrated the PAH into a fraction representing only 0.02% of the cigarette smoke condensate.

Permaphase-ODS cleanly separated some PAH standards, with aqueous methanol solvents, but its resolution of the PAH isolate was disappointing. A typical chromatogram of condensate material from gel filtration fraction 32 is shown in Fig. 3. It can be readily seen that poor resolution and the elevated baseline preclude the identification of individual components by stop-flow u.v.-spectrum scanning. For this particular chromatogram, the mobile phase was a methanol-water solvent system, but others were also investigated. Mobile phases of water modified with ethanol, isopropanol, and dioxane were all examined; the data indicated that for the Permaphase-ODS column, methanol-water was the best mobile phase for the separation of PAH standards, but it could not separate the complex PAH isolate of the condensate into identifiable compounds.

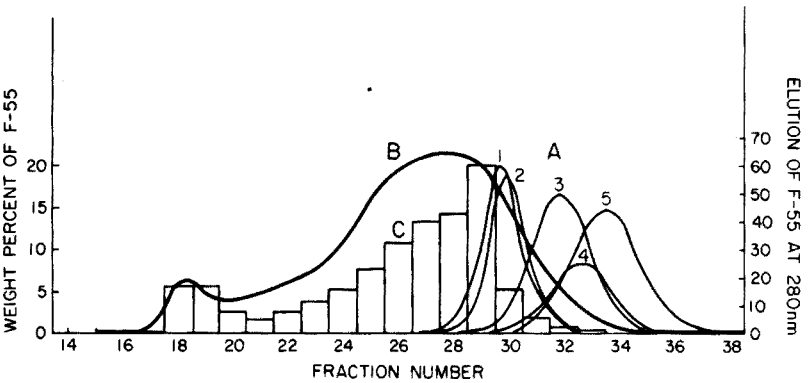

Fig. 2. Gel filtration chromatography: A, elution curves of standard PAH (1-naphthalene, fluorene; 2-anthracene, chrysene, fluoranthene; 3-pyrene; 4-BaP; 5-3,4,8,9-dibenzpyrene). B, 280 nm absorbance curve for the elution of 0.25 g of F-55 (100 = 1.28 absorbance units). C, percent weight distribution of F-55 gel fractions.

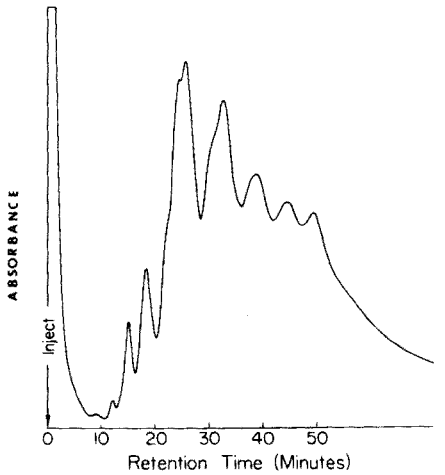

Fig. 3. H.p.l.c. chromatogram of gel filtration fraction 32 of PAH isolate on Permaphase-ODS.

Permaphase ETH (an aliphatic ether polymer bonded to Zipax glass microbeads) has many of the characteristics of Permaphase ODS, but exhibits different selectivity. For the separation of PAH on Permaphase ETH, the solvent mixture isopropanol—water was better than the methanol or ethanol—water phases. A chromatogram for PAH standards is shown in Fig. 4, where the peaks were identified by retention data and stop-flow u.v.-spectrum scanning. The peak heights or peak areas in this chromatogram do not represent the relative concentration of these standards, since their molar absorptivities differ by as much as two orders of magnitude at the monitored wavelength of 254 nm. The use of a mobile phase gradient, increasing from 25 to 40% (isopropanol—water), produced an efficient separation of the PAH without band-spreading. Moreover, the retention time of PAH was not merely a function of the mutual attraction of the non-polar stationary phase and the non-polar PAH, but also of the partitioning effects of non-polar PAH solute between non-polar stationary phase and polar mobile phase, modified with a solvent of reduced polarity. Increased percentages of modifiers improved the solubility of sample in the mobile phase. Resolution and retention were governed by the percentage of the modifier. Larger concentrations of the less polar modifier (isopropanol) in the aqueous mobile phase tended to decrease retention time and increase the efficiency of the column. The peak height of a late eluting compound may be dramatically increased by higher percentages of mobile-phase modifier, resulting in sufficient concentrations for an identifiable u.v.-spectrum.

The PAH-separating ability of Permaphase ETH is exemplified for gel filtration fraction 32 of F-55 (Fig. 5); this fraction was selected because of its maximum content of benzo(a)pyrene, which was shown to be contained

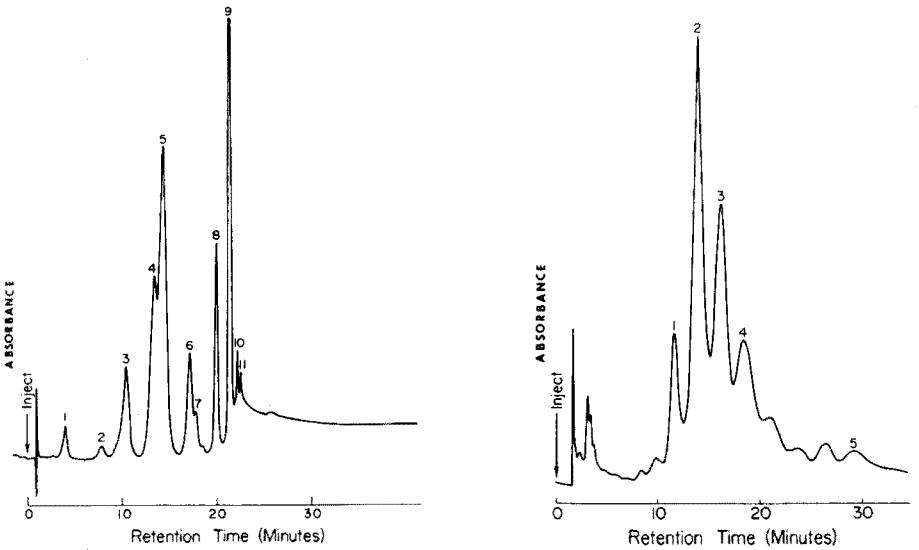

Fig. 4. H.p.l.c. chromatogram of PAH standards on Permaphase ETH. 1-Naphthalene. 2-Unknown. 3-Biphenyl. 4-Phenanthrene. 5-Anthracene. 6-Fluoranthene. 7-Pyrene. 8-Chrysene. 9-Benzopyrene.

Fig. 5. H.p.l.c. chromatogram of PAH in gel filtration fraction 32 on Permaphase ETH. 1-Pyrene. 2,4-Methylpyrene. 3-Unknown. 4-Chrysene. 5-Benzopyrene.

in gel filtration fractions 30—35. Since a gel filtration fraction contained only a small portion of the total PAH components of the smoke condensate, the chromatogram was much simpler than the one for the total PAH isolate. The separation was achieved by means of a linear mobile phase gradient of 3% min^{-1} from 25 to 40% isopropanol—water. Components were identified by the stop-flow scanning technique, which yielded a complete u.v.-spectrum of the peak components. It is most important to obtain the spectra of the standard PAH and of the unknown samples in the same solvent system and in the flow cell of the spectrophotometric detector, under identical conditions. The spectrum of peak 5 at its apex was not identifiable; however, a u.v. spectrum of the sample from the leading edge of peak 5 (at 80% total peak height), was clearly that of benzo(e)pyrene. Similarly, a sample at 80% total peak height on the trailing edge resulted in a u.v. spectrum that was unambiguously benzo(a)pyrene. The two benzopyrenes were not separated by this procedure, but could be individually detected and quantified by monitoring the effluent at other wavelengths.

Examination of the u.v. spectra of both benzopyrenes (Fig. 6) allowed the selection of a better monitoring wavelength. At 254 nm, the log ϵ values of the two compounds are of the same order, 4.6 and 4.3, respectively, and therefore, both contribute to the observed peak. However, at 332 nm

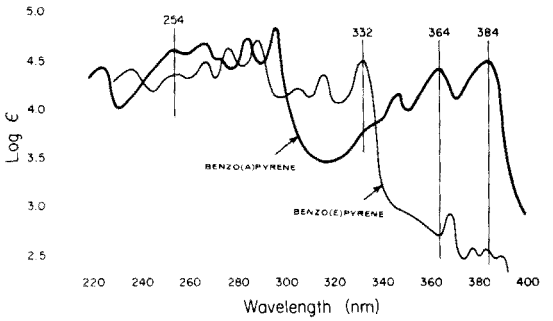

Fig. 6. U.v. spectra of benzo(a)pyrene and benzo(e)pyrene.

benz(e)pyrene has a log ϵ value of 4.5 while that of benzo(a)pyrene is only 3.7. At 364 nm, the log ϵ values are 2.65 for benzo(e)pyrene and 4.35 for benzo(a)pyrene. Therefore, benzo(e)pyrene can be detected at 332 nm without appreciable interference from benzo(a)pyrene, which may be monitored efficiently at 364 nm without interference from benzo(e)pyrene.

Quantitative data on benzo(a)pyrene and benzo(e)pyrene confirmed previous findings [9] that the benzo(a)pyrene content of fresh smoke condensates from commercial 85-mm non-filter cigarettes is about 1.3 μg/100 cigarettes and that initially there is about three times as much benzo(a)pyrene present as benzo(e)pyrene. However, the benzo(a)pyrene content was found to decrease radically with time, possibly because of oxidation. Therefore, benzo(a)-pyrene analyses on condensates should be performed as quickly as possible after sampling. The stop-flow u.v. scans of several of the examined peaks in the gel filtration fractions indicated that more than one PAH was co-eluted. No quantitative experiments were carried out on the other identified compounds, but these should be readily accomplished if the chromatographic peaks correspond to single compounds or if, at the monitored wavelength, the absorptivities of any co-eluting compounds are relatively low.

It was concluded that reverse-phase h.p.l.c. has not yet shown itself capable of separating the complex PAH isolate of cigarette smoke condensate into all its components. However, qualitative and quantitative methods for certain compounds can be developed with existing instrumentation. Thus, the combined gel filtration fractions 30—35, yielded a sample for the rapid determination of benzo(a)pyrene by this h.p.l.c. method.

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EXTRACTION OF ZIRCONIUM AND HAFNIUM FROM HYDROCHLORIC ACID SOLUTIONS WITH DERIVATIVES OF DIANTIPYRYLMETHANE

A. I. BUSEV

Department of Chemistry, M.V. Lomonosov. State University, Moscow (U.S.S.R.)

V. P. ZHIVOPISTSEV, B. I. PETROV and M. I. DEGTEV

Department of Chemistry, A.M. Gor'ki State University, Perm' (U.S.S.R.)

P. NENNING

Chemistry Section, Karl-Marx University, 701 Leipzig (German Democratic Republic)

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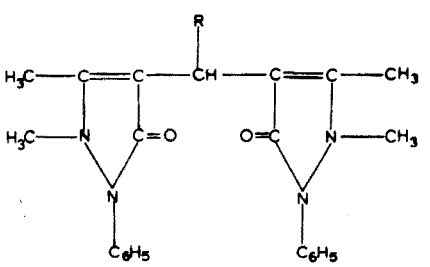
SUMMARY

Zirconium and hafnium are extracted quantitatively into chloroform, dichloroethane or nitrobenzene in the presence of hexyl-, nonyl- and *o*-nitrophenyldiantipyrylmethane from hydrochloric acid solutions (≥ 8 M) in the form of $RMeCl_4$ complexes. New methods of determining up to $1 \cdot 10^{-4}$% zirconium in materials containing Mg, Be, Al, Y, Sc, La, Ce, Tl, Th, Cr, Ni, and other elements are suggested.

Diantipyrylmethane (I) and its derivatives (II—XII) are capable of reacting with over 60 elements [1], including zirconium and hafnium, to form complexes or crystal line hydrates of the type R_nMeX_4 (where R = I; n = 1, 2, 3; Me = Zr or Hf; and X = NO_3^-, SCN^-, Cl^-, or I^-), complexes with partially hydrolyzed forms of the elements of the type $R_2Me(OH)_y(NO_3)_{4-y}$, and thiocyanate or nitrate complexes of the type $(RH)_2$ $[MeX_6]$. Methods for the recovery and determination of zirconium and hafnium on the macro or micro scale in media of diverse composition have been based on the extraction of diantipyrylmethane complexes of these two elements into chloroform and dichloroethane [2—11].



R = H (I), CH_3 (II), C_3H_7 (III), iso-C_4H_9 (IV), C_6H_{13} (V), C_9H_{19} (VI), C_6H_5 (VII), C_6H_5-CO (VIII), 2-C_5H_4N (IX), *o*-NO_2-C_6H_4 (X), *m*-NO_2-C_6H_4 (XI) and *p*-NO_2-C_6H_4 (XII).

Diantipyrylmethane and its homologs and derivatives differ widely in their properties, although they have analogous molecular structures and the same reaction centres. The present paper is concerned with the extraction of zirconium and hafnium with diantipyrylmethanes in a chloride system.

EXPERIMENTAL

Solutions of 0.1 M $ZrOCl_2$ and 0.1 M $HfOCl_2$ in 6 M hydrochloric acid were prepared.

The reagents I—XII were synthesized by condensing antipyrine with the appropriate aldehyde, then recrystallizing and drying the product [12]. Solutions (0.125 M) of the reagents were prepared in chloroform or dichloroethane.

Extraction conditions were as follows: aqueous phase = 20 ml; organic phase = 20 ml; [Me] = 0.0125 M; hence R/Me = 10. Precipitation occurred in some cases, and so zirconium and hafnium were determined after phase contact for 20 h to allow sufficient time for equilibrium to be reached.

RESULTS

Table 1 shows the pattern of the distribution of zirconium between the phases for reagents II—XII when the aqueous phase was 7—10 M in hydrochloric acid. At low acidities, the element was found predominantly in the aqueous phase. Diantipyrylmethane failed to extract zirconium and hafnium regardless of the acidity of the medium; the addition of salting-out agents and the use of a large excess of I failed to improve the recovery. With reagent II, the percentage of zirconium extracted into chloroform reached 6% when 8 M hydrochloric acid was used (see Table 1). As the acidity of the aqueous layer was increased to 9 M, a third, crystalline, phase emerged. With dichloroethane, the precipitate formed at a lower acidity (8 M); in 10 M HCl, 96% of the zirconium appeared in the precipitate. Reagent III likewise gave a poor extraction of zirconium into chloroform (12—17%) except from 10 HCl, although with dichloroethane extraction was 52—61% in 9—10 M HCl. With reagent IV, the chloroform layer was divided into two sublayers; with dichloroethane, a precipitate of the complex formed. Reagent V extracted practically all the zirconium, provided that the acidity was 7—8 M. Reagent VI behaved similarly to V. With reagent VII, over 90% of the zirconium precipitated. With solutions of VII, in dichloroethane, about 50% of the zirconium was in the organic phase when 8 M hydrochloric acid was used. With reagent VIII, zirconium was extracted to the extent of 2—3% and 15—67% by chloroform and dichloroethane, respectively. Reagent IX failed to extract any zirconium. Of the isomers, X, XI and XII, reagent X was the most effective, extracting zirconium almost completely into dichloroethane from 8—10 M hydrochloric acid.

A comparative evaluation of the extraction patterns for zirconium and

TABLE 1

Extraction of zirconium from chloride solutions with diantipyrylmethanes

Reagent	HCl, M	Distribution of zirconium in the phases, %					
		Chloroform			Dichloroethane		
		Aqueous	Organic	Precipitate	Aqueous	Organic	Precipitate
II	7	97	3		95	5	
	8	94	6		88	8	4
	9	79	3	18	10	5	85
	10	4	1	95		4	96
III	7	87	13		90	10	
	8	83	17		75	22	3
	9	70	12	18	34	61	5
	10	12	3	85	6	52	42
IV	7	30	60	10[a]	67	30	3
	8	27	50	23[a]	50	45	5
	9	20	42	38[a]	20	72	8
	10	14	45	41[a]	4	50	46
V	7	9	91		5	95	
	8	3	97		2	98	
	9		47	53	1	99	
	10		41	59		100	
VI	7				10	90	
	8				5	95	
	9				2	98	
	10				1	99	
VII	7	12	4	84	47	34	20
	8	2	4	94	7	48	45
	9	1	3	96	3	32	65
	10		1	99	2	28	70
VIII	7	11	3	86	70	15	15
	8	11	2	87	37	53	10
	9	15	2	83	29	67	4
X	7				6	89	5[a]
	8				5	95	
	9	7	85	3	2	98	
	10	5	55	40	1	99	
XI	7	3	2	95			
	8		1	99	49	47	4
	9		1	99	20	78	2
	10				2	40	58
XII	7	3	1	96	8	14	78
	8		2	98	1	4	95
	9		1	99	1	1	98
	10		1	99		1	99

[a]The third phase was liquid.

hafnium was carried out with the most effective reagents, V and X (Fig. 1). With reagent X, the organic phase was divided into two in the 4—7 M HCl range. Hafnium was extracted less efficiently than zirconium at acidities lower than 7 M; in fact, a separation of these elements seems feasible. From 8—10 M hydrochloric acid, both elements were extracted virtually completely into dichloroethane; precipitation occurred with chloroform if the medium was left to stand for some time.

When the concentration of the reagents was reduced, the extraction rate diminished abruptly to the extent that zirconium and hafnium complexes passed into a precipitate insoluble in either the aqueous or the organic phase; the sole exception was the extraction of zirconium and hafnium complexes with V or X into dichloroethane. Chloride complexes of zirconium and hafnium with V or X were also readily extracted into nitrobenzene, but the toxicity of nitrobenzene limits its application severely.

The optimal conditions for the extraction of zirconium and hafnium are therefore: 8—10 M hydrochloric acid with reagent V or X in dichloroethane. A two-fold excess of the reagent suffices for 95—97% extraction.

Composition of the complexes

The composition of the complex in the organic phase was found by the molar ratio method (Fig. 2). For both reagents, R : Me = 1 : 1. In order to clarify the extraction process, extracts obtained from aqueous phases with and without zirconium or hafnium, were analyzed for all the components present. Zirconium and hafnium ions were determined complexim etrically [1].

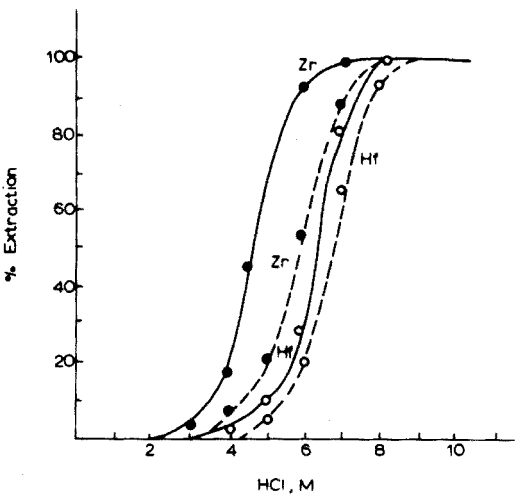


Fig. 1. Dependence of extraction rates of Zr and Hf with reagents V (—) and X (---) on the HCl concentration in the aqueous phase.

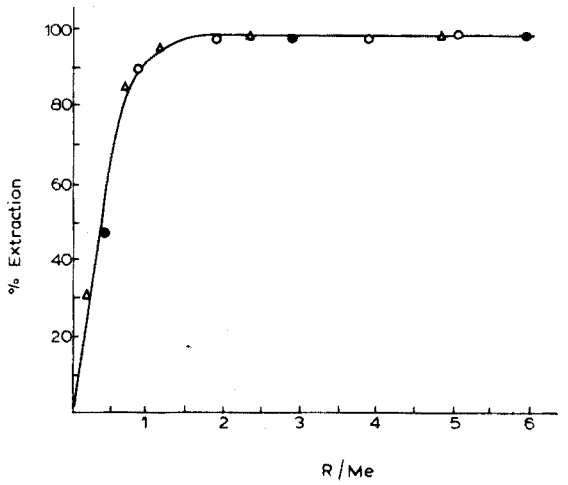


Fig. 2. Molar ratio technique (9 M HCl) (•) V. (△) X.

The acidity was determined by titration with 0.1 M sodium hydroxide to a phenolphthalein end-point; the alkali not only neutralized hydrogen ions but also formed zirconium hydroxide and this was taken into account in calculations. Chloride ions were determined by titration with silver(I) solution, and the reagent by alkalimetric titration [1]. The marked changes that occurred in the organic phase composition during the extraction of zirconium allowed conclusions to be reached regarding the nature of the complex extracted (Table 2). Thus, with reagent V, 97—98% of the reagent is present in dichloroethane in the form of an acid salt. The excessive acidity of the organic phase observed in the control experiment (32.6 ml of 0.1 M hydrochloric acid instead of 29.6 ml) may be attributed to co-extraction of the acid. If the extraction process is expressed by the equation:

$$RH_2Cl_2 \cdot nHCl_{(o)} + Zr^{4+}_{(a)} + (2-n)Cl_{(a)} \rightleftharpoons RZrCl_{4(o)} + (2+n)H^+_{(a)} \qquad (1)$$

TABLE 2

Analysis of dichloroethane extracts
($V_{aq.} = V_{org} = 50$ ml; 8 M HCl, 0.15 M V, 0.1 M X; 10-ml aliquots of the extract were analyzed)

Reagent	Zr added (ml 0.1 M)	Extract analysis (ml 0.1 M)			
		R	Zr^{4+}	H^+	Cl^-
V	—	14.8	—	32.6	32.7
V	30	14.7	5.3	21.0	42.0
X	—	1.8	—	2.3	2.2
X	12.5	5.2	2.4	3.0	12.7
X	25	7.7	4.9	2.7	22.9
X	40	8.9	7.7	1.7	32.5

(where $n < 1$ and the subscripts o and a denote the organic and aqueous phases, respectively), then the reduction of acidity observed in the extract by $11.6 \cdot 10^{-4}$ g-ions, and the increase in chloride by $9.3 \cdot 10^{-4}$ g-ions, during the extraction of $5.3 \cdot 10^{-4}$ g-ions of zirconium comply with the equation. In experiments with reagent X, a control experiment (8 M HCl) showed that only 18% of the reagent was present in dichloroethane as R · HCl. Irrespective of the quantity of zirconium ions introduced into the aqueous phase (12.5, 25 or 40 ml of 0.1 M), extraction of the element was accompanied by an increase in X by 1 equivalent and in chloride ions by 4 equivalents, whereas the hydrogen ion concentration remained practically stable, suggesting that the complex $RZrCl_4$ was also extracted.

In hydrochloric acid solutions, zirconium and hafnium react [1, 13] with reagents I—V to form complexes R_nMeCl_4, where $n = 1, 2, 3$. As the concentration of hydrochloric acid in the solution rises, the number of reagent molecules coordinated by the zirconium or hafnium ions decreases from 3 to 1, and the solubility of the complexes diminishes sharply, so that they precipitate [1]. In the concentrated hydrochloric acid solution — extractant system, the element is in the same form ($RMeCl_4$) in both phases, so that its distribution coefficient is determined essentially by the ratio of the solubilities of the corresponding complex in the organic phase and in the aqueous solution. The extraction distribution coefficients for zirconium correlated well with the ratios of the solubility of its complexes with reagents II and V in chloroform and dichloroethane to their solubility in hydrochloric acid solutions. The homologs of I become increasingly hydrophobic; accordingly, their ability to extract the compounds increases correspondingly because of the resulting increase in solubility of the zirconium and hafnium complexes in organic solvents, and the corresponding decrease in solubility in aqueous solutions. The presence of additional salt-forming groups, e.g., the nitrogen atom of the pyridine ring, as in IX, or of groups inhibiting complex formation as in VIII, reduce the efficiency of extraction.

ANALYTICAL APPLICATIONS

The extraction of zirconium by reagent V gave the basis for a recovery technique. Macro quantities of the element extracted from 8—9 M hydrochloric acid can be determined complexiметrically [14] and micro quantities by emission spectrography [15] or by a photometric method [16] with arsenazo III. The latter method is proposed here for the determination of zirconium in magnesium and aluminium alloys after extraction; the former method has the advantage of allowing zirconium to be determined in a wide range of materials.

Zirconium determination in aluminium and magnesium alloys

To a separatory funnel add a solution containing 2—30 μg Zr, and adjust the acidity to 8—9 M in hydrochloric acid; the aqueous phase volume should be 20—50 ml. Extract with 20 ml of a solution (0.5%, w/v) of hexyldiantipyrylmethane in dichloroethane. After extraction for 5—10 min, transfer the organic phase to another separatory funnel, and wash the aqueous phase with 5—7 ml of dichloroethane, which is added to the extract afterwards. Re-extract the zirconium with 10 ml of 0.5 M hydrochloric acid, remove the organic phase, and wash the aqueous phase 2 or 3 times with small portions of dichloroethane to remove traces of the reagent. Transfer the second extract and the wash liquid (12 ml of 12 M hydrochloric acid) to a 25-ml measuring flask, add 1 ml of an arsenazo III solution (0.1%, w/u) and make up to the mark with water. The absorbance of the solution is measured in a 10-mm cell at 665 nm against a valid reagent blank.

Extraction—spectrographic determination of zirconium

Extract 2—40 μg of zirconium and 200 μg of a reference element (thallium) in 20—40 ml of 8—9 M hydrochloric acid for 10—15 min with 5 ml of a solution (8%, w/v) of hexyldiantipyrylmethane in chloroform. Transfer the extract to another separatory funnel, and add 5 ml of petroleum ether. When shaken, the system divides into two phases. Transfer the lower viscous phase, several drops in volume, which contains 97—99% of the zirconium, to the flat surfaces of 12 carbon electrodes. After drying, photograph these to give one spectrum without moving the plate on the spectrograph under conventional conditions. The analytical line pair Zr, 267.86 nm and Tl, 276.78 nm was used.

To study their effect on the results, calibration curves for Mg, Be, Al, Y, Sc, La, Ce, Ti, Th, Cr, Ni and other matrices were plotted. The calibration curves were linear, with an angular coefficient close to unity. A single calibration curve obtained with synthetic standards can therefore be employed for zirconium determinations. Direct spectrography of the zirconium concentrated in the organic microphase dispensed with an ashing stage, thereby reducing the analysis time by a factor of 2—3, and increasing the accuracy and reproducibility of determinations [15].

Statistical data on the two proposed methods are given in Table 3.

TABLE 3

Statistical data for the zirconium determinations

Method	Zr taken (μg)	Zr found $\bar{x}$ (μg)	n	s	s_r
Extraction—photometric	10.0	10.1	20	0.4	4.0
Extraction—spectrographic	9.1	9.5	20	1.3	14.3

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A NEW ANALYTICAL REACTION FOR THE DETERMINATION OF PERRHENATE

PASCHOAL SENISE and LOURDES GONÇALVES SILVA
Instituto de Química, Universidade de São Paulo, São Paulo (Brazil)
(Received 21st April 1975)

SUMMARY

Perrhenate is quantitatively extracted into methyl isobutyl ketone from aqueous solutions containing copper(II), azide and an excess of 2,2′-bipyridine. Measurement of the extracted copper either by spectrophotometry or by atomic absorption spectrometry, allows the determination of perrhenate in the ranges 16—40 $\mu g\ ml^{-1}$ or 3—16 $\mu g\ ml^{-1}$ in the final dilution, respectively. The procedure is highly selective, being applicable in the presence of a large concentration of molybdate and a considerable number of foreign ions. The extracted species corresponds to the formula $CuN_3(bipy)_2\ ReO_4$.

Copper(II) can be extracted into some organic solvents from solutions containing azide and an excess of 2,2′-bipyridine when certain anions are present in the aqueous phase. Perchlorate is effective as the anion, and a qualitative test for perchlorate [1] as well as a method of determination [2] have been developed. Perrhenate ions behave in exactly the same way as perchlorate, and perrhenate can be detected and determined on this basis even in the presence of large amounts of molybdate.

The sensitivity of the qualitative test can be improved by substituting thiosulfate for azide [3] because of the deeper color of the organic extract, but, owing to the instability of this color, azide is necessary in the quantitative method.

In this paper the determination of perrhenate by spectrophotometry or by atomic absorption measurement of the extracted copper is described.

EXPERIMENTAL

Apparatus

For the spectrophotometric and reflectance measurements a Zeiss PMQ II spectrophotometer was used. A Perkin-Elmer Model 303 atomic absorption spectrophotometer and a copper hollow-cathode lamp were used with the following standard instrumental conditions: wavelength 324.7 nm; slit setting 0.4 nm; lamp current 30 mA; air pressure 9.0 psi; acetylene pressure 9.0 psi.

A 2 × 2-in. NaI(Tl) detector connected to a 512-channel analyzer (Nuclear Chicago Corporation), an Industrial Instruments model RC-168 conductivity bridge, and a Perkin-Elmer model 337 spectrometer were used to measure γ-radiation, conductance, and infrared spectra, respectively.

Chemicals

Standard perrhenate solution

Prepare a stock solution by dissolving potassium perrhenate in distilled water to give a concentration of ca. 0.013 M. This can be standardized by titration of the perrhenic acid formed by treatment of the solution with Amberlite IR-120 ion-exchange resin. Prepare working solutions by appropriate dilution.

Copper-2,2'-bipyridine reagent

To 0.3 g of 2,2'-bipyridine in a 10-ml volumetric flask add 2.5 ml of 0.15 M copper sulfate and heat carefully in a water bath at 50—60 °C to facilitate dissolution. Add 2.5 ml of 0.1 M sodium azide and dilute to the mark by adding an acetic acid — sodium acetate buffer (0.2 M) of pH 5.2. Keep under diffuse light.

Solvent

Use a recently prepared solution of 2,2'-bipyridine in methyl isobutyl ketone (15—20 mg ml^{-1}). Keep in the dark.

Spectrophotometric procedure

To 0.50 ml of sample solution (pH 4—9) in a ground-stoppered test tube, add 0.50 ml of the copper(II) reagent and 2 ml of solvent. Shake vigorously, centrifuge for a few seconds to separate the phases and transfer the organic layer to a 5-ml volumetric flask with an extraction pipet [4]. Repeat the extraction three times with 1.0 ml of solvent each time and make up to the mark with the same solvent. Keep in the dark. Read the absorbance at 415 nm against a blank run in parallel.

In the presence of nitrate, chloride or tungstate, proceed as follows.

Nitrate present

Transfer 0.50 ml of the sample solution to a ground-stoppered test tube and evaporate under an infrared lamp. Add 0.5 ml of 98% formic acid and evaporate to dryness under the lamp. Repeat this treatment four times. Add 0.50 ml of distilled water and proceed as described in the regular procedure.

Chloride present

Evaporate 0.50 ml of the sample solution under an infrared lamp. Add 0.1 ml of concentrated nitric acid and evaporate again. Add 0.5 ml of 98% formic acid and proceed as indicated for samples containing nitrate.

Tungstate present

Follow the regular procedure after a preliminary addition of 0.05 ml of 6% (w/v) sodium fluoride solution to the sample solution.

Atomic absorption procedure

The extraction procedure described above was used and copper was determined in the organic extract. Readings were found to be proportional to concentration, the optimal concentration range being 3—16 μg of ReO_4^- ml^{-1} in the final dilution, corresponding to 15—80 μg ml^{-1} in the aqueous phase.

The sensitivity was thus enhanced compared to the spectrophotometric method.

RESULTS AND DISCUSSION

Choice of solvent

Extractions with 43 different solvents were tested both in the presence and in the absence of perrhenate in aqueous solutions containing copper sulfate, azide and excess of 2,2′-bipyridine. The extraction was evaluated by the yellow color of the extract. As in the study with perchlorate [1], hydrocarbons proved ineffective as extractants. Nitromethane, nitrobenzene and acyclic alcohols with less than 7 atoms in the carbon chain, as well as cyclopentanol and cyclohexanol, provided yellow extracts regardless of the presence of perrhenate. Most of the ethers and esters tested were very poor extractants. However, several ketones and chloroform were found to be very efficient and selective extractants, since the yellow color in the organic phase was not observed in the absence of perrhenate. The final solvent was chosen after interference tests had been made with foreign ions, in particular molybdate and tungstate; determinations were completed by atomic absorption measurement of the copper in the organic phase. Best results were obtained with methyln-propyl, methyl isopropyl and methyl isobutyl ketone, the last being chosen because it is easily available.

Working conditions

A thorough study of the influence of various factors led to the following conclusions. A concentration of at least 15 mg ml^{-1} of 2,2′-bipyridine in the organic solvent is necessary in order to extract perrhenate quantitatively, because the 2,2′-bipyridine in the aqueous phase is removed by the solvent during the extraction; the reagent should preferably contain 30 mg ml^{-1} of 2,2′-bipyridine. The azide concentration must be kept at a relatively low level. The copper(II) concentration is not at all critical. The pH must be carefully controlled in the reagent which must be buffered at pH 5.20—5.50; the pH of the working solution may vary from ca. 4—9. The volume of the aqueous phase is somewhat critical and dilution above 1.00 ml should be avoided.

Extraction efficiency

Samples of the standard solution were extracted as in the recommended procedure and back-extraction of perrhenate to the aqueous phase was accomplished by shaking the organic extract with 0.5 M sodium hydroxide. After the pH had been re-adjusted with sulfuric acid, the extraction was repeated. Absorbance measurements at 415 nm were compared with those of analogous samples obtained by the regular procedure. The results (Table 1) showed quantitative recovery of perrhenate.

Spectrophotometric determination

The absorption spectrum of the organic extract shows a band with a well-defined maximum at 415 nm. Spectral characteristics did not change during 10 h when the solution was kept in diffuse light. Absorbance readings of organic extracts kept in the dark were reproducible for 30 days. Extraction in the temperature range 20—25 °C gave reproducible results.

Beer's law was obeyed up to 40 μg ReO_4^- ml^{-1} in the measured solution. A Ringbom plot of the data showed the optimal concentration range to be 16—40 μg ReO_4^- ml^{-1} in the organic solution, corresponding to 80—200 μg ml^{-1} in the aqueous phase.

Precision study

Two series of 20 independent determinations, with 64.0 and 190.0 μg of perrhenate in the aqueous phase, were performed in order to evaluate the precision of the method. The standard deviation calculated from the absorbance values was 0.0036 for the first set and 0.0030 for the second. The calculated confidence limits for a 95% confidence level were ± 0.7 μg and ± 0.6 μg, respectively.

Interference study

Nitrate interfered by increasing the absorbance of the extract, but was easily eliminated by treatment with formic acid under infrared radiation. Chloride in relatively large amounts inhibited the extraction; treatment with

TABLE 1

Extraction efficiency

	ReO_4^- in the org. phase (μg ml^{-1})[a]		
	25.4	28.6	31.8
Direct extraction	0.298	0.337	0.372
Reextraction	0.296	0.336	0.370

[a]Absorbance values are given. No significant differences in the values were found in repeated determinations.

concentrated nitric acid under infrared radiation eliminated chloride, and the excess of nitrate was removed with formic acid as before. Tungstate in amounts larger than 100 times that of perrhenate interfered, causing high absorbances; addition of fluoride prevented this interference. Table 2 shows the results obtained in the presence of these three anions after the samples had been treated as described.

It should be mentioned that vanadate is precipitated by the copper-2,2′-bipyridine complex. It could be removed by previous precipitation with barium acetate, but somewhat lower results than expected were then obtained for perrhenate. Table 3 shows the results obtained with anions that did not interfere under the working conditions. Oxalate if present in larger amounts than indicated should be removed by precipitation with calcium acetate. Precipitation occurred when chromate and tungstate were present but this was ignored because the final results were not affected.

Because of the practical importance of the determination of perrhenate in the presence of large amounts of molybdate, as well as of the Re/Mo separation, special attention was paid to the tolerance limit for molybdate. In additional experiments, perrhenate was extracted in the presence of molybdate labelled with $^{99}Mo(T_{1/2} = 66$ h); the γ-activity at the 741-keV photopeak was measured both in the aqueous and in the organic layer. The results showed that molybdate was retained totally in the aqueous phase. In these experiments, amounts up to 40,000 μg of molybdate in the working sample were taken. The perrhenate present was not affected, and the expected absorbance values were always obtained.

It was observed that some cations that react with azide or 2,2′-bipyridine interfered, leading to low results. Experiments were carried out with Fe(III), Cr(III), Co(II), Ni(II) and Mn(II) at concentrations of 10,000—20,000 $\mu g\ ml^{-1}$ in the aqueous solution. These cations were easily removed by preliminary treatment with an ion-exchange resin (Amberlite IR-120, sodium form).

TABLE 2

Removal of interfering anions

NO_3^- present μg	Cl^- present μg	WO_4^- present μg	ReO_4^- present μg	ReO_4^- found μg
12500	—	—	127.0	126.3; 126.4; 126.2
10000	—	—	89.0	88.7; 88.6; 88.6
—	13000	—	127.0	126.8; 126.5; 126.3
—	11000	—	89.0	88.5; 88.3; 88.2
		10000	89.0	89.2; 89.4; 89.2
		10000	64.0	64.0; 64.3

TABLE 3

Interference study for spectrophotometry of 89.0 μg of perrhenate

Foreign ion	μg	ReO_4^- found (μg)	Foreign ion	μg	ReO_4^- found (μg)
MoO_4^{2-}	17500	89.1	$B_4O_7^{2-}$	2000	89.2
$H_2PO_2^-$	5000	89.0	$HAsO_4^{2-}$	3250	89.1
HSO_3^-	5000	88.0	F^-	6250	88.9
BrO_3^-	12500	89.0	SeO_4^{2-}	1800	89.0
IO_3^-	12500	89.3	Ac^-	17500	89.0
AsO_2^-	5000	89.1	HPO_4^{2-}	12500	89.1
$C_2O_4^{2-}$	625	88.8	SO_4^{2-}	20000	89.0
SeO_3^{2-}	1250	89.0	TeO_3^{2-}	1250	88.5
CrO_4^{2-}	2000	89.3	TeO_4^{2-}	2000	89.2

Atomic absorption spectrometry

The effects of foreign ions on the atomic absorption method were the same as observed for spectrophotometry. Table 4 shows the results obtained in the presence of diverse ions. In the case of nitrate, chloride and tungstate, the results reported were obtained after the elimination treatments described above had been applied.

Nature of the extracted compound

The organic extract was analysed for perrhenate and for copper. Perrhenate was determined as described above after back-extraction to an aqueou solution. Copper(II) was titrated with 10^{-3} M EDTA and PAN as indicator (0.1% solution in methyl isobutyl ketone). The ratio of copper(II) to perrhenate was found to be 1 : 1.

From relatively large amounts of organic extract, small bright green crystal were obtained by slow evaporation at room temperature. The absorption spectrum of a solution of this product in methyl isobutyl ketone previously shaken with a slightly acidic aqueous solution of sodium azide, was identical to

TABLE 4

Interference study for a.a.s. of 47.0 μg of perrhenate

Foreign ion	μg	ReO_4^- found (μg)	Foreign ion	μg	ReO_4^- found (μg)
MoO_4^{2-}	12000	47.0	NO_3^-	6000	46.4
WO_4^{2-}	7500	47.1	Cl^-	6000	46.6
F^-	3000	47.0	Ac^-	10000	47.0
$B_4O_7^{2-}$	2000	47.0	HPO_4^{2-}	7000	47.0
SO_4^{2-}	15000	47.0			

that of the organic extract obtained by the analytical procedure (Fig. 1). Larger amounts of this substance were obtained from aqueous solutions of copper(II), azide and perrhenate ions with excess of 2,2′-bipyridine. The bright green crystals collected gave the same spectrum as in Fig. 1, when dissolved in the same manner in methyl isobutyl ketone.

The isolated substance was analysed not only for copper, by titration with EDTA and PAN, and for perrhenate by applying the method described here, but also for carbon, hydrogen and nitrogen. The results of these analyses agreed with the formula $CuN_3(bipy)_2ReO_4$.

Conductance measurements of a solution of the compound in nitrobenzene provided molar conductance values of 26.8 mho cm^2 mol^{-1} at 25.0 °C corresponding to a 1:1 electrolyte [5].

The infrared absorption spectra of the compounds isolated from aqueous solution and from the organic extract were identical. A band at 2030 cm^{-1} corresponds to the stretching frequency of the azide ion; a symmetric band at 910 cm^{-1} corresponding to the perrhenate ion, shows that the Td symmetry was not affected, so that the ion is not coordinated to the central nucleus. There was no evidence for water molecules. The reflectance spectrum of the solid compound was also determined, and comparison with the absorption spectrum showed no significant differences.

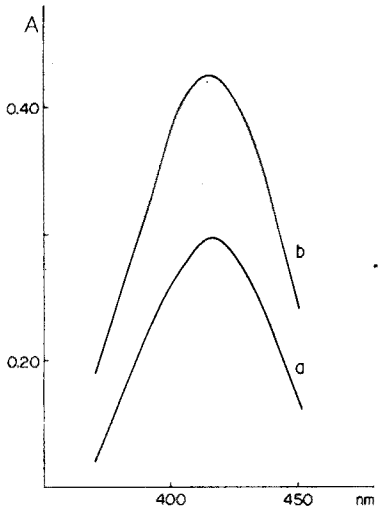

Fig. 1. Absorption spectra: a, organic extract obtained from the analytical procedure (25.4 μg ReO_4^- ml^{-1}); b, solution of $CuN_3(bipy)_2ReO_4$ in methyl isobutyl ketone previously treated with slightly acidic aqueous sodium azide.

CONCLUSIONS

The results reported confirm that perrhenate behaves exactly like perchlorate in the reaction studied. The extracted compound must correspond to the formula $CuN_3(bipy)_2ReO_4$ similarly to the perchlorate compound [2], involving a penta-coordinated copper(II) central ion such as that present in a number of species described in the literature, e.g., $CuX(bipy)_2ClO_4$, $CuX(phen)_2ClO_4$, $CuX(bipy)_2X$, $CuX(phen)_2X$ (X being Cl^-, Br^-, I^-, SCN^-, NO_2^-, $HCOO^-$, H_3CCOO^-, $C_6H_5COO^-$) as well as $CuL(bipy)_2ClO_4$ and $CuL(phen)_2ClO_4$ (L being NH_3 or C_6H_5N) [6–8].

From the analytical standpoint, the selectivity of the procedure, which allows the separation of perrhenate from large amounts of molybdate, seems worth noting, because this goal is achieved only by a few methods reported in the literature.

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THE RAPID SEPARATION OF TRACE METALS FROM NEUTRON-ACTIVATED SALINE MATRICES BY CHELATION ON A POLY-5-VINYL-8-HYDROXYQUINOLINE COLUMN*

JOHN A. BUONO*, RUDOLPH W. KARIN** and JAMES L. FASCHING

Department of Chemistry, University of Rhode Island, Kingston, Rhode Island 02881 (U.S.A.)

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SUMMARY

Instrumental neutron activation analysis of biological and environmental samples suffers from interferences caused by high salt concentrations. Poly-5-vinyl-8-hydroxyquinoline coated on controlled pore diameter glass beads is suggested as a chelating column for the rapid removal of aluminum, vanadium, copper and manganese from neutron-activated sea-water samples. Separation from bulk elements is satisfactory at flow rates of 20 ml min^{-1}. With addition of carriers and with chemical yield determinations, relative standard deviations of 2—10% can be achieved for spike concentrations of 0.1 μg Mn ml^{-1}, 0.3 μg V ml^{-1}, 20 μg Al ml^{-1} and 1.0 μg Cu ml^{-1}.

8-Hydroxyquinoline (oxine) has been widely used as a precipitating reagent and as an extractant since it was first examined in 1910 [1, 2]. As early as 1939, Erlenmeyer and Dahn [3] attempted to chromatograph cations on columns of oxine, but the slight water solubility of oxine at pH 7.0 and the rapid increase in this solubility with an increase or decrease in pH rendered this technique unfeasible. After Meinhard [4] had suggested attaching the chelating group to a solid substrate, Parrish [5] incorporated oxine into a resorcinol-formaldehyde condensation polymer, and later [6] coupled oxine to a diazotized polyaminostyrene. Pennington and Williams [7] synthesized a formaldehyde—oxine—resorcinol condensation resin similar to the resin formed by Parrish. Vernon and Eccles [8] have reviewed and evaluated these and other chelating resins and found that all of the oxine-containing resins suffered from low metal chelation capacities and/or lengthy equilibrium periods for chelation. Because of these problems, oxine-containing resins have not been useful for the rapid separation of trace metals from saline matrices.

Sugawara et al. [9] chemically bonded oxine to controlled pore diameter glass (cpg) beads, and evaluated the ability of the immobilized chelate to

*Present address: AID Division of Fisher Scientific, 590 Lincoln Street, Waltham, Massachusetts 02154, U.S.A.

**Present address: New England Nuclear Corporation, Atomlight Place, North Billerica, Massachusetts 01862, U.S.A.

extract metals from aqueous solutions. The effects of pH, the time of metal—oxine contact and the presence of sodium chloride were also studied. CPG—oxine is not a feasible chelating agent for the rapid removal of trace metals from saline matrices. This separation is extremely critical to the determination of short half-lived trace metals in high salt matrices by instrumental neutron activation analysis (i.n.a.a.).

Tang and Maletskos [10] developed a heterogeneous isotopic exchange separation for the removal of sodium and potassium from activated samples. Unfortunately, this procedure required 1-h separation times, which preclude its use with short half-lived isotopes.

The purpose of this paper is to report the use of a new methylenic linear polymer, poly-5-vinyl-8-hydroxyquinoline (PVO), which retains the chelating capabilities of oxine while remaining insoluble in water. Since this linear polymer is not rigid enough to be used in a column by itself, PVO was coated on glass beads. The PVO-beads were packed into chromatographic columns and used for the rapid separation of copper, manganese, vanadium and aluminum from high salt matrices.

EXPERIMENTAL

Reagents

All chemicals were of analytical-reagent grade. Standard solutions of trace metals were prepared from Fisher Atomic Absorption Stock Standards. The concentration of all the metal salt solutions used was between 1 and 10 p.p.m. PVO was prepared by the method of Vittimberga and Vijayaraghavan [11]. Porous glass beads (40—80 mesh; 500 Å pore diameter, Bio Rad Corp.) were used as a solid substrate.

Apparatus

Polyethylene chromatographic columns with porous polyethylene chromatographic filter discs (Bel-Art Co.) were used for the column separations. A 2-MW swimming pool reactor (Rhode Island Nuclear Science Center) with a $4 \cdot 10^{12}$n cm^{-2} s^{-1} thermal neutron flux was used to irradiate samples and standards before the separation studies. The γ-ray activity of the activated samples was counted on a 40-cm^3 Ge(Li) (Ortec Co.) coaxial detector (resolution 2.3 keV for the 1332-KeV Co γ-ray) coupled to a 4096-channel analyzer (Nuclear Data Corp.) with a computer-compatible magnetic tape output. All spectra were processed by a PIDAQ program [12] with an IBM 360-50 computer. The following γ-rays were used for the activity determinations: 1779 keV for 2.23-min ^{28}Al; 1434 keV for 3.75-min ^{52}V; 1039 keV for 5.10-min ^{66}Cu; 847 keV for 2.58-h ^{56}Mn; 1370 keV for 15.0-h ^{24}Na; 1642 keV for 3.75-min ^{38}Cl; and 616 keV for 17.6-min ^{80}Br.

Column preparation

PVO was dissolved in benzene (1 mg ml^{-1}). Porous glass beads (10 g) were placed in 20 ml of this solution under vacuum. After the solution rapidly filled the void volume (ca. 36% of total volume of the beads), the benzene was evaporated off leaving a thin PVO coating on and within the beads. The PVO-beads were washed with several portions of distilled-deionized water under vacuum. Aliquots (10 ml) of the washed PVO-beads were slurry-packed into polyethylene chromatographic columns which were prebuffered with 10 ml of an acetate buffer.

Batch procedure

A Cu, Mn, Al, V, Cl and Br standard solution (1 ml) was irradiated for 15 min. The sample was buffered to the desired pH with 10 ml of buffer and placed in a snap-top polyvial containing 10 ml of previously buffered PVO-beads. The mixture was shaken for 30 s and filtered. After the PVO-beads had been washed with 10 ml of buffer, the desired metals were stripped off the beads with 1 M nitric acid. The activities of the acid wash and of the original filtrate were then measured.

To determine the relative ease of chelate formation between the PVO and the trace metals (as well as the salts), the distribution coefficient (K_d) was determined from the equation: K_d = (g of solute/g of polymer)/(g of solute/ml of solution). A high K_d would indicate that the particular cation forms a PVO chelate more easily than a cation with a low K_d at the same pH. Neutron activation analysis was used to determine the K_d terms because of the ease of simultaneously determining relative amounts of trace elements.

Column procedure

An irradiated standard (1 ml) was buffered to the desired pH, and half of this sample was immediately counted for 200 s. The other half was placed on a previously buffered 10-ml PVO-beads column. The column was eluted with 30 ml of pH 9.0 buffer under pneumatic pressure at a flow rate of 20 ml min^{-1}. The column was flushed dry, disassembled and washed with 1 M nitric acid. The column and acid wash were then counted for 200 s. After corrections for detector dead time and sample decay time, the activities of both halves of the sample were compared.

A similar procedure was used for the analysis of spiked sea-water samples. A spiked sea-water sample and an aqueous standard were irradiated together for 15 min. After irradiation, the sample and standard were passed through separate PVO-beads columns at the same pH and flow rate. The activities of the sample and standard were counted, corrected for dead time and decay time, and compared. The amounts of copper, manganese, vanadium and

aluminum in the sea-water sample were determined from its activity relative to the activity of the standard.

RESULTS AND DISCUSSION

PVO-beads are useful for the extraction of trace amounts of aluminum, copper, vanadium and manganese from aqueous and high saline aqueous matrices.

Batch experiments

The K_d terms for copper, manganese, aluminum, vanadium, sodium, chloride and bromide were determined as a function of pH over the pH range 4.0—9.0 (acetate buffers). The PVO washed off the beads below pH 4.0 and above pH 9.0. The optimal extraction of vanadium occurs at pH 4.0—6.0 (Fig. 1(a)), whereas the optimal pH range for the extraction of copper is 7.0—9.0 (Fig. 1(b)). Both manganese and aluminum are extracted best at pH 9.0 (Fig. 1(c, d)). These data follow the pH ranges of precipitation published by Goto [13].

Since the aim of this work was to develop a fast separation procedure for the removal of Cu, Mn, V and Al from salts, the K_d values for the extractions of Na, Cl and Br were also determined as a function of pH; the results are shown in Fig. 1(e, f, g). The increase in the K_d of sodium with increasing pH indicates a greater electrostatic attraction for the sodium ion as the oxinate becomes more negative in basic solutions. The slight variation in the K_d values for chloride and bromide as a function of pH may also be explained in terms of electrostatic attraction.

A comparison of the relative magnitudes of the K_d values of the elements of interest to the K_d values of salts indicates the feasibility of using the PVO-beads system for the separation of the four metals from salts. Figure 2 shows the ratio of the K_d values of the elements of interest to the K_d value of sodium at pH 9.0. Since the K_d values of Cl and Br are less than that of sodium, any element which can be separated from sodium can also be separated from these halides. At pH 9.0 the K_d values of all four metals are between 8 and 67 times that of sodium. It should therefore be easy to separate quickly trace quantities of these metals from high amounts of salts.

Column experiments

The elution of a salt matrix from a 10-ml PVO-beads column was studied as a function of eluant volume. The column was eluted with 100 ml of an ammonia-ammonium acetate buffer (pH 9.0) at a flow rate of 20 ml min^{-1}. Sodium (95%) was removed after elution with three column volumes (30 ml) of buffer; 99% was removed with 50 ml of buffer. Chloride and bromide (95%) were removed after elution with three column volumes (30 ml) of pH

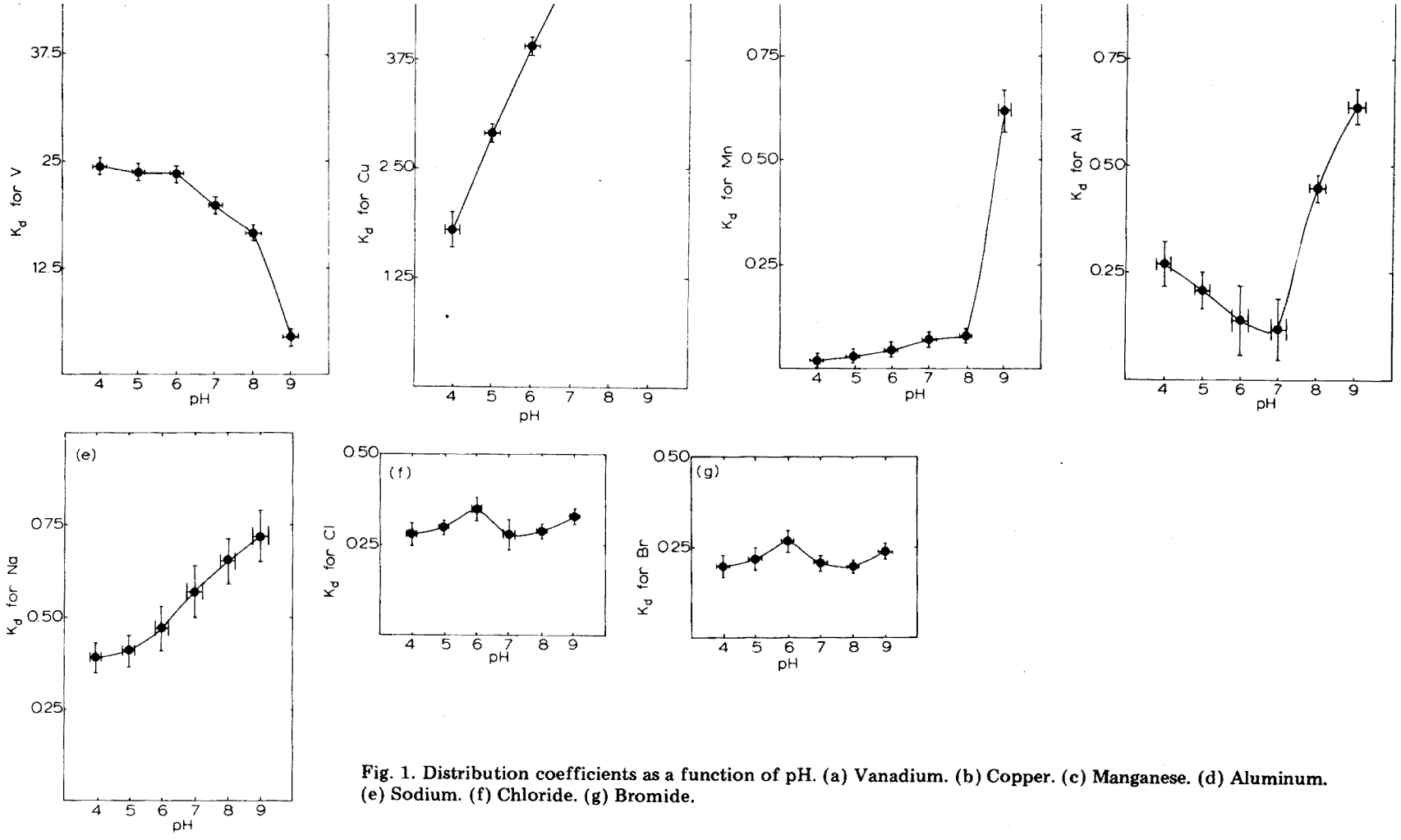

Fig. 1. Distribution coefficients as a function of pH. (a) Vanadium. (b) Copper. (c) Manganese. (d) Aluminum. (e) Sodium. (f) Chloride. (g) Bromide.

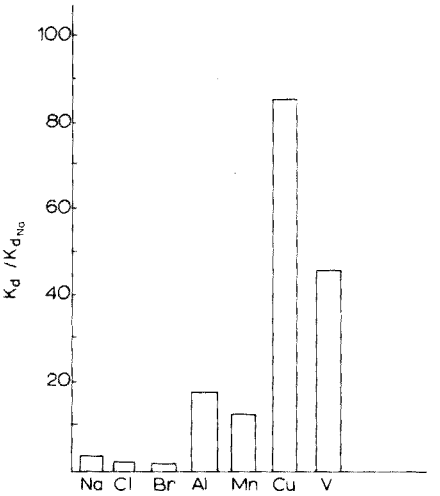

Fig. 2. A comparison of the distribution coefficients of copper, aluminum, vanadium, manganese, chloride and bromide to the distribution coefficient of sodium at pH 9.0.

9.0 buffer. Only three column volumes of buffer were used in the actual separations, in order to minimize the time of separation. The separation of trace amounts of the four metals from salts is feasible based on the above data.

A spiked sea-water sample (1 ml) was irradiated for 15 min, and the γ-ray activity of half the sample was counted for 200 s. The resultant spectrum is shown in Fig. 3(a); the only identifiable peaks arise from the high salt concentration, particularly the 1368-keV ^{24}Na, the 616-keV ^{80}Br, and the 1642-keV ^{38}Cl peaks. The second half of the sample was buffered to pH 9.0 and separated on a 10-ml PVO-beads column. After elution with 30 ml of pH 9.0 buffer at 20 ml min^{-1} the column was flushed dry and its γ-ray activity was counted for 200 s. The resultant spectrum is shown in Fig. 3(b). The ^{24}Na, ^{80}Br and ^{38}Cl peaks have been reduced by a factor of 10^2 compared to Fig. 3(a), and the 1039-keV ^{66}Cu, the 1434-keV ^{52}V, the 847-keV ^{56}Mn and the 1778-keV ^{28}Al peaks are all clearly distinguishable.

Several aliquots of the above spiked sea-water samples were irradiated for 15 min and separated by the column technique. These samples were compared to aqueous standards which were irradiated simultaneously with the samples and carried through similar separation procedures. The relative standard deviations of four replicate analyses of these samples varied from 30.5% for copper to 61% for manganese. Subsequent studies showed that this lack of precision was caused by the relatively low effective 8-hydroxyquinoline concentration of the PVO-beads and too short metal chelate contact times caused by the need for fast flow rates. The precision improved markedly when the eluant flow rate was reduced to 5 ml min^{-1}, but the separation time was increased to 9 min. Attempts to increase the

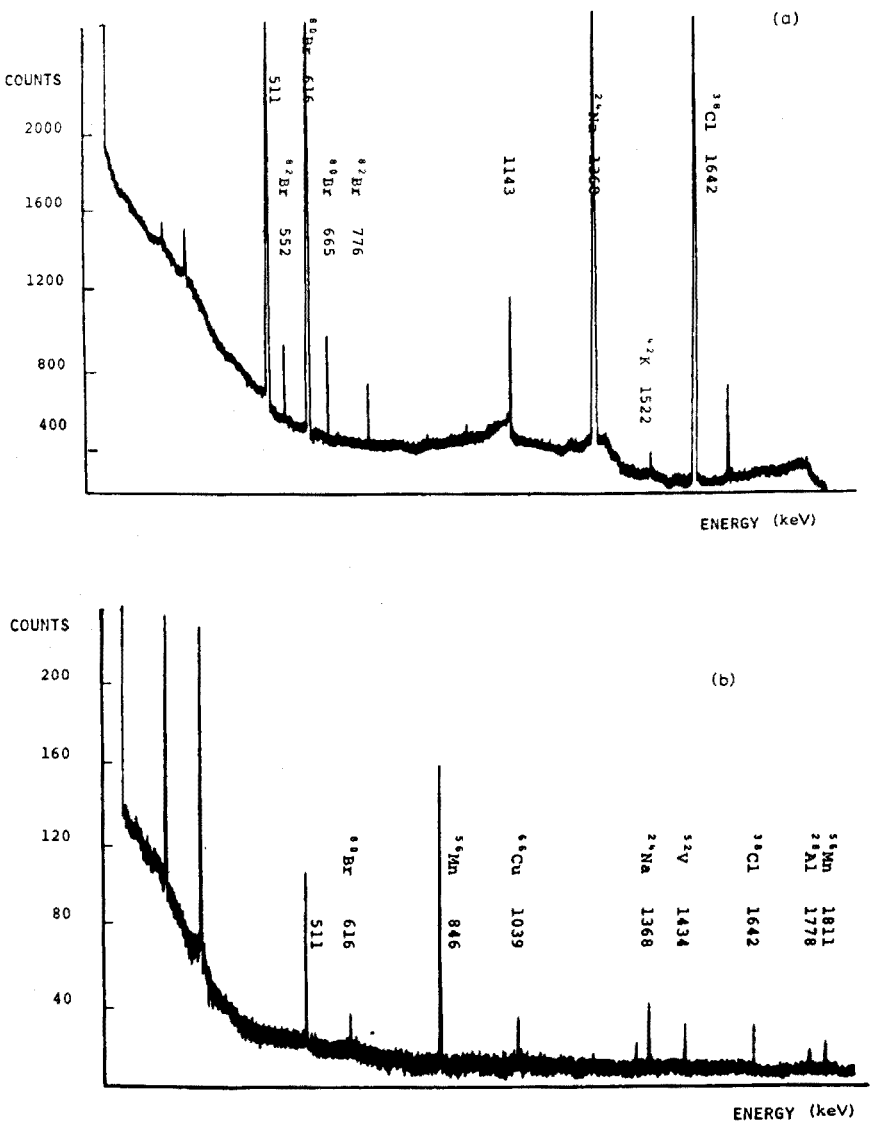

Fig. 3. γ-Spectrum of an activated spiked sea-water matrix (a) without separation, (b) after a PVO column separation. For the spike concentrations, see Table 1.

effective chelating reagent concentration by coating more of the polymer on the glass beads were unsuccessful. This problem was overcome by the addition of a carrier solution of the four desired metals to each sample and standard before the separation procedure and the determination of the chemical yields of both the sample separation and the standard separation. Table 1 presents the results of the analysis of the same spiked sea-water standards after the determination of chemical yields.

TABLE 1

Analysis of a spiked sea-water sample

Element	Spike concn. $\mu g\ ml^{-1}$	Mean concn. found[a] ($\mu g\ ml^{-1}$)	s_r
Mn	0.10	0.098 ± 0.008	8.8
V	0.33	0.33 ± 0.03	9.1
Al	2.00	1.87 ± 0.18	9.6
Cu	1.00	0.96 ± 0.02	1.8

[a]Mean of 4 separate determinations and standard deviation.

Poly-5-vinyl-8-hydroxyquinoline is not intended to replace ion exchange or chelating resins. However, when coated on solid substrates such as controlled-pore-diameter glass beads, PVO may be used for the rapid separation of trace metals from high saline matrices. Further studies of the chelating properties of this polymer will be directed towards a rapid quantitative separation procedure which will not require the determination of chemical yields.

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SUBSTOICHIOMETRIC EXTRACTION OF GOLD(III). DETERMINATION OF THE EXTRACTION CONSTANT OF GOLD DICHLORIDE DIETHYLDITHIOCARBAMATE

H. CHERMETTE, J. F. COLONAT and J. TOUSSET

Institut de Physique Nucléaire, Université Claude Bernard Lyon-I and IN2P3, 43 Bd du 11 novembre, 69621 Villeurbanne (France)

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SUMMARY

The extraction constant of gold dichloride diethyldithiocarbamate has been determined (log K = 68.2 ± 0.2) by studying the competition from palladium in the substoichiometric extraction of gold with copper diethyldithiocarbamate in chloroform. Experimental conditions that allowed the equilibrium to be reached for any palladium concentration were chosen so that the influence of the palladium concentration on the extraction kinetics has also been studied. A program for the computation of any stability constant from extraction data for mixtures of three cations has been written.

The gold(III)—diethyldithiocarbamate system, first mentioned [1] by Bode and Neumann, was studied by Beardsley et al., who reported [2] a substoichiometric determination of traces of gold by neutron activation analysis, in which the separation was performed after the addition of ca. 1 mg of gold carrier, and also reported [3] an isotopic dilution determination of gold(I) with diethyldithiocarbamate ion $(C_2H_5)_2\ NCS_2^-$ (denoted by DDC). Evidence of two compounds, $Au(DDC)Cl_2$ and $Au(DDC)_2\ Cl$, was first given by Kukula et al. [4]. Kukula and Simkova described [5] a substoichiometric separation of gold diethyldithiocarbamate for activation analysis.

The determination of gold(III) by isotope dilution analysis has not been investigated, however, probably because values for the stability constants of the complexes involved are not available. Although the stability constants of the complexes of palladium or mercury with DDC have been determined [6—8] only the qualitative order given by Stary et al. [9, 10]

Au^{3+} Pd^{2+} Hg^{2+} Ag^{+} Cu^{2+} ...

allows higher values for the gold—DDC stability constants to be predicted.

For a substoichiometric isotope dilution determination, only the complex $Au(DDC)Cl_2$ is involved. To determine its stability constant, the competition from palladium in the extraction of gold by substoichiometric quantities of copper diethyldithiocarbamate in chloroform has been studied. The extractant selected was $Cu(DDC)_2$ because its stability is much lower than those of

gold and palladium diethyldithiocarbamates (Table 1) although it is more stable than NaDDC or HDDC in acidic solutions [2, 4].

EXPERIMENTAL

Apparatus

The scintillation counter for the gold-194 and -196 activities was a 102 × 102-mm NaI(Tl) crystal associated with a single-channel γ-ray spectrometer. For palladium-103, a 44 × 5 mm NaI(Tl) crystal was used.

Reagents

Water and all reagents used had the required purity for trace analysis.

Copper diethyldithiocarbamate

Sodium diethyldithiocarbamate (Prolabo) was dissolved in distilled water, an excess of copper sulphate (Prolabo) solution was added, the precipitate of $Cu(DDC)_2$ was extracted into chloroform, and the solution was evaporated to dryness. Dilute $Cu(DDC)_2$ solutions were prepared just before use.

TABLE 1

Extraction constants of DDC—metal complexes in chloroform

Metal	Complex	Constant	Log (constant)	Ref.
Cu	$Cu(DDC)_2$	$\frac{[Cu(DDC)_2]_{org}}{[Cu^{2+}]\,[DDC^-]^2}$	26.6 27.1	11 5
Hg	$Hg(DDC)_2$	$\frac{[Hg(DDC)_2]_{org}}{[Hg^{2+}]\,[DDC^-]^2}$	45.3	5.8
	Hg(DDC)Cl	$\frac{[Hg(DDC)Cl]_{org}}{[Hg^{2+}]\,[DDC^-]\,[Cl^-]}$	29.8	7
Pd	$Pd(DDC)_2$	$\frac{[Pd(DDC)_2]_{org}}{[Pd^{2+}]\,[DDC^-]^2}$	69.8	6
	Pd(DDC)Cl	$\frac{[Pd(DDC)Cl]_{org}}{[Pd^{2+}]\,[DDC^-]\,[Cl^-]}$	47.2	6
Au	$Au(DDC)_2^+Cl^-$	$\frac{[Au(DDC)_2^+Cl^-]_{org}}{[Au^{3+}]\,[DDC^-]^2\,[Cl^-]}$		
	$Au(DDC)Cl_2$	$\frac{[Au(DDC)Cl_2]_{org}}{[Au^{3+}]\,[DDC^-]\,[Cl^-]^2}$	68.2 ± 0.1	This work

Gold and palladium chloride solutions

Weighed amounts of pure palladium (or gold) metal (Comptoir Lyon Allemand) were dissolved in aqua regia; the solution was evaporated repeatedly to dryness with hydrochloric acid to remove the nitric acid, and finally diluted to the required strength with 1 M hydrochloric acid.

Radioisotopes

Labelled gold was prepared by irradiating pure platinum with deuterons (20—27 MeV) in the Synchrocyclotron at Lyon. (The main reaction is $^{194}Pt(d, 2n)^{194}Au$). The platinum target was dissolved in aqua regia; the resulting solution was evaporated to dryness with hydrochloric acid to remove the nitric acid. The product was then dissolved in 5 M hydrochloric acid and gold was extracted into a mixture (1 + 1) of chloroform and methyl ethyl ketone (MEK). The organic solution was evaporated to dryness with hydrochloric acid to remove the organic solvents, and the residue was finally dissolved in 0.1 M HCl. The purity of gold was checked by γ-spectroscopy: gold-199, -198, -195, -193, but mainly the -194 and -196 radioisotopes, were detected when platinum radioisotopes were kept in the aqueous phase.

Palladium-103 was obtained by irradiation of pure rhodium with deuterons (20—27 MeV) in the Synchrocyclotron at Lyon. The rhodium target (powder), attacked by chlorine in molten potassium chloride in a quartz tube, gave K_3RhCl_6 and $K_2Pd^*Cl_6$ in a few hours, and these salts were dissolved in 0.1 M HCl. Palladium was extracted into a saturated solution of dimethylglyoxime in chloroform [12]; the organic phase was evaporated to dryness repeatedly with sulphuric acid, and with hydrochloric acid, to destroy the dimethylglyoxime. The palladium chloride obtained was dissolved in 0.1 M HCl. Only palladium-103 could be detected, by means of its 20-keV rhodium x-rays, in this solution.

Procedures

After the gold carrier had been added to the labelled solution, extractions were performed on the gold—palladium mixtures by substoichiometric amounts of $Cu(DDC)_2$ in chloroform. The molar ratio of Au/Cu $(DDC)_2$ was ca. 4—6:1, and equal volumes of the aqueous and organic phases were always shaken mechanically for a constant time (ca. 30 min). The activity of a 5-ml aliquot of the organic phase was measured.

The kinetics of the extraction of palladium was studied by shaking labelled palladium solutions in 1 M HCl with a fixed substoichiometric amount of $Cu(DDC)_2$ in chloroform for various lengths of time. The molar ratio of $Pd/Cu(DDC)_2$ was ca. 4:1 and equal volumes of the organic and aqueous phases were taken. Aliquots (5 ml) of the organic phase were evaporated to dryness on Whatman paper (55 mm) and counted, since the 20-keV rhodium x-rays (from ^{103}Pd) are strongly absorbed by chlorinated solvents.

RESULTS AND DISCUSSION

Great care is necessary to avoid incorrect interpretation of the results, arising from kinetic effects. The choice of extraction conditions is discussed below.

Palladium concentration

The complexation of palladium(II) by DDC has been investigated by Briscoe and Humphries [6]. The normal reaction of palladium with $Cu(DDC)_2$ is

$$Pd^{2+} + Cu(DDC)_{2\,(org)} \rightleftharpoons Pd(DDC)_{2\,(org)} + Cu^{2+} \quad . \qquad (1)$$

Under substoichiometric conditions, ([DDC] < [Pd]) the reaction is

$$Pd^{2+} + Pd(DDC)_{2\,(org)} + 2Cl^- \rightleftharpoons 2Pd(DDC)Cl_{(org)} \qquad (2)$$

Reaction (2) is slow, and depends on the concentration in the aqueous phase: for a given stoichiometry (i.e. a given concentration ratio of Pd^{2+}/DDC), the formation of palladium chloride diethyldithiocarbamate increases with the palladium concentration as shown in Fig. 1.

pH Value

Strongly acidic conditions are necessary to avoid colloid formation in aqueous gold or palladium solutions, and trace determinations involving solutions obtained by the dissolution of samples in acids are often required.

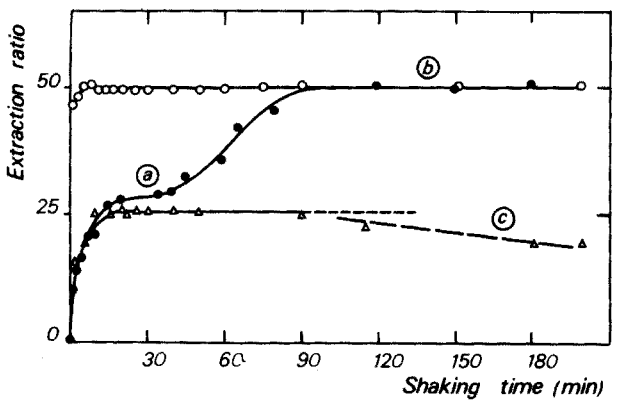

Fig. 1. Influence of palladium concentration on its substoichiometric extraction by $Cu(DDC)_2$ in chloroform: (○) 10^{-4} M palladium chloride, 0.1 M Cl^-, 0.5 M H^+ (H_2SO_4); (●) 10^{-5} M palladium chloride, 0.1 M Cl^-, 1 M H^+; (△) 10^{-6} M palladium chloride, 0.1 M Cl^-, 1 M H^+. Stoichiometry $[Pd]/[Cu(DDC)_2] \simeq 4$. (a) Extraction of $Pd(DDC)_2$. (b) Extraction of Pd(DDC)Cl. (c) Degradation of DDC vs. time.

However, the decomposition of diethyldithiocarbamic acid increases with increasing acidity, and extractions [2, 4] are usually done with $Cu(DDC)_2$ or $Zn(DDC)_2$, instead of with NaDDC, HDDC or the diethylammonium salt. The decomposition of $Cu(DDC)_2$ cannot be neglected [1] when the concentration of acid exceeds 5 M.

The formation of Pd(DDC)Cl is pH-dependent; Briscoe and Humphries [6] have shown that the rate increases with the acidity. An optimal hydrogen ion concentration (ca. 1 M) was chosen.

Chloride concentration

Chloride is necessary for the formation of the gold diethyldithiocarbamate dichloride complex; high concentrations of chloride ion should make gold extraction easier. Chloride ion also prevents the formation of colloidal gold and palladium by formation of the chlorinated complexes $AuCl_x^{3-x}$ and $PdCl_x^{2-x}$, which are much less stable than the DDC complexes [13].

Reaction [2] is also dependent on the chloride concentration; high chloride concentrations should make the formation of the chlorinated complex easier. However a kinetic effect occurs [6]; the more chlorinated the solution, the slower the reaction, with the formation of Pd(DDC)Cl. This can be explained by the formation of chloro complexes

$$Pd^{2+} + n\ Cl^- \rightleftharpoons Pd\ Cl_n^{2-n} \quad n = 1, 2, 3 \text{ or } 4 \qquad (3)$$

which cannot react directly on $Pd(DDC)_2$ to give Pd(DDC)Cl.

A chloride concentration of ca. 0.1 M was chosen for experiments on the competition from Pd^{2+} in gold extraction.

Shaking time

The time required to reach equilibrium depends on the efficiency of the shaking process and on the shape of the separatory funnels, but semi-quantitative results can be given. The extraction of gold-DDC complexes is rapid; equilibrium is reached [4] in a few minutes. The slowest reaction is the complexation of palladium, as has been explained above; this is critical when the concentration of palladium added is smaller than that of DDC. Fortunately, the results show that, in these conditions, extraction of gold is not affected by palladium, regardless of whether $Pd(DDC)_2$ or Pd(DDC)Cl is involved.

Stoichiometry Au/DDC

A concentration ratio for DDC/Au of less than 0.7 is necessary to prevent the formation of $Au(DDC)_2^+$ close to (and evidently above) stoichiometric proportions as can be seen from the results of Kukula et al. [4]. Different values were chosen for different gold concentrations. Some results are shown in Fig. 2.

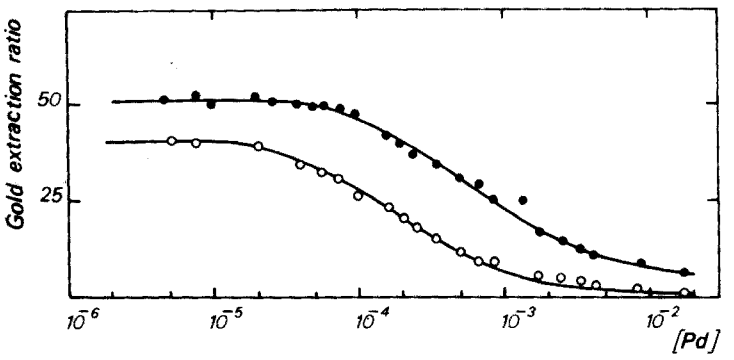

Fig. 2. Influence of palladium concentration on the substoichiometric extraction of gold by $Cu(DDC)_2$ in chloroform: (•) $1.2 \cdot 10^{-5}$ M $Cu(DDC)_2$, $4.56 \cdot 10^{-5}$ M Au; (○) $1.18 \cdot 10^{-6}$ M $Cu(DDC)_2$, $5.07 \cdot 10^{-6}$ M Au, 0.91 M HCl.

NUMERICAL TREATMENT

This is performed on competition curves such as given in Fig. 2. These equilibria can be described by the equations

$$\begin{aligned} &2\,Au^{3+} + Cu(DDC)_{2(org)} + 4\,Cl^- \leftrightharpoons Cu^{2+} + 2\,Au(DDC)Cl_{2(org)} \\ &Pd^{2+} + Cu(DDC)_{2(org)} \leftrightharpoons Cu^{2+} + Pd(DDC)_{2(org)} \\ &Pd^{2+} + Pd(DDC)_{2(org)} + 2\,Cl^- \leftrightharpoons 2\,Pd(DDC)Cl_{(org)} \\ &Au(DDC)Cl_{2(org)} + Pd^{2+} \leftrightharpoons Au^{3+} + Cl^- + Pd(DDC)Cl_{(org)} \end{aligned} \qquad (4)$$

Different values of the extraction constant

$$K = \frac{[Au\,(DDC)Cl_2]_{org}}{[Au^{3+}]\,[Cl^-]^2\,[DDC^-]}$$

are tested to find if they fit roughly on the experimental curve. Values differing by a factor of $10^{0.2}$ are then checked and the best fit (least squares of differences between experimental and calculated extraction ratios) is kept. Then a second curve-fitting is performed to calculate the best value of the total concentration of DDC (solutions could decompose slowly, mainly at high dilutions [14], see Fig. 1). Generally a correction of 1—3 % of the previous concentration is obtained. A final fitting of the stability constant value, involving steps of 0.02 in the logarithm of the constant, was carried out by a program written in Fortran and computed on a Control Data CDC 6600, which takes all DDC complexes and metal—chloride complexes into account, and is sufficiently general to compute any stability constant of this kind of complex from extraction data for mixtures of three cations, chloride, and an extractant such as DDC. (This program, with a sample calculation, is available on request).

The values obtained by this method for different curves are identical,

which is proof of the correctness of the description of the system by eqn. (4). However, the accuracy of the value (Table 1) is dependent on the stability constants of the other complexes involved in the equilibrium, mainly that for Pd(DDC)Cl.

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DIFFUSE AND INTERNAL REFLECTANCE SPECTROSCOPY FOR THE DETERMINATION OF TRACE METALS IN POWDERED SOLIDS: COBALT AND COPPER COLLECTED WITH ZINC TETRATHIOCYANATOMERCURATE

D. E. RYAN, O. LIARDON and R. YOUNG

Trace Analysis Research Centre, Chemistry Department, Dalhousie University, Halifax, Nova Scotia, B3H 4J1 (Canada)

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SUMMARY

Small amounts of cobalt and copper can be determined directly, after coprecipitation with zinc tetrathiocyanatomercurate, by diffuse reflectance spectroscopy. The limits of detection are about 1 μg of metal per 40 mg of collector; if 100—200-ml sample volumes are available, 5—10 ng of metal ion per ml can be determined, but these limits are improved by about 50 % if the powdered solid samples are wetted before measurement.

Recent studies have been directed toward the possibility of determining trace amounts of metal ions by direct measurement of some property of the solid state. Some success has been achieved, after coprecipitation of the trace element, by measurement of the luminescence of inorganic phosphors [1—3] and fluorescent chelates [4, 5] or of the reflectance of coloured chelates [6] and inorganic compounds [7].

Zinc tetrathiocyanatomercurate(II) can collect certain metal ions and form characteristic intensely coloured precipitates; this property has long been used for the sensitive detection of copper(II) and cobalt(II). The ability of zinc tetrathiocyanatomercurate to collect substances at concentrations much lower than their natural solubility is based on the formation of solid solutions [8].

This paper describes the determination of trace amounts of cobalt(II) and copper(II) by diffuse reflectance spectroscopy, after collection from solution with zinc tetrathiocyanatomercurate, and the results of internal reflectance spectroscopy studies aimed at achieving increased sensitivity.

EXPERIMENTAL

Apparatus, reagents, solutions

Measurements were made with a Farrand Optical Co. VIS-UV Chromatogram Analyzer, a Zeiss PMQ II Chromatogram Spectrophotometer, or a Unicam SP 8000 Spectrophotometer.

The sample cell for the chromatogram instruments was an aluminium sheet painted white with drilled holes (5 mm diam.) and covered with a thin (1mm) glass plate. It was packed with powdered sample held in place by masking tape; the glass was removed before measurement.

The cells for internal reflectance studies were 41 × 14 × 1 mm u.v.-grade sapphire or quartz single-pass parallelepiped plates with bevels of 60° at each end. These cells were mounted in Twin-Parallel-Mirror-Reflectance Attachments (Harrick Scientific Corporation, Ossining, N.Y.) in the Unicam SP 8000 spectrophotometer.

A solution of tetrathiocyanatomercurate was prepared by dissolving 8 g of mercury(II) chloride and 9 g of ammonium thiocyanate in 100 ml of distilled water. A zinc(II) solution containing 1 g of zinc acetate in 100 ml was used. Stock solutions containing 1000 μg ml^{-1} of copper(II), cobalt(II), and nickel(II) were prepared from the chlorides. All chemicals used were reagent grade.

Initial procedure

To 100 ml of solution, containing 1—20 μg of metal ion, add 2 ml of the 1 % zinc acetate solution. Adjust the pH within the range 1.0—6.0 and add 2 ml of tetrathiocyanatomercurate reagent with rapid stirring (magnetic stirrer). Filter the precipitate through a crucible after stirring for 2—3 min. Wash with distilled water and dry in vacuo for 1 h at room temperature. Pack the cell and measure the reflectance at 540 nm for copper, at 600 nm for cobalt and at 400 nm for nickel.

RESULTS AND DISCUSSION

Figure 1 shows the reflectance spectra for 10 μg of copper, cobalt, and nickel collected by the above procedure. The separate maxima for cobalt and nickel permit their simultaneous determination. Copper absorbs over a wide wavelength range and must be masked if it is present, when analyzing for cobalt or nickel. At pH 5.6—6.0, 600 μg of copper does not interfere in the determination of 10 μg of cobalt or nickel (collected from 100 ml of solution) if 10 ml of a 0.5 M solution of glycine are added before the pH adjustment and the addition of thiocyanatomercurate.

Figure 2 shows typical calibration curves. The standard deviation for ten determinations of cobalt and copper (3 μg in 100 ml of solution) was 10 %.

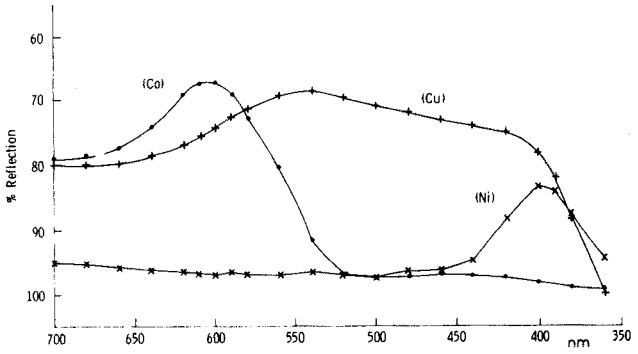

Fig. 1. Spectra of 10 μg Cu(II), Co(II) and Ni(II) coprecipitated with 45 mg of $ZnHg(SCN)_4$. (Zeiss PMQ II Chromatogram Spectrophotometer).

The sensitivity is significantly lower for nickel and there was low reproducibility for concentrations below 5 μg per 100 ml of solution. Collection of trace amounts of nickel with α-benzildioxime, however, permits nickel to be determined at the 1 ng l^{-1} (and less) level per ml [6].

A 1000-fold weight excess of the following ions does not interfere in the analysis of copper: bismuth(III), calcium(II), iron(II), lead(II), lithium(I), magnesium(II), manganese(II), mercury(II), potassium(I), silver(I), sodium(I), acetate, bromide, chloride, citrate, fluoride, nitrate, perchlorate, phosphate, sulfate, tartrate and thiocyanate. A 100-fold excess of antimony(III), barium(II), cadmium(II), strontium(II), cyanide, iodide, and a 10-fold excess of aluminium(III), cobalt(II), nickel(II) and zinc(II) also do not interfere. Iron(III) and sulfide interfere.

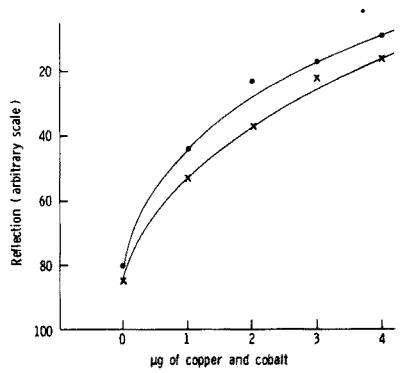

Fig. 2. Calibration curves for cobalt (•) and copper (×), coprecipitated with $ZnHg(SCN)_4$.

In the determination of cobalt, a 1000-fold excess of the following ions does not interfere: barium(II), bismuth(III), calcium(II), chromium(III), lead(II), lithium(I), magnesium(II), mercury(II), potassium(I), silver(I), sodium(I), tin(IV), acetate, bromide, chloride, citrate, fluoride, iodide, nitrate, perchlorate, phosphate, sulfate, tartrate and thiocyanate. A 100-fold excess of antimony(III), cadmium(II), zinc(II), cyanide, sulfide, and a 10-fold excess of aluminium(III), and nickel(II) do not interfere, but copper(II) and iron(III) must be masked.

Fluoride can be used to prevent interference by a 10000-fold weight excess of iron(III) up to pH 5.5; above this pH, hydrolysis occurs and the precipitate has a slightly yellow colour (maximum absorption occurs about 480 nm). Copper(II) is masked with glycine above pH 5.5. Simultaneous masking of copper(II) and iron(III) with glycine and fluoride is therefore difficult. When both iron and copper are present, iron should be removed by extraction with ether from 7 M hydrochloric acid before analyzing for cobalt.

To demonstrate the potential of the method, cobalt was determined in a stainless steel (containing 18.6 % Cr, 9.38 % Ni, 0.89 % Mn, 0.82 % Si, 0.32 % Ti, 0.11 % Cu and lesser amounts of V, Mo, Al, C, As and P) after removal of iron (by extraction with ether from 7 M hydrochloric acid) and nickel (by precipitation with dimethylglyoxime) in 1 and 2-ml aliquots of the final 100 ml of solution. The value found was 0.053 % Co (cobalt present = 0.056 %).

Copper and cobalt were also determined in synthetic blood containing 19,900 mg $Na\,l^{-1}$, 16900 mg $K\,l^{-1}$, 620 mg $Ca\,l^{-1}$, 410 mg $Mg\,l^{-1}$, 4750 mg $Fe\,l^{-1}$, 130 mg $Zn\,l^{-1}$, 10 mg $Cu\,l^{-1}$ and 5 mg $Co\,l^{-1}$. A 5-ml sample was evaporated to dryness, 50 ml of 7 M hydrochloric acid were added, and the iron(III) was extracted with portions (2 × 100 ml) of diethyl ether. The ether was back-washed with 30 ml of 7 M HCl, the washings and solution were evaporated to dryness, and the solid was dissolved in water and diluted to 100 ml. Copper was determined in aliquots containing ca. 1 and 2 μg of copper by collection with zinc thiocyanatomercurate from 100-ml volumes at pH 4; found 10 mg l^{-1} of copper. The same procedure was applied for cobalt but 1 ml of 0.5 M glycine was added to mask copper and precipitation was done at pH 5.7; found, 5.4 mg l^{-1} of cobalt.

Factors affecting method

Zinc tetrathiocyanatomercurate is not very insoluble so that the amount of precipitate will depend upon solution volume. The weights of precipitate obtained from 50, 100, 150 and 200-ml volumes were 43.8, 43.0, 41.9 and 40.9 mg, respectively. These differences correspond to ca. 1 mg per 50 ml change in volume and have little effect on the results obtained. Relative peak heights for 3 μg of cobalt collected from 50, 100, 150 and 200 and 250 ml

of solution, by addition of the same volume of reagents, were 22, 30, 30, 28 and 30 respectively and the reflectance is essentially constant from 100 to 250 ml volumes.

Precipitation at elevated temperatures (75—80 °C) results in more crystalline precipitates and a higher absorption (lower reflectance); collection of the trace element is the same, but the amount per unit volume is increased. The reproducibility of results, however, was poor.

The quantity of precipitate depends on the amount and nature of the reagent added in excess. A two-fold excess of thiocyanatomercurate gives maximum precipitation, but a larger excess gives more rapid precipitation; a six-fold excess was used to obtain the present results. Two ml of 1 % zinc acetate was used to give sufficient precipitate to pack the holes in the cell.

Precipitation (and collection) is essentially independent of pH over the range 1.0—3.0. Above pH 3.0 the absorption decreases but satisfactory results are obtained at pH 5.5—6.0 (recommended when masking copper). Above pH 6.0 there is a rapid decrease in the amount of precipitate obtained.

Multiple internal reflectance

The results reported above were obtained by ordinary reflection from the surface and from radiation penetrating below the surface and re-emerging after being scattered several times. The use of internal reflectance spectroscopy for the determination of trace elements collected by solids was also investigated.

Internal reflection spectroscopy is based on the total reflection which occurs in a higher refractive index medium, relative to a lower refractive index one, over a particular angle range. Light entering the plate (or cell) emerges from the other end after making a certain number of reflections against both faces of the plate (Fig. 3). The material whose spectrum is being taken is placed on the plate face(s) and, at every reflection point, absorption can take place if sample and plate are in intimate contact.

Figure 4 shows the internal reflectance spectrum for a $2 \cdot 10^{-5}$ M solution of safranine dye in water. The quartz cell was dipped into the dye solution and allowed to drip dry; the homogeneous film remaining on the plate face is not visible to the eye when viewed by transmitted light but is readily seen by internal reflectance. Comparison with a normal transmission spectrum for 10^{-6} M aqueous solution of the dye shows they are essentially the same.

Internal reflection analyses, at 560 nm, for $5.5 \cdot 10^{-7}$—$5.5 \cdot 10^{-5}$ M safranine in 1:1 benzene—acetone were satisfactory; the volatility of benzene—acetone gave fast drying of the plate. Reproducibility was good and the internal reflectance technique can be used for the determination of absorbing molecules in volatile organic solvents. There appears to be no advantage over transmission techniques, however, with the possible exception that only small solution volumes are required.

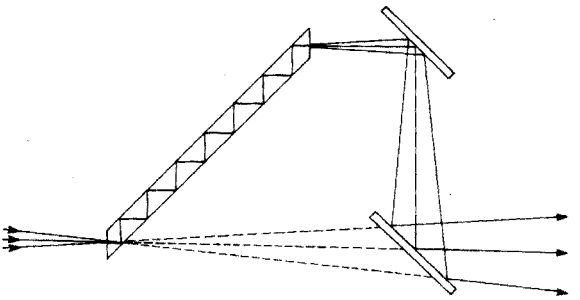

Fig. 3. Light path in the internal reflectance cell.

Similar attempts to determine copper or cobalt collected by zinc tetrathiocyanatomercurate were unsuccessful; significant loss of light occurred through scattering by the powdered solid. With no sample on the face plate of the cell, light entering the cell is not diffused but leaves the cell as a small beam; the quartz cell is almost invisible and the bevelled end face shows shadows. With white powder on the plate face, the cell is illuminated as a result of scattering and the angled face outlet is homogeneously bright. This large amount of light scattering from the surface of the powdered solid makes internal reflectance, by this method, of little value for increasing the sensitivity of the ordinary reflection process.

Internal reflectance can, however, increase sensitivity by 50 % if the solid packed into the holes of the aluminium plate cell is wetted. Figure 5 shows calibration curves for copper collected with $ZnHg(CNS)_4$. Curve (a) is for samples measured in the usual way by direct reflectance. Curve (b) is for the same samples after wetting with 0.01 ml of Nujol; $ZnHg(CNS)_4$, wetted with Nujol because of its low volatility was used as a blank. The sensitivity is increased because the light is reflected internally by the liquid, resulting in more absorption by the "coloured" solid. The extent of such internal reflectance depends upon the angle of incidence and the refractive index of the liquid used for wetting. The standard deviation for 10 samples, each containing 3 μg of copper, was the same for dry and wetted powders

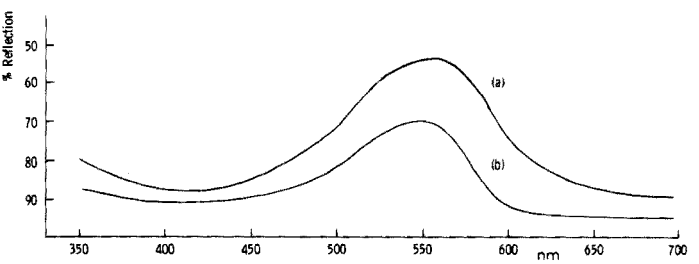

Fig. 4. Spectra of safranine for (a) a $2 \cdot 10^{-5}$ M solution by internal reflectance spectroscopy and (b) for a 10^{-6} M solution by normal transmission spectroscopy.

but the increased sensitivity with wetted samples results in a relative standard deviation improvement from 10 % to 4.5 %.

Recommended procedure

To 100 ml of solution containing 1—20 μg of metal ion add 2 ml of the 1 % zinc acetate solution. Adjust the pH within the range 1.0—3.0 (unless copper is to be masked) and add 2 ml of tetrathiocyanatomercurate reagent with rapid stirring (magnetic stirrer). Filter the precipitate through a crucible after stirring for 2—3 min. Wash with distilled water and dry in vacuo for 1 h at room temperature. Pack the cell, wet with ca. 0.01 ml of Nujol, and measure the reflectance at 540 nm for copper and at 600 nm for cobalt.

This work was supported by a grant from the National Research Council of Canada.

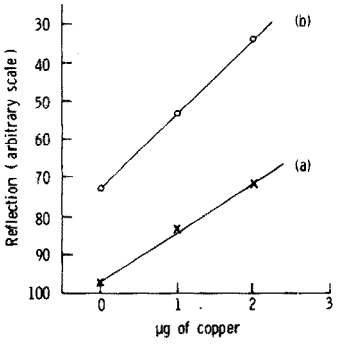

Fig. 5. Calibration curves with (a) dry powdered solid and (b) with the same samples wetted by Nujol.

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A KINETIC-CATALYTIC METHOD WITH REPEATED ADDITION OF ONE REACTANT — THE DETERMINATION OF MANGANESE, IODIDE, UREASE AND CADMIUM

H. WEISZ and K. ROTHMAIER

Lehrstuhl für Analytische Chemie, Chemisches Laboratorium der Universität Freiburg i. Br. (German Federal Republic)

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SUMMARY

The use of a new kinetic-catalytic method with repeated addition of one reactant in the same system is described. The determination of manganese (periodate—malachite green; photometric observation), iodide (cerium(IV)—arsenic(III); potentiometric observation) and urease (hydrolytic splitting of urea; potentiometric observation) serves to illustrate the procedure.

At the end of a catalyzed reaction of two reactants

$$A + B \xrightarrow{K} P$$

the catalyst per definitionem is present in its unchanged original form and amount. Therefore, the measurements necessary for the determination of the catalyst can be repeated several times; it is then possible to calculate a mean value and consequently to reduce the errors by a reasonable degree.

It should be possible to achieve this by repeatedly adding the reactant (A) discontinuously in small but equal volumes to a solution containing the second reactant B in high concentration as well as the catalyst to be determined. This addition should always be done at the very moment when the last added amount of reactant A had just been consumed by the reaction. This procedure can be repeated, of course, only as long as the concentration of reactant B can be regarded as practically constant. The reaction product P which accumulates in the system must not exert a visible influence on the reaction. A further condition for this method is that the freshly added volumes must be very small compared to the total volume of the system so that they can be neglected. The concentration of the added reactant A in these small volume-increments must therefore be sufficiently high to allow for a measurable phenomenon in the total system.

Preliminary experiments showed that it is better always to add the reactant A at a certain defined point before the previously added amount of A has been completely consumed because otherwise the reaction times become

impracticably long.

If the reaction is followed by observing the decrease in the concentration of reactant A, an equal volume of a defined solution of this reactant must be added when its concentration in the system has reached a certain value.

If the reaction is followed by observing the formation of the reaction product P, a defined amount of an auxiliary reagent R must be added at the moment when the concentration of product P in the system has reached a certain value. This reagent R must react very quickly with the product P, so that reactant A, which is added once only at the beginning of the reaction, is completely regenerated (P + R → A). This is necessary, otherwise in the succesive measurements, the reaction rates would no longer be constant because of the decreasing concentration of reactant A.

In some cases it is possible to keep the concentration of reactant A constant by applying it right away in a high concentration as with the reactant B; in this case, the regeneration of A is not necessary (see Determination of urease).

In all these cases, the time Δt necessary to achieve a certain predetermined change in the concentration of either reactant A or product P in the various single "reaction runs" is a measure of the concentration of the catalyst to be determined (fixed-concentration method).

DETERMINATION OF MANGANESE(II) BY USE OF THE PERIODATE–MALACHITE GREEN REACTION

The oxidation of malachite green by periodate to colorless products of unknown structure is catalyzed by manganese(II) [1]. The course of this reaction can be followed photometrically.

Experimental

A photometer ("Universal-Kolorimeter Modell J", Lange, Berlin) was used to follow the course of the reaction, the absorbance being measured at 617 nm. The 20-mm pathlength optical cell used was jacketed for temperature control. The temperature of the thermostat was here, and in all other methods in this paper, maintained at 25 °C.

Solutions

For the buffer solution, dissolve 276 g of NaH_2PO_4 and 120 ml of glacial acetic acid and dilute to 1 l with double-distilled water, and add 210 ml of 0.1 M sodium hydroxide. Use a manganese(II) chloride stock solution containing 100 μg Mn ml^{-1}.

Procedure

Into the cell, place x ml of manganese sample solution, $(8-x)$ ml of double-distilled water, 1 ml of sodium periodate solution (2 % w/v) and 1 ml of buffer solution. After 10 min, start the reaction by adding 50 μl of

malachite green solution (0.2 mg ml^{-1}) to the thermostated reaction mixture with an Eppendorf pipette. The percentage absorption increases to about 50 %. As soon as the absorption of the malachite green, consumed by the reaction, decreases to 35 %, add another aliquot (50 μl) of the malachite green solution; this brings the absorption to a value of about 67 %. From this point onwards, always add 50 μl of this reactant when the absorption just reaches a value of 35 %. The change in absorbance with time is recorded (Fig. 1).

With this mode of operation it is necessary to observe constantly the course of the reaction. This can be avoided by using an instrument with which the addition of the reactant, at a required and preselected absorption value, is done automatically. The apparatus (Fig. 2) consists of a photometer (see above), a mV/pH meter, a controller ("Impulsomat"), an instrument for the preselection of the volume ("Dosifix"), and an automatic 5-ml burette (all from Metrohm, Herisau, Switzerland). The method of operation of this apparatus is as follows. Place the optical cell (3) with all necessary solutions in the photometer. The signal of the photocell (5) is amplified (6). The resulting actual potential, measured by the mV meter, is compared in the controller (Imp.) with the preset working potential (400 mV), which in turn corresponds to a certain working absorption (35 %). As soon as these two potentials are equal a signal is automatically passed on to the automatic burette, which in turn delivers a certain preselected volume (50 μl) of the reactant to the reaction mixture.

This automatic procedure was used in all the other determinations described in this paper. Obviously all examples mentioned below could likewise be carried out manually.

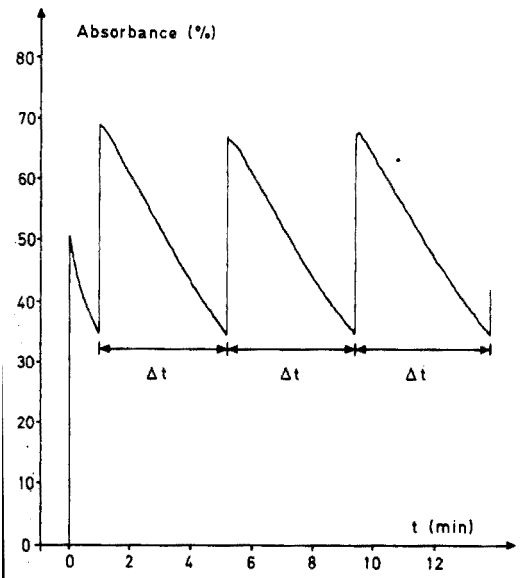

Fig. 1. Recorder graph. 1.0 μg Mn/10 ml.

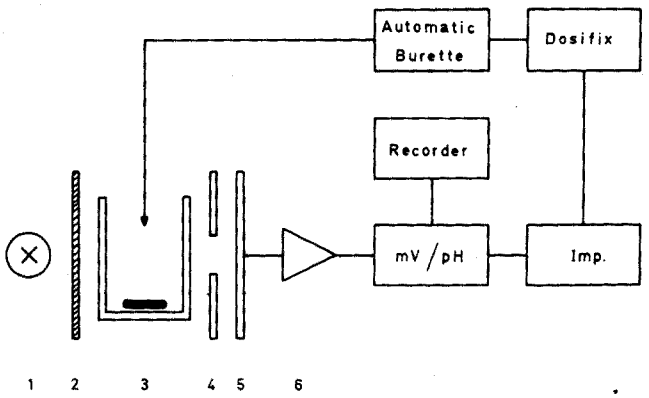

Fig. 2. Apparatus. 1, Source of light; 2, monochromatic filter; 3, thermostated reaction cell with magnetic stirrer; 4, iris diaphragm; 5, photocell; 6, amplifier (Z_f/Z_i = 10 $k\Omega$/ 1000Ω).

Evaluation and results

In the determination of manganese, the time Δt (min) between two consecutive maximum points of the curve (corresponding to the single additions of malachite green solution) is taken from the recorded graphs (Fig. 1). The calibration curve (Fig. 3) is prepared by plotting the reciprocal of the average of three single measurements ($1/\overline{\Delta t}$) against the catalyst concentration. The plot is linear for the range, 0.7—2.0 μg Mn/10 ml, but at low concentrations there is a slight curvature. Some results are listed in Table 1.

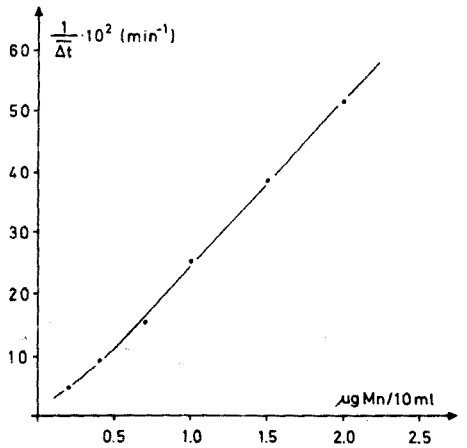

Fig. 3. Calibration graph for the determination of manganese.

TABLE 1

Determination of manganese

Manganese (μg/10 ml)		Rel. error %	Manganese (μg/10 ml)		Rel. error %
Given	Found		Given	Found	
0.200	0.207	+3.5	0.780	0.790	+1.3
0.210	0.220	+4.8	0.800	0.810	+1.2
0.250	0.250	±0.0	0.872	0.830[a]	−4.8
0.300	0.290	−3.3	0.960	0.934	−2.7
0.308	0.320[a]	+3.9	1.04	1.08	+3.8
0.500	0.490	−2.0	1.40	1.41[a]	+0.7
0.568	0.560[a]	−1.4	1.60	1.65[a]	+3.0
0.648	0.630	−2.8	2.00	1.99	−0.5

[a]These determinations were done manually.

DETERMINATION OF IODIDE BY USE OF THE CERIUM(IV)—ARSENIC(III)—REACTION

The reaction between cerium(IV) and arsenic(III) is catalyzed by iodide [2]. The course of the reaction for this determination can be followed potentiometrically.

Experimental

The apparatus used was the same as in the photometric determination (Fig. 2), but instead of the photometer and the amplifier, a platinum and a saturated calomel electrode were used for controlling the addition of the reactant.

Solutions

For the arsenic(III) solution (0.2 M) dissolve 9.892 g of As_2O_3 in 200 ml of 0.1 M sodium hydroxide, mix with 35 ml of 10 M sulphuric acid, and dilute to 500 ml with double-distilled water. Use a potassium iodide stock solution containing 1 mg I ml^{-1}.

Procedure.

Before the determinations, preset the working potential at 810 mV.

In a 20-ml beaker, place x ml of iodide sample solution, 1 ml of arsenite solution, and 1 ml of 4 M sulphuric acid. To achieve a reasonably reproducible starting potential (ca. 290 mV), add 0.1 ml of $3.75 \cdot 10^{-3}$ M cerium(III) sulphate solution in 0.5 M sulphuric acid and dilute the mixture to 10 ml with double-distilled water. Lower the electrodes and the tip of the automatic burette (glass-capillary) into the solution. After 10 min of thermostating, start the reaction by the addition of two drops (0.11 ml) of 0.1 M

cerium(IV) sulphate solution in 0.5 M sulphuric acid. Whenever the actual measured potential reaches the working potential (810 mV), add one drop (55 μl) of the cerium(IV) solution via the automatic arrangement. The recorded curve is similar to that shown in Fig. 1.

Evaluation and results

For the evaluation, the time Δt (min) between two consecutive additions of the cerium(IV) solution is measured from the recorded graph. A calibration graph (Fig. 4) is prepared, as before, by plotting $1/\overline{\Delta t}$ (three single measurements) against the iodide concentration. The graph is a smooth curve over the range 0.1—1.0 μg I^-/10 ml. Some results are listed in Table 2.

DETERMINATION OF UREASE BY HYDROLYTIC SPLITTING OF UREA

The hydrolytic splitting of urea to form ammonia and carbon dioxide is catalyzed by urease [3]. The course of the reaction is followed by measuring the pH value of the system. As soon as the predetermined pH value has been reached (corresponding to a definite amount of ammonia formed by the reaction), a defined volume of standard acid (auxiliary reactant, see above) is added so that the pH is never lower than 7. This avoids partial denaturation of the enzyme. This procedure can be repeated several times, because both reactants, i.e. urea and water, are present in high concentrations.

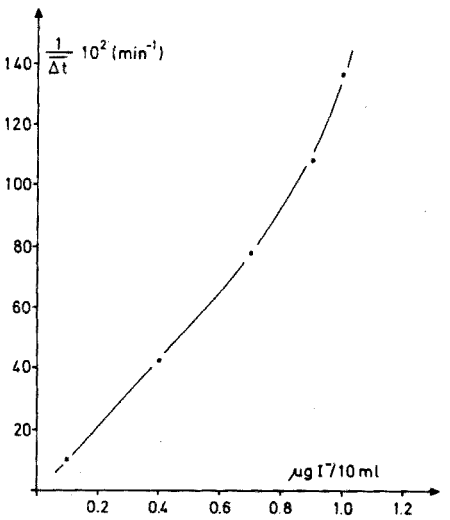

Fig. 4. Calibration graph for the determination of iodide.

TABLE 2

Determination of iodide

Iodide (μg/10 ml)		Rel. error %	Iodide (μg/10 ml)		Rel. error %
Given	Found		Given	Found	
0.114	0.106	−7.0	0.645	0.650	+0.8
0.150	0.146	−2.7	0.650	0.677	+4.2
0.238	0.222	−6.7	0.850	0.886	+4.2
0.300	0.318	+6.0	0.900	0.895	−0.6
0.490	0.470	−4.1	0.960	0.940	−2.1

Experimental

For the determination of the activity of urease, a micro glass electrode and a saturated calomel electrode as reference electrode are used. The instrumental arrangement is the same as in the case of the iodide determination.

Solutions

For the urea solution, mix 3 g of urea and 0.04 ml of 0.74 M ammonium carbonate solution and dilute to 100 ml with double-distilled water.

For the urease stock solution, dissolve 11.6 mg of lyophilized enzyme (specific activity, 100 I.U. mg^{-1} enzyme; Boehringer, Mannheim) and 0.12 ml of 0.74 M ammonium carbonate solution, and dilute to 20 ml with double-distilled water. This stock solution, standardized as described by Gorin and Chin [4], had an activity of 14.5 I.U. ml^{-1}.

Procedure

Adjust the working potential so that the automatic addition of the 0.05 M sulphuric acid (55 μl) takes place at a pH of 8.25.

To a 20-ml beaker, transfer 1 ml of urea solution and 8 ml of water. Dip the electrodes and the tip of the automatic burette into the solution and maintain the temperature of the reactants at 25 °C. After 10 min start the reaction by the addition of 1 ml of urease sample solution. As soon as the pH of the system has reached the value of 8.25, always add 55 μl of the 0.5 M sulphuric acid by the automatic arrangement. Record the change of the pH value against the time.

Figure 5 shows that the peaks become lower with consecutive additions of the acid, because of the accumulated ammonium salts. Nevertheless, the time intervals Δt remain equal, except for the first measurement.

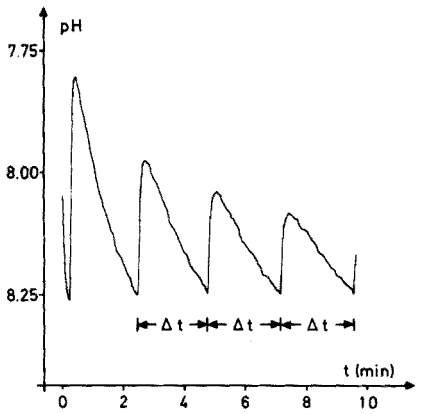

Fig. 5. Recorder graph. Activity of the urease 4.35 I.U./10 ml.

Evaluation and results

For the evaluation, the time Δt (min) between two consecutive additions of sulphuric acid is taken from the recorded graph. For each single determination four measurements should be made, but only the last three should be used for calculation of the average.

The calibration graph is prepared by plotting the reciprocal of the average $1/\overline{\Delta t}$ against the activity of the urease. A linear calibration graph is obtained for urease activities in the range 0.725—14.50 I.U./10 ml.

Some results are given in Table 3.

A number of metal ions show a strong inhibiting action on urease [5], and these ions can be determined by measuring the residual activity of the enzyme. Cadmium can be determined in the range 1—10 μg Cd/10 ml. The reaction conditions are the same as those described for the urease determination.

In some cases, where a reaction product is removed by an auxiliary reagent R, it is possible to add an excess of the latter. This not only removes the reaction product, formed up to this moment, but also adds a definite

TABLE 3

Determination of urease

Urease (I.U./10 ml)		Rel. error %	Urease (I.U./10 ml)		Rel. error %
Given	Found		Given	Found	
0.812	0.754	−7.1	7.250	6.989	−3.6
1.450	1.450	±0.0	8.700	8.874	+2.0
2.523	2.494	−1.1	9.889	9.396	−5.0
4.350	4.495	+3.5	11.60	12.11	+4.4
6.554	6.467	−1.3	13.05	12.62	−3.3

amount of the auxiliary reagent R. That means that the excess of the reagent R causes an additional induction period, forming a kind of Landolt-type reaction. For the evaluation, the time Δt is again a measure of the concentration of the catalyst. Here very distinct peaks are always formed on the recorded graphs. In this way, it is possible to determine 1—10 μg Cu/10 ml by means of the peroxodisulphate—iodide reaction (auxiliary reagent: thiosulphate) [6] with photometric observation, and 1—10 μg V/10 ml by means of the bromate—iodide reaction (auxiliary reagent: ascorbic acid) [7] with potentiometric observation.

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A RAPID METHOD OF SEPARATION AND DETERMINATION OF QUATERNARY AMMONIUM COMPOUNDS INVOLVED IN CARNITINE METABOLISM

J. SCOTT HAYES, MIGUEL A. ALIZADE and KLAUS BRENDEL

Department of Pharmacology, University of Arizona Medical School, Tucson, AZ 85724 (U.S.A.)

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SUMMARY

The rapid separation of several quaternary ammonium compounds involved in carnitine metabolism is reported. Two convenient methods based on spectrophotometry and liquid scintillation counting are employed to determine the eluting compounds. The application of the techniques to biological determinations is discussed.

Carnitine (γ-trimethylamino-β-hydroxybutyric acid) is chemically similar to choline and is distributed ubiquitously in the tissues of almost all organisms, the highest concentrations being found in muscle. Its rôle in fatty acid metabolism has been elucidated and reviewed [1]. Carnitine is involved in the transport of mainly long chain fatty acids across the inner mitochondrial membrane to the site of β-oxidation. Carnitine levels vary with physiological state and disease [2—5]. It was assumed until recently that carnitine, although participating in reactions of lipid metabolism, was not itself metabolized. It is now clear that carnitine is derived from lysine via butyrobetaine [6] and is decarboxylated to β-methylcholine [7]. The carnitine pool is regulated by synthesis and degradation. Investigations of the activities of the synthetic and degradative pathways under specific conditions such as starvation, diabetes mellitus and diphtheria become increasingly important.

Several techniques for the separation and determination of quaternary ammonium salts such as carnitine and its metabolites have been described [8—11], but these methods have some inherent drawbacks. Sodium triphenylcyanoborate forms relatively insoluble complexes with cesium and quaternary ammonium salts; these complexes can be used quantitatively [12—14]. The procedures involve either gravimetric determinations or relatively complex titrations. In studies of the metabolism of carnitine it was necessary to determine carnitine and several of the intermediates in its synthesis and degradation. All the compounds in question were quaternary ammonium compounds which had been separated previously on large

cation-exchange resin columns [15, 16]. A more rapid and complete separation of these compounds, followed by a simple assay for their determination, was required. A high-pressure ion-exchange column separation, followed by determination of the eluting quaternary ammonium salts with unlabeled or sodium triphenyl-(^{14}C)-cyanoborate, was therefore developed. The precipitated sodium triphenylcyanoborate complexes are filtered, dissolved, and measured spectrophotometrically or by scintillation counting.

EXPERIMENTAL

Materials

DL-Carnitine (Sigma) and choline chloride (Eastman Kodak) were used. Butyrobetaine was prepared from 4-aminobutyric acid with methyl iodide and silver oxide in methanol-water. [17]. 4-Trimethylaminocrotonic acid [18], β-Methylcholine [19] and trimethylaminoacetone [19] were synthesized; the compounds were recrystallized until literature melting points were obtained. Sodium triphenylcyanoborate was purchased from K & K Laboratories, Inc. Sodium triphenyl-(^{14}C)-cyanoborate was synthesized from the non-labeled compound by exchange with ^{14}C-NaCN at pH 8 in a closed vessel. DL-Carnitine (methyl-^{3}H, 250—1000 mCi/mmole) (Amersham/Searle Corporation), Triton X-100 (Rohm and Haas) and Omnifluor (New England Nuclear) were used. Chromatography equipment was obtained from Chromatonix, and Aminex A-5 cation-exchange resin from Bio-Rad Laboratories.

Separation technique

To reduce the time required to separate several of the quaternary ammonium compounds involved in carnitine metabolism (butyrobetaine, carnitine, β-methylcholine), and to increase the resolution, a jacketed, high-pressure glass column 0.63 × 58 cm was employed. The column was packed with 45 cm of Aminex A-5 and equilibrated with the starting buffer. The separation was accomplished most efficiently at 48 °C with various gradients of sodium chloride — sodium citrate buffers having molarities between 0.2 and 0.4 and pH values between 2 and 5 (see Fig. 1); 100 fractions, each of 1.4 ml, were collected. The entire separation procedure required 2.5 h; with proper gradient choice no overlapping peaks were observed. The recovery of the separated quaternary ammonium salts was found by the addition of radioactive tracers to be 95—98 %.

Assay procedure

A solution of 187 g of sodium chloride and 78.4 g sodium citrate (1 M), adjusted to pH 4, 6 and 8 with 10 M HCl or 1 M NaOH, was adjusted to 0.4

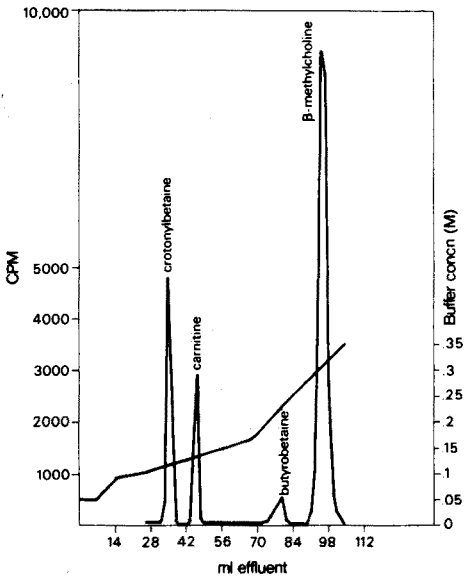

Fig. 1. Separation of radioactive quaternary ammonium salts related to carnitine on a 58 × 0.6 cm column of Aminex A-5 cation exchanger pumped at 350 psi with the indicated NaCl—Na-citrate gradient of pH 4.4.

equivalent of Na^+. Sodium triphenylcyanoborate (200 mg) was dissolved in 1 ml of distilled water. Solutions of quaternary ammonium salts were adjusted to 20 mole ml^{-1} with distilled water.

Each sample tube contained 0.5 ml of sodium citrate buffer or, alternatively, 0.5 ml of the sodium citrate eluate, to which 0.05 ml of sodium triphenylcyanoborate solution was added. After mixing, 0—3 moles of the quaternary ammonium salt to be assayed were added to the reaction mixture, and the total volume was adjusted to 1 ml, stirred, and cooled in ice water for 15 min. The precipitates were filtered on Millipore glass fiber pads (12 mm diam.) utilizing a micro Buchner filter set. The resulting filter cake was dissolved in 1 ml of methanol, 2 ml of water were added, and the absorption was read at 265 nm in a Gilford Spectrophotometer (see Fig. 2). No significant difference in absorption maxima for the different precipitates could be detected (see Fig. 3). Alternatively, sodium triphenylcyanoborate labeled in the cyano-group was reacted with various quaternary ammonium salts, and the filtered precipitates were transferred to scintillation vials containing 1 ml of methanol. After the precipitates were dissolved, 10 ml of a solution containing 333 ml of Triton X-100 and 667 ml of toluene and 3.8 g of Omnifluor were added, and the vials were counted in a Beckman LS 250 scintillation counter. When quaternary ammonium salts were eluted from Dowex 50 columns with up to 2 M HCl or acidic citrate buffers, sodium acetate was a suitable alternative buffer; 0.5 ml of eluate was added to 0.5 ml

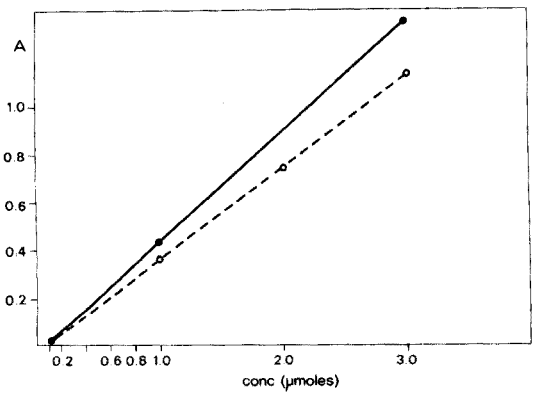

Fig. 2. Quantitation of (•) carnitine HCl and (○) choline HCl following precipitation with triphenylcyanoborate at their optimal pH. The absorbance of the redissolved filter cakes at 265 nm is plotted against concentration in the original solution.

of 5 M sodium acetate buffer and the assay was carried out as described above. Phosphate and Tris buffers were unsuitable.

The absorption at 265 nm is related linearly to the concentration of each quaternary ammonium salt (precipitated and filtered) in the range ca.

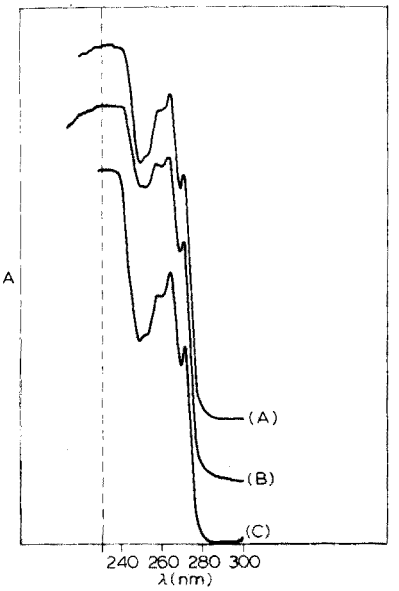

Fig. 3. Absorption spectra for the indicated triphenylcyanoborate complexes. λ_{max} = 265 nm. (A) Carnitine or butyrobetaine. (B) Crotonylbetaine. (C) β-Methylcholine.

0.1—3.0 μ mole ml^{-1} in the original reaction mixture (Fig. 2) for all compounds assayed at the optimum pH for precipitation. The time for maximum precipitation was similar for all compounds tested, and cooling for longer than 15 min did not significantly increase the amount of precipitate formed; it is necessary to filter the solution while it is cold.

Similar results were obtained with sodium (^{14}C) triphenylcyanoborate (specific activity 20 μCi/mmole), counting the precipitate collected on the filters. Figure 4 shows a linear relationship between the concentration of carnitine and counts min^{-1} on the filters over a 30 fold concentration range of DL-carnitine.

pH-Solubility profile in 0.4 M sodium citrate buffer

The 0.4 M sodium citrate buffer was adjusted with 6 M HCl or 0.4 M NaOH to pH 4, 6 and 8. To 0.5 ml of the adjusted buffer 0.5 ml of water and 0.5 ml of sodium triphenylcyanoborate solutions (200 mg ml^{-1}) were added, the reagents were mixed well, and the tubes cooled in an ice bath for 15 min. While the reaction media were still cold, the ensuing precipitates were filtered. The precipitates were dissolved in 1 ml of methanol and read at 265 nm after the addition of 1 ml of water. Choline, β-methylcholine and trimethylaminoacetone form precipitates which are least soluble at pH 8; the carnitine, butyrobetaine and crotonylbetaine complexes are completely soluble at this pH and the precipitates of the carnitine group are least soluble at pH 4. Both groups show linear increases in absorbance at 265 nm with

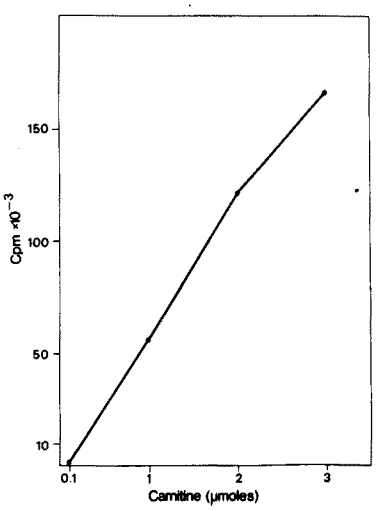

Fig. 4. Increasing concentrations of carnitine HCl were complexed with sodium triphenyl-(^{14}C)-cyanoborate. The resulting filter cakes were dissolved and counted by liquid scintillation.

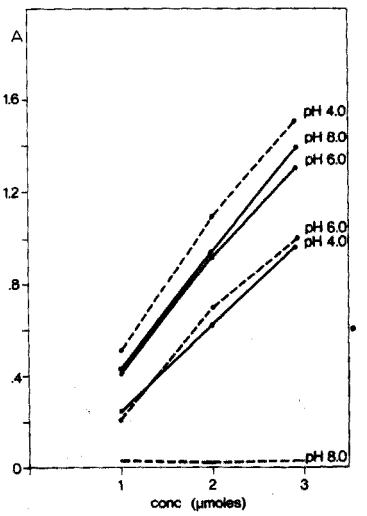

Fig. 5. pH profile of β-methylcholine and carnitine precipitates in 0.4 M sodium citrate buffer. Choline and trimethylaminoacetone have the same profile as β-methylcholine whereas butyrobetaine and crotonlylbetaine follow the pattern of carnitine. (———) β-Methylcholine-HCl. (---------) Carnitine-HCl.

increasing concentration from 0.1 to 3 μmoles ml^{-1} in the original reaction mixture at the optimum pH values (Fig. 5).

Measurement of carnitine pool size in rats

Rats (150—250 g) were extracted with 1 l of methanol in a Waring blender. After filtration, the methanol fraction was taken to dryness on a rotary evaporator. The residue was hydrolyzed with 50 ml of 0.2 *M* NaOH containing 0.005 M Na_2SO_3, for 12 h at 45 °C. This solution was subsequently acidified with 2 M HCl, and extracted with chloroform (3 × 100 ml). The aqueous phase was evaporated (rotary evaporator) and the residue was dissolved in 5—10 ml of distilled water. A 0.250-ml aliquot was loaded on the Aminex A-5 high-pressure cation-exchange column. The elution of the carnitine with citrate buffer was followed by the procedure described above. The average carnitine pool size per 100 g for male 150—250 g fed Sprague Dawley rats was 12.7 ± 2.4 mg; for starved rats of the same weights, the pool size per 100 g was 19.8 ± 2.7 mg. These values are within the ranges previously determined by isotope dilution methods [4, 5, 20]. The carnitine determination of Tsai et al. [5] were conducted by a modification of the method of Cederblad and Lindstedt [21].

DISCUSSION

Precipitation of quaternary ammonium salts with triphenylcyanoborate constitutes a rapid and convenient method for the determination of these compounds when combined with either scintillation counting of the radioactive precipitate or measurement of the absorbance at 265 nm. Both methods are equally convenient, and of comparable sensitivity. The optimal pH range for precipitation of an individual compound depends on the presence of other functional groups, which should be in their non-ionized form. High-pressure ion-exchange column chromatography for the separation of quaternary ammonium compounds, combined with the described assay procedure, gives a rapid and convenient analytical tool for studies of carnitine metabolism.

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Short Communication

DETERMINATION OF LEAD IN COAL AND COAL ASHES BY FLAMELESS ATOMIC ABSORPTION SPECTROMETRY

C. BLOCK*

Institute for Nuclear Sciences, Rijksuniversiteit Gent, Proeftuinstraat 86 B-9000 Ghent (Belgium)

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The determination of trace elements in coal and coal ash is important from a geochemical point of view, and in connection with combustion processes, which can be an important source of airborne particulates. A multi-element determination of trace elements in coal by thermal neutron-activation analysis [1], and a separate determination of silicon and oxygen by 14-MeV neutron activation analysis [2] have recently been developed. However, these methods do not allow the determination of lead, which is of great concern because of its toxicity.

Several methods, such as spark-source mass spectrometry [3, 4], x-ray fluorescence [5], optical emission spectrometry [6, 7], atomic absorption spectrometry [7, 8], etc., have been applied for the determination of lead in coal and coal ashes; atomic absorption spectrometry is probably most widely accepted as appropriate. In the work described here, the reproducibility and accuracy of the determination of lead in coal, coal ash and fly ash by flameless atomic absorption spectrometry were critically studied. A recent method for the decomposition of coal in a concentrated acidic medium in a high-pressure bomb [8] was slightly modified for these coal and coal ash samples. The influence of the acid concentration on the absorbance measurement was thoroughly investigated. Lead was also determined in fly ash after collection on filter papers and removal by ultrasonic vibration [9].

Experimental

Apparatus

A Perkin-Elmer model 503 double-beam atomic absorption spectrophotometer was used with an Intensitron hollow-cathode lamp, a deuterium background corrector, a HGA 2100 graphite furnace and a Hitachi Perkin Elmer Model 56 recorder. Coal and coal ash samples were decomposed in an acid digestion bomb, consisting of a teflon crucible encased in a metal body with a screw cap.

*Research associate of the I.I.K.W.

A Branson Ultrasonic cleaner [9], consisting of an ultrasonic generator Model LG-150 and an ultrasonic tank-type transducer LTH-60, was used to remove fly ash from the filter paper.

Influence of acidity

Coal and coal ash samples are usually decomposed by digestion with mixtures of concentrated acids such as perchloric, sulfuric and nitric acids, etc. The decomposition of such samples in a teflon pressure digestion bomb requires strong acids [8, 10], hence a nitric acid—hydrofluoric acid mixture was used.

Since the influence of acidity on the lead absorption cannot be neglected [8, 9], this effect was thoroughly investigated; both nitric and hydrofluoric acids decreased the net absorbance, but the influence of nitric acid was more significant; a 2.8 M nitric acid concentration decreased the absorbance by 17.3%, where as a 2.8 M hydrofluoric acid solution decreased the absorbance by 13.5% (Fig. 1). Since the sensitivity of the method is decreased at high acidities, the acid concentration of the test solution must be kept as low as possible. Figure 1 shows that the absorbance was only slightly influenced by small changes in acidity up to 0.7 M nitric acid or 1.5 M hydrofluoric acid. The amounts of acids for the decomposition should therefore be selected appropriately. For the present samples, the amounts required were minimized by increasing the temperature and time of the digestion. It is obvious from Fig. 1 that the acidities of unknown and standard solutions should be as similar as possible.

Procedure for coal and coal ash

Crush and homogenize the coal samples, and ash them in platinum crucibles in an electric furnace at 900°C [11]. Transfer about 200 mg of coal or about 30 mg of coal ash to an acid digestion bomb, add a mixture of 3 ml of 14 M nitric acid (Merck suprapur) and 4 ml of 30 M hydrofluoric acid,

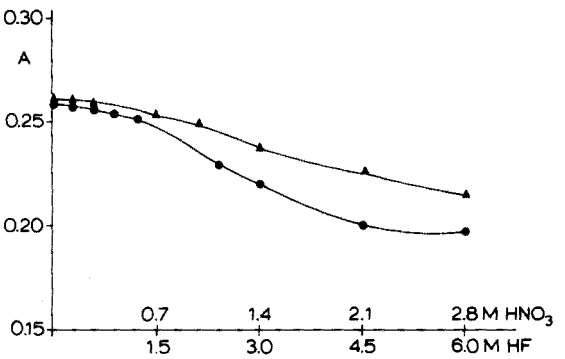

Fig. 1. Influence of acidity on the absorbance measurement. (▲) Increasing HNO_3 concentration with constant 1.2 M HF. (●) increasing HF concentration with constant 0.42 M HNO_3.

and heat at 230°C for 6 h. After cooling, dilute the solution to 100 ml with distilled water and store in polyethylene containers. The final solution contained ca. 0.42 M nitric acid and 1.2 M hydrofluoric acid.

Prepare a lead stock solution from reagent-grade lead nitrate and dilute to concentrations varying from 10 to 400 $\mu g\ l^{-1}$, with a solution whose acidity is similar to that of the unknown samples.

Procedure for fly ash

Collect the fly ash samples on Whatman 41 filter papers [12]. Remove the collected dust from the filter by ultrasonic vibration in a 0.1 M nitric acid solution as described previously [9] for aerosol samples. Use dust fractions corresponding to about 10 mg of fly ash. Prepare standard solutions of lead nitrate dissolved in 0.1 M nitric acid, in concentrations ranging from 0.025 to 5 $mg\ l^{-1}$.

Absorption measurements

Use the following optimal apparatus settings: lamp current, 10 mA; slit, 4; drying, charring and atomization temperatures of 100°C, 400°C and 2700°C, respectively [9]. Inject 10—75 μl of sample solution into the graphite tube with Eppendorf micropipettes, and measure at the lead 283.3-nm line. Use the deuterium background corrector to compensate for scattering and broadband absorption.

After subtraction of the blank value for the reagents, calculate the concentrations of lead in the coal, coal ash and fly ash from the appropriate calibration curves.

Results and discussion

Reproducibility

The reproducibility of the sampling and measuring procedure was investigated in detail. The results obtained for solutions corresponding to 21, 75 and 443 $\mu g\ Pb\ g^{-1}$ of coal or ash to five successive injections of single sample solutions based on the same calibration curve, showed relative standard deviations of 3.6—5.6 from the mean. When five different calibration curves were used for similar measurements, the relative standard deviations found varied from 3.8 to 5.6, when each determination was the mean value of 4 injections. The relative standard deviation of the complete procedure, i.e. sampling, dissolution and measurement, (based on four different solutions of the same coal sample, with different calibration curves and four successive injections) was found to be 3.6 for a sample containing 74 $\mu g\ Pb\ g^{-1}$, and 4.1 for a sample containing 296 $\mu g\ Pb\ g^{-1}$. This unexpectedly good reproducibility, is probably due to the fact that each individual value is already the mean of 12 injections.

Accuracy

The accuracy was investigated by analysing NBS coal and fly ash standard reference materials. For each material four solutions were prepared and analyzed in triplicate. The results (Table 1) agree, within the indicated uncertainty limits, with the certified values and with more recent values [13]. The large uncertainties in the results for coal can be attributed to inhomogeneity [13].

Analysis of coals and ashes

Ten coal samples (from different Belgian mines) and their respective ashes were analyzed (Table 2). The lead concentrations found are in the 7—110

TABLE 1

Analyses of NBS Standard Reference Materials ($\mu g\ g^{-1}$)

	Certified value	Photon activation [13]	This work
SRM 1632 (coal)	30± 9	—	28 ± 5[a]
SRM 1633 (fly ash)	70 ± 4	75 ± 5	74 ± 4

[a]90% confidence limits on the mean of 4 determinations.

TABLE 2

Lead concentrations in coal and coal ash ($\mu g\ g^{-1}$)

Type	Coal	Coal ash	Expected conc. in coal[a]
Home heating			
A	16.0 ± 1.7[b]	450 ± 22	17.1
B	10.7 ± 0.8	395 ± 16	10.9
C	7.6 ± 0.5	282 ± 13	8.0
D	9.3 ± 0.9	292 ± 20	8.8
E	16.1 ± 0.7	430 ± 30	15.5
F	10.2 ± 0.7	180 ± 15	10.6
Cokes			
G	10.2 ± 0.7	155 ± 15	10.8
H	10.0 ± 0.8	120 ± 8	9.4
Electric power station			
I	110 ± 7	270 ± 14	104
J	60 ± 5	235 ± 15	61

[a]Calculated from the concentration in coal ash and the experimentally determined ash content.

[b]90% confidence limits on the mean (2—4 determinations).

$\mu g\ g^{-1}$ range, and agree with literature values [3—6, 13—15], of 2—110 $\mu g\ g^{-1}$. The lead concentrations in home heating and coking coal are 5—10 times lower than those in power station coal. The lead contents of the coal ashes agree with literature data (95—572 $\mu g\ g^{-1}$) [16] and seem to be independent of the ash content, the highest concentrations in the ash being encountered in low-ash coals (home heating). Table 2 also compares the lead concentration in coal, expected from the experimentally determined ash content and the lead concentration in coal ash, with the determined concentrations; the deviations between these sets of values are of the same order of magnitude as the confidence limits, proving that there was no significant loss of lead on ashing.

The lead concentrations in fly ash, collected in a large combustion facility [12] burning low-ash coal, were also determined. A mean value of 1520 μg Pb g^{-1} was found for 6 samples (range 1170—1810 $\mu g\ g^{-1}$). Compared with some literature values ranging from 15 to 1000 $\mu g\ g^{-1}$ [3, 6, 14, 17, 18], this value is high, but a concentration of 1600 $\mu g\ g^{-1}$ has been mentioned [7] for fly ash particles of 1.1—2.1-μm diameter. The large range for lead in fly ash can probably be ascribed to different operating parameters in the installations.

The fly ash concentrations encountered here suggest that coal may be an important source of toxic lead.

Grateful acknowledgement is made to Professor J. Hoste and Dr. R. Dams for their interest, to Dr. M. Janssens for helpful discussions, and to the I.I.K.W. and the F.K.F.O. for financial support.

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Short Communication

NEUTRON ACTIVATION ANALYSIS FOR THORIUM IN MONAZITE SAND WITH ARSENIC AS INTERNAL-STANDARD

TONG-CHUIN PUNG, HUI-TUH TSAI and SHAW-CHII WU

Institute of Nuclear Energy Research, Atomic Energy Council, Lung-Tan, Taiwan 325 (Republic of China)

(Received 25th April 1975)

Neutron activation analysis of thorium is mostly based on the nuclear reaction:

$$^{232}\mathrm{Th}\ (n, \gamma)\ ^{233}\mathrm{Th} \xrightarrow[22.4\ \mathrm{min}]{\beta^-} {}^{233}\mathrm{Pa} \xrightarrow[27.0\ \mathrm{d}]{\beta^-} {}^{233}\mathrm{U}$$

Thorium has been determined in minerals [1] and cerium matrices [2] by β^- counting of chemically separated ^{233}Th. Aluminum [3] and biological materials [4] have been analyzed by counting the ^{233}Pa γ-emission after chemical separations. Thorium has also been determined by delayed neutron counting [5], and by nondestructive neutron activation analysis with high-resolution Ge(Li) detectors [6, 7].

In this communication, a convenient and precise internal-reference method [8, 9] is proposed for the determination of thorium in monazite sand; arsenic serves as the internal standard, and no chemical separations are needed.

Experimental

Reagents

Thorium and arsenic oxides were of high-purity grade (Johnson Matthey). Monazite sand was mined from south Tung-Sen-Chou of Taiwan.

Preparation of samples

A series of synthetic mixtures of thorium and arsenic were prepared as follows: thorium oxide was dissolved in 12 M hydrochloric acid containing several drops of 1 M hydrofluoric acid. Arsenic(III) oxide was dissolved in hydrochloric acid. Mixtures of these solutions with known thorium-to-arsenic weight ratios ranging from $2.59 \cdot 10^{-1}$ to $8.13 \cdot 10^{2}$, were prepared by pipetting aliquots on to Whatman filter papers, adding 0.16 μg of arsenic to each sample, drying in a desiccator, and wrapping in aluminum foil.

Black and yellow monazite sands were used as unknown samples; 0.26—3.97 mg of each sand was weighed into polyethylene tubes (i.d. 4 mm), and

the same arsenic solution was added, before the mixture was dried in a desiccator. The tubes were then sealed.

Irradiation and measurement

Irradiation was done in the TRR (Taiwan Research Reactor) at the Institute of Nuclear Energy Research, in a neutron flux of $1.8 \cdot 10^{13}$ n cm^{-2} s^{-1}. The synthetic mixtures and sands were placed together in an aluminum capsule, and irradiated for 1 h.

The γ-ray measurements were made with a 35-cm^3 Ge(Li) detector, coupled to Hewlett-Packard 1024-channel pulse-height analyzer. The counting system had a resolution of 5 keV for the 661.6-keV γ peak of ^{137}Cs.

Results and discussion

The nuclear properties of the measured radionuclides are readily available [10, 11]. Figure 1 shows a typical γ-ray spectrum of the synthetic sample of Th—As mixture. The principal ^{233}Pa photopeaks (299.9, 311.8 and 340.3-keV) and ^{76}As photopeaks (559.2 and 657.0-keV) stand out clearly; among the ^{233}Pa photopeaks, the 311.8-keV peak is the most intense, and was therefore used for the determination of thorium. The 559.2-keV photopeak of ^{76}As was used as the internal standard. In previous reports [8, 9], gold was used as the internal standard, but in the present study, the 411.8-keV photopeak of ^{198}Au could not be used because it was affected by the ^{233}Pa 415.6-

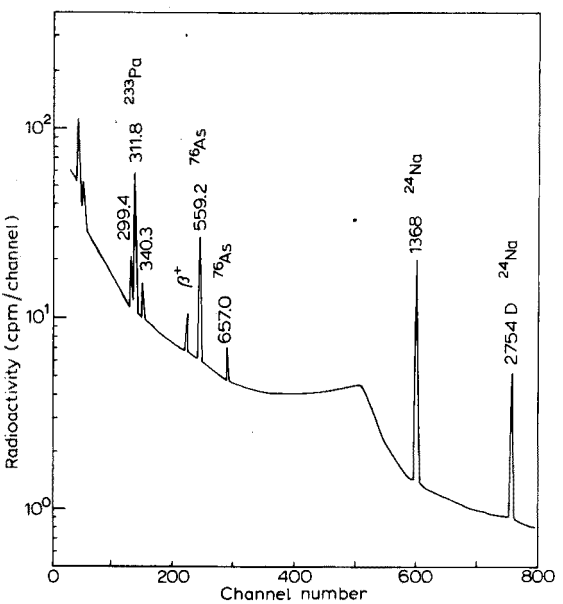

Fig. 1. γ-Ray spectrum of the synthetic sample, 44 h after the end of irradiation.

keV photopeak. However, the 559.2-keV photopeak of ^{76}As is free from interference, and can therefore be used.

For the determination of thorium, the areas of the 311.8-keV ^{233}Pa peak and the 559.2-keV ^{76}As peak were compared. A good proportionality was obtained between R_w (the weight ratio of determined element to reference element) and R_{A_0} (the activity ratio of determined element to reference element). The results are given in Table 1. Thorium can be determined down to $4 \cdot 10^{-2}$ μg by this relationship, with a relative error within ±2 %.

TABLE 1

Weight ratio (R_w) vs. activity ratio (R_{A_0}) for Th—As mixtures

R_w	R_{A_0}	R_w/R_{A_0}
$8.13 \cdot 10^{2}$	$3.37 \cdot 10^{1}$	$2.41 \cdot 10^{1}$
$1.62 \cdot 10^{2}$	$6.69 \cdot 10^{0}$	$2.42 \cdot 10^{1}$
$3.24 \cdot 10^{1}$	$1.38 \cdot 10^{0}$	$2.35 \cdot 10^{1}$
$3.24 \cdot 10^{0}$	$1.37 \cdot 10^{-1}$	$2.36 \cdot 10^{1}$
$2.59 \cdot 10^{-1}$	$1.11 \cdot 10^{-2}$	$2.33 \cdot 10^{1}$
	Mean value:	$2.37 \cdot 10^{1}$
	s:	$\pm 0.04 \cdot 10^{1}$
	s_r:	±1.7 %

Figure 2 shows the γ-ray spectrum of yellow monazite sand irradiated for 1 h. After a decay period of about 9 days, the shortlived nuclides had decayed, so that the ^{233}Pa γ-ray could be observed. The prominent 311.8-keV photopeak of ^{233}Pa was measured. The 559.2-keV photopeak of ^{76}As ($t_{\frac{1}{2}}$ = 26.3 h) was measured about 30 h after the end of irradiation. The results (Table 2) showed that the thorium content in yellow monazite sand is almost seven times higher than that in black monazite sand.

TABLE 2

Thorium content of monazite sand

Monazite sand	Weight taken (mg)	Thorium found (%)
Black	2.90	0.66
Black	0.33	0.69
Yellow	3.97	4.6
Yellow	0.26	4.8

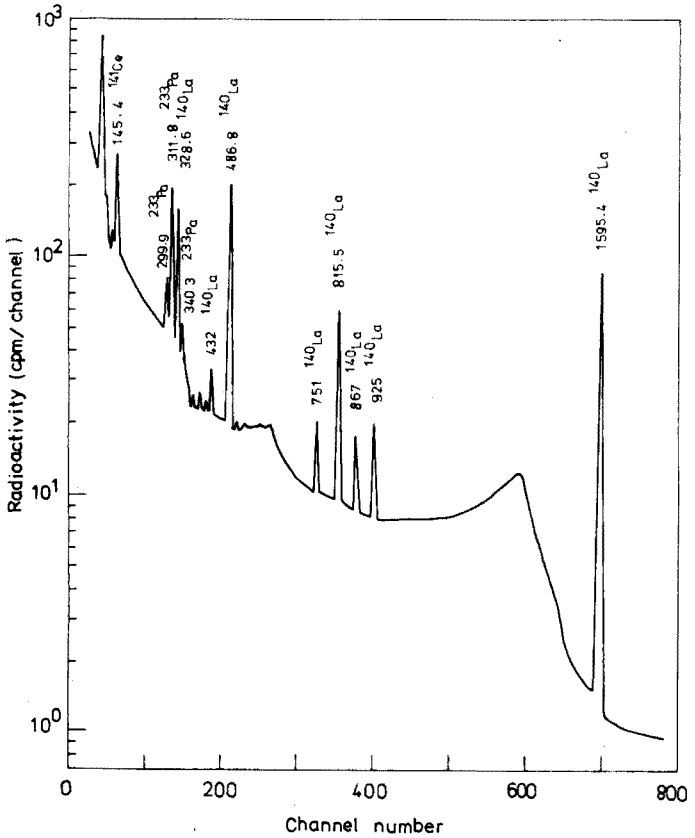

Fig. 2. γ-Ray spectrum of the yellow monazite sand, 9 days after the end of irradiation.

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Short Communication

ISOLATION OF RADIOTUNGSTEN FROM FISSION PRODUCTS AND CORROSION PRODUCTS

M. EJAZ

Pakistan Institute of Nuclear Science and Technology, Nilore, Rawalpindi (Pakistan)

(Received 2nd July 1975)

Tungsten-187 is a commonly encountered neutron-induced contaminant in nuclear reactor coolants, being introduced by corrosion of the stainless steel reactor components and fuel claddings. In addition to tungsten-187, chromium-51, iron-59 and manganese radionuclides formed by different nuclear reactions on manganese, iron and cobalt are also common contaminants. Isolation of the tungsten before measurement is usually necessary, and the method described below — based on 4-(5-nonyl)pyridine oxide [1, 2] — is applicable to a wide range of samples containing fission products.

Experimental

Nuclides and reagents.

^{185}W was obtained as a sodium tungstate solution in sodium hydroxide (Radiochemical Centre, Amersham). ^{187}W was obtained by neutron activation of reagent-grade tungsten trioxide in the research reactor (PARR) of this Institute; irradiation of 1 mg of tungsten for 1 h in a thermal neutron flux of 10^{13}n s^{-1} cm^{-2} produced 0.27 Ci of ^{187}W. 4-(5-Nonyl)pyridine oxide was prepared [1, 2]; all other chemicals used were of analytical grade.

Measurements of distribution coefficients

Extraction and distribution data were obtained as described previously [1, 2].

Separation of tungsten(VI) from stainless steel

Dissolve 20 mg of neutron-irradiated steel in 10 ml of 10 M hydrochloric acid. Equilibrate the hydrochloric acid solution once with 10 ml of a 0.1 M 4-(5-nonyl)pyridine oxide solution in xylene. Wash the organic phase three times with barren aqueous phase, and then with 0.5 M nitric acid (2 × 10 ml). Back-extract tungsten from the organic phase with 10 M sulphuric acid (3 × 5 ml).

Separation of tungsten from stainless steel and fission product mixtures

After scrubbing the organic phase with 0.5 M nitric acid as described above, wash it once with 5 ml of 1 M sulphuric acid and then back-extract

tungsten in 10 M sulphuric acid. Add 5—10 mg of potassium periodate to the back-extract and distil for 1—2 h.

Results and discussion

Tungsten is extracted almost completely by 4-(5-nonyl)pyridine oxide in xylene from 9—10 M hydrochloric acid ($D \approx 100$) [1]. Among the other constituents of steel, only iron(III) is coextracted almost quantitatively ($D > 10$); extraction of manganese(II) and chromium(III) is very poor. Because of the high distribution coefficient for tungsten, a single equilibration suffices, and the subsequent scrubbing stages barely affect the recovery of tungsten whilst eliminating chromium(III) and manganese(II) from the organic phase. Stripping the organic phase with 0.5 M nitric acid removes all the iron(III) ($D > 10^{-3}$), but not the tungsten ($D \approx 10$), which can be back-extracted with 10 M sulphuric acid. If separation from fission products is also required, another stripping step is essential. Stripping the organic phase, after removal of iron(III), with 1 M sulphuric acid removes uranium(VI), zirconium(IV) and niobium(V). Yttrium(III), cerium(III), lanthanum(III), other rare earths and niobium are stripped along with iron(III) on equilibration with 0.5 M nitric acid. Ruthenium, technetium, iodine and tellurium are only partially extracted from 10 M HCl and can be removed by distillation from the final 10 M sulphuric acid extract in the presence of potassium periodate. Clean separations of tungsten from irradiated steel and fission products were easily achieved; Fig. 1 shows the separation obtained from irradiated

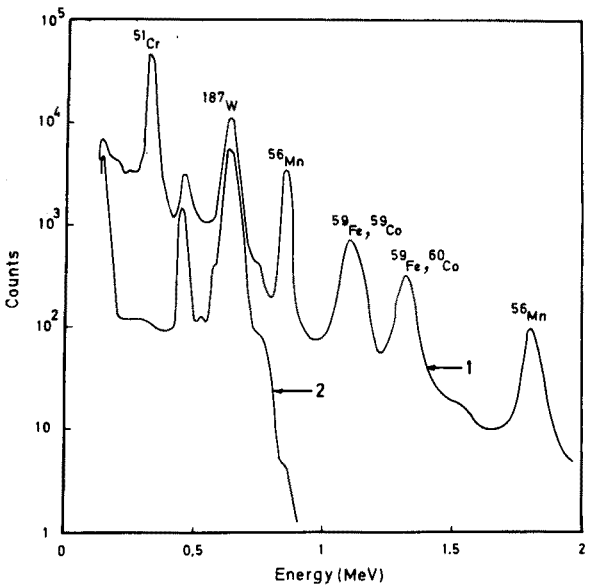

Fig. 1. γ-Spectra of an irradiated and cooled (1d) sample of stainless steel before extraction (curve 1), and of the separated tungsten extract (curve 2).

stainless steel. Similar clear separations were obtained when ^{187}W was added to 6-month old fission products and the whole procedure was applied.

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Short Communication

SEPARATION DES RADIONUCLEIDES ^{95}Zr et ^{95}Nb PAR EXTRACTION LIQUIDE—LIQUIDE EN PRESENCE DE β-ISOPROPYLTROPOLONE

L. SCHRIVER

Laboratoire de Chimie XIII, Université de Paris VI, 8 rue Cuvier, 75005 — Paris (France)

(Reçu le 27 Mai 1975)

La détection d'un radioisotope émetteur-γ tel que le ^{95}Zr en équilibre avec son isobare ^{95}Nb, produit direct de filiation, est possible en solution, les effets d'absorption des photons-γ étant négligeables dans la plupart des liquides. Cependant, dans ce cas, l'utilisation d'un cristal NaI(Tl) dont le pouvoir de résolution est de 50 keV, couplé avec un compteur à scintillation, ne permet pas de résoudre les pics correspondants à ^{95}Nb et ^{95}Zr, les énergies des photons-γ de chacun des composants étant très proches.

La purification par voie chimique du zirconium est alors indispensable toutes les 24 h; diverses méthodes on été mises au point, à l'échelle des traceurs, pour résoudre ce problème [1—4]. Dans tous ces procédés, le niobium n'est pas extrait et on obtient le zirconium dans la phase organique, ce qui nécessite sa réextraction quantitative par une solution aqueuse acide avant son utilisation.

Ce travail a montré que la β-isopropyltropolone en solution dans le chloroforme permet une purification rapide et simple du ^{95}Zr par extraction quantitative du ^{95}Nb dans la phase organique. Les proprietés de ce réactif sont très bien connues. [5]

Conditions experimentales.

L'isotope ^{95}Zr nous a été fourni par le commissariat à l'Energie Atomique. La concentration de la β-isopropyltropolone (IPT; Koch Light) dans le chloroforme, toujours en grand excès devant celle du ^{95}Zr, est déterminée par spectrophotomètrie ($\epsilon = 6 \cdot 10^3$ ml^{-1} cm^{-1} à 350 nm), l'étalonnage étant fait à partir d'une solution de titre connu par pesée.

Les extractions sont réalisées sur des volumes égaux de solution aqueuse et de chloroforme après un temps d'agitation de 10 min.

Les activités du zirconium-95 et du niobium-95, ont pu être déterminées simultanément dans les deux phases grâce à l'équipement pour spectrophotomètrie-γ mis à notre disposition au laboratoire Pierre Sue à Saclay. Cet équipement comprend un sélecteur multicanaux (4000). Intertechnique relié à un détecteur à semi conducteur Ge—Li dont le pouvoir de résolution

inférieur à 5 keV permet de visualiser directement les pics relatifs du ^{95}Zr au ^{95}Nb comme le montre la Fig. 1.

Description de la méthode

La solution aqueuse contenant les radionucléides à la concentration 10^{-6} M en milieu acide perchlorique 4 M ou plus est agitée successivement deux fois avec un volume égal d'une solution chloroformique de IPT à la concentration $9 \cdot 10^{-5}$ M. Après centrifugation, il reste dans la phase aqueuse 80 % du ^{95}Zr initial alors que tout le niobium (99,8 %) est dans la phase organique. On peut alors utiliser directement la solution purifiée de ^{95}Zr.

Resultats

Influence de l'acidité

La concentration de tropolone étant constante et égale à $9 \cdot 10^{-5}$ M dans le solvant, celle du ^{95}Zr en phase aqueuse de l'ordre de 10^{-6} M pour éviter sa polymérisation [6], nous avons fait varier l'acidité de la phase aqueuse de 1 à 4M, à force ionique constante à l'équilibre ($HClO_4 + NaClO_4 = 4$). Quand l'acidité augmente, nous constatons que l'extraction du ^{95}Zr diminue alors que celle du niobium augmente, comme le montrent les résultats ci-dessous

H^+ aqueux	D_{Nb}	% Nb extrait	D_{Zr}	% Zr extrait
1 M	6	85,5	3,5	77
2 M	11	91,7	0,6	37,5
3 M	15	93,5	0,2	17
4 M	19	95,05	0,09	8,2

Une forte acidité en phase aqueuse favorise donc la séparation entre ^{95}Zr et ^{95}Nb.

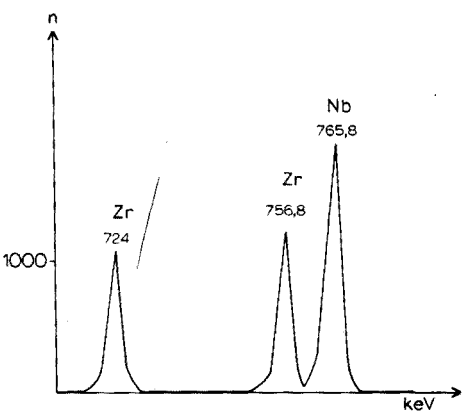

Fig. 1. Variations du nombre d'impulsions en fonction de l'énergie, pour un échantillon de ^{95}Zr en présence de son produit de désintégration ^{95}Nb, obtenues avec un détecteur GeLi.

Les résultats ci-dessus vérifient nos résultats antérieurs relatifs à l'extraction du zirconium par la IPT; à concentration constante de chélatant, la courbe log $D_{Zr} = f \log(H^+)$ est une droite de pente égale à -3 [7]. Par contre nous constatons ici, que pour le niobium, la pente de la droite log $D_{Nb} = f(\log(H^+))$ est une droite de pente égale à $+1$.

Influence de la concentration en tropolone

Avec une solution aqueuse d'acide perchlorique 4 *M* de concentration 10^{-7} à 10^{-6} M en zirconium-95, nous avons réalisé une série d'extractions avec des concentrations de tropolone dans le chloroforme variant de $5 \cdot 10^{-6}$ à $3 \cdot 10^{-4}$ M.

Les pourcentages des tropolonates de ^{95}Zr et de ^{95}Nb extraits en fonction de la concentration en tropolonone dans le solvant sont reportés sur la Fig. 2. L'extraction du niobium est supérieure à celle du zirconium; pour une concentration en tropolone de $9 \cdot 10^{-5}$ M, 95 % du niobium sont extraits et seulement 10 % du zirconium; après une deuxième extraction le niobium est extrait à 99,75 %, alors qu'il reste en phase aqueuse, 80 % du zirconium initial. Cependant pour une concentration en tropolone supérieure à $4 \cdot 10^{-4}$ M, les deux métaux sont extraits quantitativement ce qui limite l'utilisation de la IPT à une séparation ^{95}Zr—^{95}Nb à l'état de traces.

Influence de quelques anions

Nous avons étudié l'influence de différents anions en phase aqueuse sur l'extraction des deux radionucléides, en maintenant constantes à l'équilibre, l'acidité en phase aqueuse (2M), la concentration en tropolone dans le solvant ($8 \cdot 10^{-5}$ M) et la force ionique à 4 avec du perchlorate de sodium. Les résultats montrent que les anions sulfate, oxalate et phosphate qui complexent fortement les deux cations métalliques empêchent l'extraction du niobium et sont donc défavorables, même à des concentrations de 10^{-3} M. Par contre, la séparation reste quantitative en présence des ions chlorure et

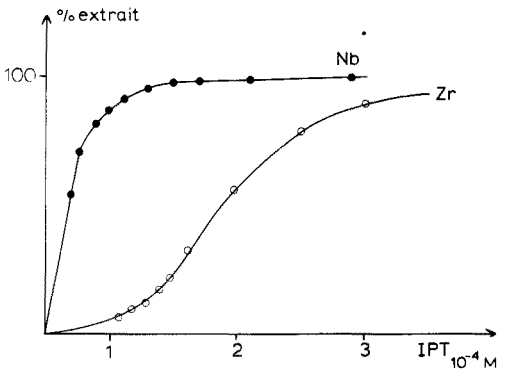

Fig. 2. Variations des pourcentages extraits de ^{95}Nb et de ^{95}Zr en fonction de la concentration en IPT dans le chloroforme. (H^+ = 4 M; I = 4).

nitrate même à 3 M. Les ions thiocyanate qui complexent plus le zirconium que le niobium favoriseraient la séparation mais du fait de leur décomposition en milieu très acide [8], ils ne sont guère utilisables pour augmenter le coefficient de séparation.

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Short Communication

ISOTOPIC EXCHANGE AT THE GAS CHROMATOGRAPHIC ELECTRON CAPTURE DETECTOR CONTAINING A TRITIUM SOURCE

SUEO NISHI

National Chemical Laboratory for Industry, 1-chome, Honmachi, Shibuya-ku, Tokyo (Japan)

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Tritium emanation from gas chromatographic electron capture detectors (e.c.d.) with tritium sources is well known. Shoemake et al. [1] indicated that the tritium emanation increased rapidly when the temperature exceeded 200 °C. Kahn and Goldberg [2] discussed the level of tritium eluted from the detector, and the necessity of proper laboratory ventilation. Fenimore et al. [3] and Hartmann [4] reported that the emanation of tritium at elevated temperatures could be reduced by the use of a scandium tritide source.

However, little attention has been given to possible interactions between tritium in the e.c.d. and organic compounds being eluted from the gas chromatographic column. This paper describes the isotopic exchange between tritium in the e.c.d. and hydrogen atoms in the chromatographed compounds.

Experimental

The JGC 810 gas chromatograph (Japan Electron Optics Laboratory) used was equipped with an e.c.d. containing 300 mCi of tritium embedded in titanium plated on one side of a 1 × 3 cm stainless steel foil; the foil was rolled to a cylinder with the treated surface facing inwards.

The radioactivity of the effluent from the e.c.d. was measured by both radio gas chromatography and the liquid scintillation counting method. For the former type of measurement, the proportional gas flow counter JGC RAD 750 was connected to the gas chromatograph as shown in Fig. 1. The radioactivities of the effluents were measured quantitatively by liquid scintillation counting; the effluents were trapped in 10 ml of toluene at dry ice—acetone bath temperatures, and aliquots were added to a PPO—POPOP—toluene liquid scintillator. Separately, the background activity was measured by trapping an effluent without sample injection, for 1 min; this was subtracted from the activity measured with the samples. An Aloka 601 liquid scintillation counter (Nippon Electric Co.) was employed. In order to

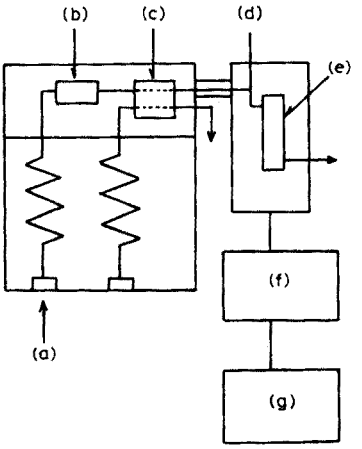

Fig. 1. Schematic diagram of radio gas chromatograph: (a) sample inlet; (b) e.c.d. (tritium source); (c) thermal conductivity detector; (d) propane; (e) proportional flow counter; (f) rate meter; (g) recorder.

monitor the effluents, a thermal conductivity detector was connected in series with the e.c.d. Dual 2-m columns packed with 10 % SE-30 on Chromosorb W, were used with helium as the carrier gas.

Results and discussion

Results obtained by radio gas chromatography for four compounds are shown in Fig. 2, which indicates clearly that organic compounds with reactive hydrogen atoms such as alcohols or acetone, partly exchange their hydrogen with tritium as they flow through the e.c.d. Other aliphatic or aromatic alcohols, aldehydes, ketones and carboxylic acids behaved similarly. In the gas chromatography of aliphatic and aromatic hydrocarbons, no exchange phenomena were observed.

Duplicate experiments were carried out with 10-μl injections of various organic compounds, the radioactivities of the trapped effluents being measured; significant differences were observed in the radioactivities depending on the nature of samples (Table 1). Compounds containing hydrogen in polar functional groups such as hydroxyl or carboxylic groups, gained higher levels of radioactivity. The temperature affected the exchange reaction markedly; the logarithm of the activity gained by the effluent increased proportionally with increase in detector temperature (Fig. 3). The activity was also affected by the carrier gas flow rate; at low flow rates, higher activity was found in the effluent (Fig. 4), as would be expected for longer contact times between the sample and the tritium source.

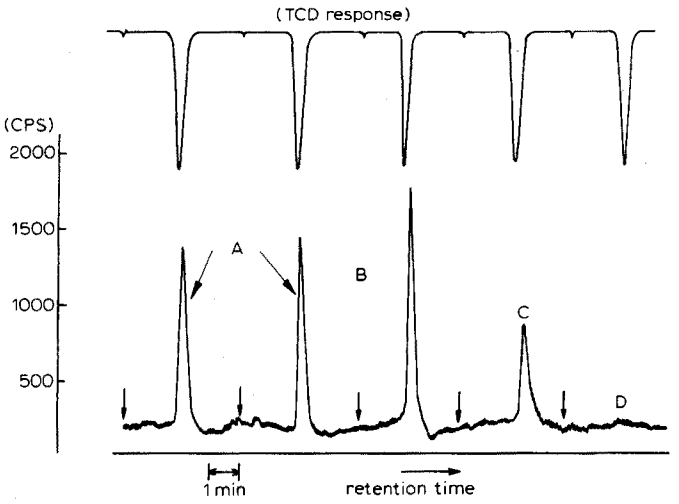

Fig. 2. Radio gas chromatogram of e.c.d. effluent. A, Ethanol. B, Methanol. C, Acetone. D, Benzene. Samples (2μl) were injected at the points indicated by arrows.

TABLE 1

Radioactivities of effluents

Compound	Radioactivity (nCi)	
	150 °C	185 °C
Methanol	79	—
	67	590
Ethanol	76	340
	62	390
Isopropanol	55	380
	48	400
Acetone	38	120
	32	130
Valeraldehyde	30	130
Acetic acid	82	440
	82	470

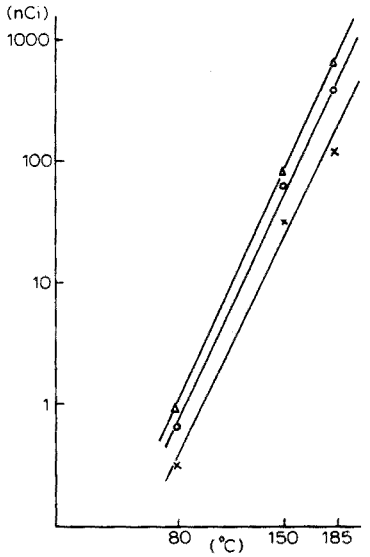

Fig. 3. Effect of e.c.d. temperature on radioactivity of effluent. Sample injection: 10 μl. (△) Methanol. (○) Ethanol. (X) Acetone.

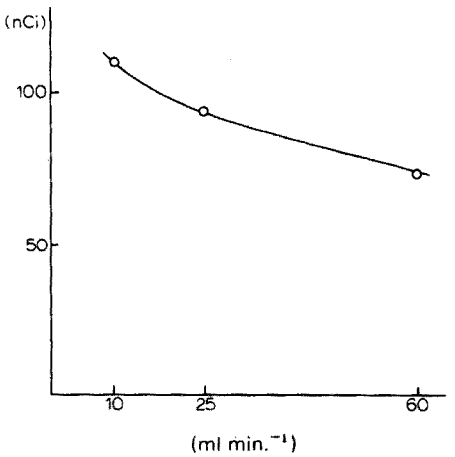

Fig. 4. Effect of carrier gas flow rate on the radioactivity of the effluent. Sample: 10 μl of ethanol. Detector temperature: 150 °C.

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Short Communication

THE VOLATILITY OF CHROMIUM FROM BREWERS YEAST DURING ASSAY

G.B. JONES, R.A. BUCKLEY and C.S. CHANDLER

CSIRO, Division of Human Nutrition, Kintore Avenue, Adelaide, South Australia 5000 (Australia)

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Recently dietary chromium has been recognized as an essential trace element for human and animal nutrition [1]. Mertz et al. [2] and Wolf et al. [3] have drawn attention to the possible losses of chromium as volatile organic complexes during assay. Cary and Allaway [4] have claimed that the addition of silver nitrate to wet digestion mixtures prevents the loss of chromium as chromyl chloride; they also drew attention to possible losses of chromium in dry ashing procedures by fusion or adsorption on the walls of vessels.

Brewers yeast contains relatively high levels of chromium (0.5—1.2 $\mu g\ g^{-1}$) of which a considerable proportion is present as the biologically active glucose tolerance factor (GTF), which is soluble in 50% (v/v) ethanol [5]. In this investigation brewers yeast was grown in a medium containing ^{51}Cr(III). The total chromium content of this yeast was found to be 1.0 $\mu g\ g^{-1}$, of which 26% was soluble in 50% (v/v) ethanol. Possible loss of ^{51}Cr was examined in samples during freeze-drying, oven-drying at 100 °C, wet digestion and dry ashing at temperatures up to 800 °C.

Experimental

Culture medium

An aqueous solution (3 l) containing 2.0% (w/v) Bacto-Peptone, 0.5% (w/v) Bacto yeast extract, 5.0% (w/v) glucose and 0.5% (w/v) sodium chloride was diluted to 3 l with water, and sterilized by autoclaving. ^{51}Cr of specific activity 2 $\mu Ci\ \mu g^{-1}$, as sodium chromate, was dissolved in 5% (v/v) nitric acid. Chromium (VI) was reduced to Cr(III) with sodium sulphite before it was added to the yeast culture.

Apparatus

A γ-scintillation counter was used for all radioactivity measurements.

Procedure

^{51}Cr-labelled brewers yeast (Saccharomyces carlsbergensis) was grown under CO_2 in a medium containing 40 μCi of ^{51}Cr(III) by incubation at 28 °C for 6 days with an extra 100 g of glucose added on each of the first three days.

The yeast cells were separated by centrifugation and washed with water until the radioactivity in the supernate was reduced to twice the background count. Radiation counts were recorded on weighed samples of this damp ^{51}Cr-labelled yeast which were then either freeze-dried or oven-dried (24 h at 100 °C). To correct for quenching, the amount of water originally present in the yeast was added to each dried sample and allowed to equilibrate before the post-drying count.

There were no significant losses of ^{51}Cr during drying by either method. Percentage recoveries of ^{51}Cr from 13 estimations averaged 101.8 ($s \pm 1.3$). The bulk of the yeast was then freeze-dried and used in the remainder of the experiment.

Wet digestion

In order to ascertain whether any loss of ^{51}Cr occurs by volatilization, during wet digestion, weighed samples (ca. 0.1 g) were placed in Pyrex digestion tubes (15 × 125 mm) and the ^{51}Cr activity was measured. The samples were then digested in the same tubes, with various combinations of acids, sodium chloride and silver nitrate (see Table 1). The digests were diluted to 3 ml with water and the ^{51}Cr activity measured. Corrections were made for decay of the isotope and quenching. No residual ^{51}Cr activity was found in the tubes after emptying and thorough washing with water. In all cases, satisfactory recoveries of ^{51}Cr were obtained (Table 1).

TABLE 1

Recovery of ^{51}Cr from yeast after wet digestion

Digestion mixture	No. of samples	Mean % recovery ($\pm s$)
$H_2SO_4 + HNO_3 + HClO_4$	6	97.3 ±1.7
$H_2SO_4 + HNO_3$	4	100.75 ±3.7
$HNO_3 + HClO_4$	6	97.1 ±0.2
$H_2SO_4 + HNO_3 + HClO_4 + AgNO_3$[a]	4	102.2 ±3.2
$H_2SO_4 + HNO_3 + HClO_4 + AgNO_3$[a] + NaCl[b]	5	102.0 ±4.0
$H_2SO_4 + HNO_3 + HClO_4 + NaCl$[b]	4	98.65 ±4.3
$HNO_3 + HClO_4 + AgNO_3$[a]	4	101.3 ±2.6

[a] 0.4 ml 10% of (w/v) $AgNO_3$.
[b] 0.2 ml of 2% (w/v) NaCl.

Effect of dry ashing up to 800 °C

Weighed samples (0.03 g) were placed in Vitreosil silica tubes (12 × 55 mm) and counted. They were then heated to graded temperatures from 100 °C to 800 °C, rising in 100 °C increments, in a thermostatically controlled muffle

furnace with air drawn through it; the temperature was maintained at each level for 2 h. The tubes were cooled and counted after each heating increment to determine the ^{51}Cr activity. The samples were completely ashed at 600 °C. Recoveries of ^{51}Cr were also determined on samples of yeast heated rapidly to 700 °C. Other silica tubes containing yeast (0.03 g) and 0.004 g of sodium chloride were counted to determine the ^{51}Cr activity, heated at 600 °C for 7 h, cooled and counted again. Table 2 shows that there were no losses of ^{51}Cr for any ashing conditions tested.

TABLE 2

Recovery of ^{51}Cr from yeast after dry ashing

Temperature (°C)	No. of detns.	Mean % recovery ($\pm s$)
100—800° (stepwise)	3	100.5 ±0.5
700° (rapid rise)	3	100.9 ±1.7
600° (rapid rise)	10	100.5 ±1.7
600° (rapid rise with NaCl)	2	99.5

TABLE 3

Residual ^{51}Cr in silica tubes after dry ashing and dissolution of ash in acids

Acid used	No. of detns.	Mean % ^{51}Cr retained ($\pm s$)
Conc. HNO_3	7	85.8 ±6.0
Conc. H_2SO_4	5	0.7 ±0.3
6 M HCl	8	4.1 ±3.4
Conc. $HClO_4$	4	8.4 ±5.7

Dissolution of ^{51}Cr from yeast after ashing

After the final heatings at 600 °C or 800 °C, the contents of the tubes were dissolved by boiling in 1 ml of concentrated sulphuric, nitric, or perchloric acid or 6 M hydrochloric acid, and the tubes were rinsed thoroughly inside and out with water and dried. They were again counted to determine any residual ^{51}Cr in the tubes. Table 3 shows that only concentrated boiling sulphuric acid satisfactorily removed the ^{51}Cr from the tubes.

Discussion

The quantitative recovery of inorganic chromium added to biological samples does not necessarily indicate complete recovery of the total endogenous

chromium [2,4]. This uncertainty was studied by incorporating ^{51}Cr into plant cells during growth; brewers yeast which grows rapidly and contains a considerable proportion of GTF-chromium, proved useful test material.

No significant losses of ^{51}Cr by volatilization from brewers yeast were found with oven-drying, freeze-drying, wet digestion (even with high levels of chloride) or dry ashing. No evidence was found for the retention of ^{51}Cr on the walls of wet digestion vessels. However, ^{51}Cr could be retained on the surfaces of silica tubes after dry ashing, unless the ash was dissolved in boiling concentrated sulphuric acid; other mineral acids were unsatisfactory. Similar results were obtained after dry ashing in platinum crucibles. Brewers yeast is high in both GTF-chromium and total chromium, and it seems probable that the above results would be applicable to other biological materials.

The authors wish to thank the management of the South Australian Brewing Co. Ltd. for the supply of the yeast cultures.

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Short Communication

DETERMINATION OF NAPHTHACENE BY SENSITIZED FLUORESCENCE ON FILTER PAPER

I. HORNYÁK

Research Institute for Technical Physics of the Hungarian Academy of Sciences, Ujpest I.P.O. Box 76. 1325 Budapest (Hungary)

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It is well known that naphthacene shows a very weak fluorescence in u.v. radiation; the quantum efficiency is 0.002 [1]. However, when trace quantities of naphthacene (10^{-4}—10^{-3} M) are built into the crystal lattice of another polycyclic aromatic hydrocarbon, e.g. 2,3-benzfluorene, and excited at the absorption band of the energy donor, the characteristic yellow green sensitized fluorescence of naphthacene appears [2]. Some of the excitation energy absorbed by the donor is transferred by radiationless transition to the naphthacene molecules, so that the naphthacene decreases strongly the fluorescence of the energy donor; this change is proportional to the concentration of naphthacene [3]. The sensitized fluorescence of naphthacene has already been applied analytically in solution [4], in colloidal solution [5] and on Whatman paper [6], but the sensitized fluorescence with 2,3-benzfluorene (11-H-Benzo(b)-fluorene) has not been studied previously. The application of this phenomenon to the determination of traces of naphthacene on Whatman paper is described in this communication.

Experimental

Analytical-grade reagents (Ferak) were used. A Hitachi MPF-2A spectrofluorimeter was employed with a correction accessory, and for measurements at 77 K, with the Dewar unit of the phosphorescence accessory.

2,3-Benzfluorene and naphthacene were dissolved in benzene, to give solutions which were $5 \cdot 10^{-5}$ M, and 10^{-8}—$5 \cdot 10^{-5}$ M, respectively. Whatman paper strips were immersed in these solutions and after 10 min the solvent was evaporated, and the fluorescence intensities of both 2,3-benzfluorene and naphthacene were read off at the appropriate peak maxima with constant slit conditions and geometrical arrangements. Excitation was done at 328 nm; the excitation bandwidth was 10 nm, and the emission bandwidth 2 nm.

Results

The fluorescence spectrum of the 2,3-benzfluorene-naphthacene system shows nine bands (Fig. 1). In the shorter wavelength range, six bands pertain to 2,3-benzfluorene (361, 380, 396, 419, 445 and 473 nm); the other three relate to naphthacene fluorescence (491, 527 and 566 nm). Depending on the quantity of naphthacene present, the shape of the fluorescence spectrum changes, but the characteristic peak maxima do not. For quantitative evaluation of naphthacene, the fluorescence intensities are measured at 491 nm (I_N^{491}) and 396 nm (I_{BF}^{396}). The ratio of these intensities plotted against the naphthacene concentration shows a linear portion over the range $5 \cdot 10^{-5}$—$3 \cdot 10^{-7}$M naphthacene; this calibration curve can serve for the determination of naphthacene. The plot curves slightly at lower concentrations probably because of trace contaminants; however, this curve is reproducible and can be used analytically in the range $3 \cdot 10^{-7}$—10^{-8} M. Over the linear range the standard deviation is 1—2%, but for naphthacene concentrations below $3 \cdot 10^{-7}$ M, the standard deviation increases to 6—8%. If the fluorescence is measured at 77 K, the sensitized fluorescence intensity of naphthacene increases about twofold.

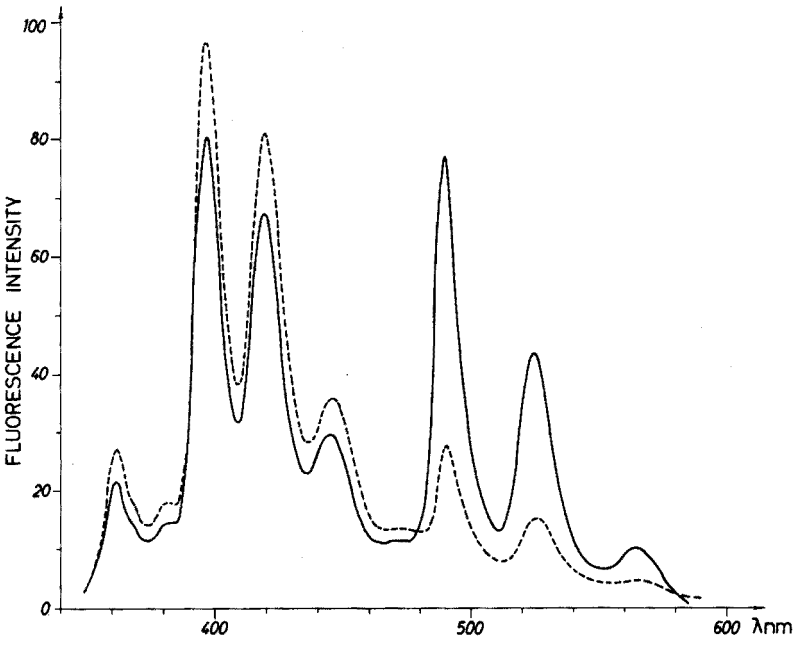

Fig. 1. Change in fluorescence intensity of 2,3-benzfluorene by the naphthacene concentration on Whatman paper. Concentration of naphthacene: (—) $5 \cdot 10^{-6}$ M; (- - -) 10^{-6} M.

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Short Communication

A SPOT TEST FOR THE DETECTION OF PERIODATE

PASCHOAL SENISE and LOURDES GONÇALVES SILVA
Instituto de Química, Universidade de São Paulo, São Paulo (Brazil)
(Received 21st April 1975)

Copper(II) can be extracted into methyl isobutyl ketone from aqueous solutions containing azide and an excess of 2,2′-bipyridine, when certain anions, such as perchlorate and perrhenate, are present [1, 2]. Periodate behaves similarly to these anions, but further studies have shown that substitution of manganese(II) for copper(II) in the aqueous medium enhances the selectivity for periodate, especially because iodate does not promote the extraction. It was thus considered of interest to develop an identification test based on the yellow color formed in the organic phase when periodate is present; for selective and sensitive tests for periodate are still scarce.

Feigl [3] based tests on the oxidation of manganese(II) to permanganate detected with tetramethyl-*p*-diaminodiphenylamine, and on the reaction with manganese(II) in phosphoric acid solution leading to the colored $Mn(PO_4)_2^{3-}$ ion. Feigl and Uzel [3, 4] utilized the formation of a red complex anion of copper(III) when periodate is present in the reaction of copper(II) with persulfate. They also based a test on the interference of periodate with the catalytic effect of copper(III) in the reaction of manganese(II) with hypobromite, leading to permanganate [4]. Other tests for periodate have been based on luminescence [5], the use of plasmochin [6] and paper chromatography [7].

Preliminary studies

Mixtures containing manganese sulfate, azide ions and an excess of 2,2′-bipyridine were tested with and without very low concentrations of periodate; extraction was evaluated by the yellow color of the organic phase. Of the many solvents tested, methyl isobutyl ketone proved most satisfactory. The effects of pH and reactant concentrations were studied quantitatively by spectrophotometric measurements of the organic extract. As observed in a similar test for perchlorate [1], it was necessary to use a buffered reagent and a solution of 2,2′-bipyridine in methyl isobutyl ketone instead of the pure solvent as extractant.

Experimental

Manganese—2,2′-bipyridine reagent

To 0.25 g of 2,2′-bipyridine in a 5-ml volumetric flask, add 1.3 ml of 0.5 M manganese sulfate solution, and dissolve by heating carefully in a water bath at 50—60 °C. Add 1.3 ml of 0.15 M sodium azide solution and dilute to the mark with an acetic acid—sodium acetate buffer of pH 4.6. Prepare daily and keep under diffuse light.

Solvent

Use a recently prepared solution of 2,2′-bipyridine in methyl isobutyl ketone (15—20 mg ml^{-1}). Keep in the dark.

Procedure

To a drop of the sample solution (pH 4—9) in a microtube, add 3 drops of the reagent and 3 drops of the solvent, and shake gently. A yellow color appears in the organic layer when periodate is present. Compare with a blank run in parallel. Limit of identification: 0.5 μg IO_4^-. Limit of dilution: 1 : 100000.

Interference study

The influence of foreign anions on the above procedure was studied, with different amounts of the ions and with strict blank comparisons; the results are shown in Table 1. It is of interest that large amounts of iodate are not extracted at all.

TABLE 1

Tolerance limits for diverse anions
(1 μg IO_4^- present)

Ion	Tolerance limit (μg)
IO_3^-	5000
NO_3^- [a], SO_4^{2-}	3000
ClO_3^-, BrO_3^-, Br^-	2000
Cl^-	1500
Ac^-	1000
$B_4O_7^{2-}$, SeO_3^{2-}, SeO_4^{2-}	500
ClO_4^- [a], ReO_4^- [a]	400
$HAsO_4^{2-}$, $H_2PO_2^-$, $S_2O_3^{2-}$, TeO_4^{2-}	250
$C_2O_4^{2-}$	200
CO_3^{2-}, F^-	100
HPO_4^{2-}, HPO_3^{2-}	50
AsO_2^-, MoO_4^{2-}, WO_4^{2-}	10

[a] After standing for 2—3 min a yellow color appears in the organic phase.

CONCLUSIONS

The recommended test is reliable, very simple to perform, and sensitive. These features and its high selectivity, especially with respect to iodate, make it preferable to the existing tests in the literature.

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Short Communication

CYCLOPENTANONE-2-CARBOXYANILIDE AS A GRAVIMETRIC AND SPECTROPHOTOMETRIC REAGENT FOR URANIUM(VI)

S. K. MANDAL, N. K. CHAUDHURI* and J. DAS

Department of Chemistry, The University of Burdwan, Burdwan 713101, West Bengal (India)

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Several β-diketones, have been used [1—4] in the solvent extraction and spectrophotometric determination of uranium(VI) and various acetanilide derivatives containing the 1,3-diketo group have been used [5—7] gravimetrically or spectrophotometrically. Cyclopentanone-2-carboxyanilide is a superior reagent for the determination of beryllium [8]. It is shown here that this reagent is also useful in the gravimetric and spectrophotometric determination of uranium(VI), being selective in the presence of the Mg—EDTA complex. The procedures are rapid, and can be used for the analysis of uranium ores.

Experimental

Reagents and chemicals

The preparation of cyclopentanone-2-carboxyanilide has already been described [8].

For the standard uranium(VI) solution, precipitate uranium from a solution of uranyl nitrate (Merck) with dilute ammonia solution in the presence of Na_2-EDTA. Wash the precipitate, dissolve in dilute nitric acid and standardize by the N-benzoyl-N-phenylhydroxylamine method [9]. Dilute this solution appropriately for the gravimetric and spectrophotometric methods.

Prepare stock solutions of diverse ions from the nitrates, chlorides and sulphates of cations, or the sodium or potassium salts of anions. Prepare magnesium—EDTA solutions in the conventional manner; use a freshly prepared 0.1 M solution for gravimetry and a 0.01 M solution for spectrophotometry.

Apparatus

A Cambridge pH meter, a Hilger—Watts H 700 Uvispek spectrophotometer with 1-cm quartz cuvettes, and an Aminco Thermoanalyser were used.

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*Present address: Radiochemistry Division, Bhaba Atomic Research Centre, Trombay, India.

Gravimetric procedure

Dilute the solution containing 30—45 mg of uranium(VI) to 150 ml, adjust the pH to ca. 5.0 with aqueous ammonia (2 M) and heat to 50 °C. Add the reagent (0.35—0.40g in 5 ml of 50% ethanol) slowly with stirring and let the mixture cool to 35—40 °C. Adjust the pH to 6.2—8.0 with aqueous 10% (v/v) pyridine solution. The brown-red complex precipitates within 30 min. Filter, wash with warm water (35—40 °C), dry at 120 °C for 1h and weigh. The conversion factor is 0.3352.

For the range of concentration specified above, the relative error did not exceed ± 0.6%. When uranium(VI) was determined in the presence of diverse ions, 10 ml of 0.1 M Mg—EDTA solution was added before the addition of the reagent.

Spectrophotometric procedure

Dilute an aliquot of the uranium(VI) solution (containing 0.1—0.6 mg) to 10 ml and heat to 50 °C on a water bath. Add 5 ml of the freshly prepared 1% (w/v) reagent solution, in 50% ethanol and adjust the pH to 6.5—8.5 with fresh aqueous 10% (v/v) pyridine solution from redistilled reagent. Cool the mixture to room temperature and extract with two 5-ml portions of isobutyl-methyl ketone. Measure the absorbance of the combined extract at 360 nm with the pure solvent as reference.

In the presence of diverse ions, add 4 ml of 0.01 M Mg—EDTA solution before the reagent and adjust the pH to 7.0—8.0.

Procedure for uranium ores

Treat about 0.5 g of the ore with dilute nitric acid and then with 5 ml of 18 M sulphuric acid. Evaporate to dryness, cool and dilute with water. Filter, and retain the filtrate. Ignite the residue, add hydrofluoric acid, heat gently and finally add 5 ml of 16 M nitric acid. Filter; combine the filtrates, and dilute to 250 ml. Determine uranium(VI) gravimetrically in an aliquot (25—75 ml) after adding Mg—EDTA solution.

For the spectrophotometric method, decompose 0.2—0.5 g of the ore as above and dilute the resulting solution to 500 ml. Treat a suitable aliquot (2—10 ml) as described above, after adding Mg—EDTA solution.

Results obtained for uraninite are given in Table 1.

Results and discussion

Uranium(VI) cyclopentanone-2-carboxyanilide complex

The brownish-red complex was dried at 120 °C and analysed for uranium by conversion to U_3O_8 and for nitrogen by the Kjeldahl method (found: 33.1%, 33.2% U; 4.0% N; $UO_2(C_{12}H_{12}O_2N)_2$, $2H_2O$ requires 33.5% U; 3.9% N). Thermogravimetric analysis confirmed that both water molecules were lost on heating to 180 °C; at 215 °C the anhydrous complex started to decompose to U_3O_8. The complex was slightly soluble in ethanol and

TABLE 1

Determination of uranium in ores

Sample no.	U_3O_8 by standard method (%)	U_3O_8 found, (%)	Rel. error (%)
Gravimetry			
1	52.51	52.45, 52.55	−0.11, +0.07
2	24.70	24.65, 24.68	−0.20, −0.08
Spectrophotometry			
2		24.43, 25.20	−1.49, +2.02
3	8.60	8.67, 8.82	+0.81, +2.24
4	2.29	2.30, 2.24	+0.42, −2.18

freely soluble in chloroform or isobutyl methyl ketone.

The composition of the uranium(VI) complex in isobutyl methyl ketone was also examined by the mole-ratio method [10]. A series of solutions, prepared by mixing a $0.0848 \cdot 10^{-3}$ M, solution of uranium(VI) with various concentrations of cyclopentanone-2-carboxyanilide ($4.92 \cdot 10^{-2}$ M to $2.95 \cdot 10^{-1}$ M), were extracted with isobutyl methyl ketone. The absorbances, measured at 360 nm, were plotted against the mole-ratio of the ligand to metal. The results indicated that the complex has a metal : reagent ratio of 1 : 2. This conclusion was confirmed by the slope-ratio method. The value of the dissociation constant calculated [11] from the mole-ratio curve was $5.68 \cdot 10^{-11}$.

Absorbance curves and validity of Beer's law

The absorption spectra of the uranium(VI)—cyclopentanone-carboxyanilide complex in isobutyl methyl ketone showed a broad peak with a plateau at 358—362 nm where the reagent did not absorb. The extraction could also be carried out with diethyl ether, amyl alcohol and tributyl phosphate; isobutyl methyl ketone was selected because it is easy to purify and recover, and does not absorb at 360 nm.

Beer's law was valid from 10.0 to 60.0 μg U(VI) ml^{-1}. The optimum concentration range [12] was 10.5—27.0 μg U(VI) ml^{-1}, the standard deviation being about 1.2%. The Sandell sensitivity [12] was 0.01 $\mu g\,cm^{-2}$. The molar absorptivity was $5821 \cdot 3\ mol^{-1}\ cm^{-1}$.

Effect of experimental variables

The uranium(VI) complex was precipitated at pH 7.0 with different amounts of the reagent dissolved in ethanol. For quantitative precipitation, the supernate should be at least 0.20% with respect to the reagent. For the extraction procedure, 50 mg of the reagent gave at least 99% transfer if the

complex was first formed in aqueous solution; a second extraction with pure solvent ensured complete transfer. Much larger quantities of the reagent were required if it was dissolved in isobutyl methyl ketone.

Uranium(VI) was precipitated from solutions adjusted to different pH values with pyridine solution. Precipitation commenced at pH 4.5 and was quantitative at pH 6.2—8.0. Above pH 8.0, colloid formation occurred. Quantitative extraction of the complex in the presence of magnesium—EDTA, was obtained at pH 7.0—8.0.

Effect of diverse ions

When the gravimetric procedure was used in the presence of magnesium-EDTA, uranium(VI) could be separated from approximately equal weights of Cu(II), Cd(II), Co(II), Hg(II), Pb(II), Ni(II), Zn(II), Bi(III), Fe(III), Al(III), Ce(IV), Th(IV), V(IV) and chromate.

The spectrophotometric procedure was tested for 30 μg U ml^{-1}; in the presence of magnesium—EDTA, there was no interference from 100-mg amounts of Ag(I), Tl(I), Hg(II), Cu(II), Cd(II), Bi(III), Pb(II), Ni(II), Co(II), Ce(IV), Th(IV), Zr(IV), Zn(II), V(IV), chromate or molybdate, or from 50-mg amounts of Al(III) or Fe(III)

The authors gratefully acknowledge the help of Dr. A. J. Bhattacharyya and (Miss) K. Ghosh, Organic Chemistry Department, in the preparation of the reagent.

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Short Communication

REDOX POTENTIAL OF THE Tl(III)—Tl(I) COUPLE IN ALKALINE MEDIUM

J. K. BHADRA, B. K. SEN and P. BANDYOPADHYAY

Chemistry Department, University College of Science, Calcutta 700009 (India)

(Received 8th June 1975)

Thallium(III) is a strong oxidant in aqueous medium (E_0 = 1.252 V) and in acidic solution [1, 2], but precipitation of thallium(III) occurs above pH 2. Because thallium(III) is complexed [3] in alkaline medium by triethanolamine, so that no precipitation of Tl_2O_3 occurs even in strongly alkaline solutions, the formal potential of the Tl(III)—Tl(I) couple in alkaline medium can be measured.

Experimental

Apparatus

A Cambridge portable pH meter was used with a Cambridge alkali-range (pH 1—13) glass electrode, and the e.m.f. of redox couples was measured between a bright platinum indicating electrode and a saturated calomel electrode through a double bridge of agar—sodium perchlorate (2 M). All measurements were made at 30 ± 0.5 °C.

Reagents

Thallium(III) and thallium(I) perchlorate were prepared from the corresponding reagent-quality carbonates (BDH) and perchloric acid (E. Merck). Analar triethanolamine was used. Care was taken to exclude carbon dioxide from the solutions.

Formal potential as a function of pH

A constant ionic strength (μ = 1) was maintained with sodium perchlorate. Thallium(III) perchlorate was added dropwise from a microburette, with constant stirring, to accurately known quantities of triethanolamine and aqueous sodium perchlorate. Thallium(I) perchlorate was then added similarly so that the solution had the same final analytical concentrations of Tl(III) and Tl(I). Either perchloric acid or sodium hydroxide was added to maintain the required pH value. After dilution to 25 ml, the pH and e.m.f. of the solutions were measured; mean values are listed in Table 1.

TABLE 1

Formal potential of Tl(III)—Tl(I) as a function of pH
([Tl(III)] = $2 \cdot 10^{-3}$ M; [Tl(I)] = $2 \cdot 10^{-3}$ M; [TEA] = 0.3 M)

pH	E vs. SCE[a] (mV)	pH	E vs. SCE (mV)
12.45	−121		
		9.00	+275
11.54	−14	8.54	+328
10.85	+81	8.00	+362
		7.65	+395
9.98	+162		
9.34	+233	6.70	+477

[a]Mean of 3 independent determinations.

Effect of triethanolamine concentration on the redox couple

The effect of varying triethanolamine concentrations on the magnitude of the formal potential was studied for constant thallium concentrations; no extra perchloric acid or sodium hydroxide was added, and the pH values varied within the range 8.90—10.14. The results are shown in Table 2.

Effect of thallium concentration

Measurements were made with Tl(III) and Tl(I) concentrations in the range 1.0—2.0 · 10^{-3} M; in all cases, [Tl(III)]/[Tl(I)] = 1, and a 0.3 M triethanolamine medium was used at pH 9.34. There was no significant trend in the results over the specified range, the mean E value being 0.235 ± 0.002 V vs. SCE.

Discussion

The experimental values show that thallium(I) behaves as a fairly good reductant in the presence of triethanolamine, because of the great stability of the thallium(III) complex with the amine. The gradual decrease in the E_0 value with increasing triethanolamine concentration (Table 2) may result from more extensive complexation of Tl(III) at higher ligand concentrations. There is a slight break at pH 8.6 in the pH—E plot of the values given in Table 1; the reason for this is obscure.

TABLE 2

Formal potential of Tl(III)—Tl(I) as a function of triethanolamine concentration ([Tl(III)] = $2 \cdot 10^{-3}$ M, [Tl(I)] = $2 \cdot 10^{-3}$ M)

[TEA]	E vs. SCE (mV)	[TEA]	E vs. SCE (mV)
0.300	288	0.775	205
0.469	248	0.926	185
0.594	223	1.080	172
		1.240	154

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BOOK REVIEWS

James M. Miller, *Separation Methods in Chemical Analysis*, Wiley—Interscience, New York, 1975, x + 309 pp., price £ 8.10.

Over the past 30 years, development in separation techniques has proceeded rapidly, and to a great extent this has stemmed from the advent of the theory of chromatographic separations. Unfortunately, however, there has been a lack of communication between practitioners of the various techniques and this has led to various closely related separation methods being viewed as completely separate techniques in their own right. The author of this book has attempted, quite successfully, to reverse this trend, and groups separation methods according to the basic physical processes involved. The first two chapters provide a justification for the grouping proposed; these chapters outline some of the major processes involved and, most importantly, provide a rationale for the symbols used. The next four chapters present the fundamental physico-chemical processes which provide the basis of separation techniques. Much of this information is covered in the normal undergraduate physical chemistry courses, but these chapters emphasize the application of the principles to separation. The remaining eleven chapters of the book deal in more detail with individual separation methods, but always with an emphasis on comparison by pointing out how the controlling factors vary as one passes from one technique to another.

A significant portion of the book deals with chromatographic methods of separation, and there is an excellent chapter on gas chromatography, which, while it is obviously not as complete as a book devoted to this technique, nevertheless provides much valuable information. The chapter on high-pressure liquid chromatography provides a basis for the use of this technique but is unavoidably already out of date.

Zone electrophoresis is treated rather sketchily and surprisingly no mention is made of the extremely powerful and fast isotachophoresis.

There are relatively few mistakes in the book which has been extremely well conceived and written for so broad and complex a subject. For example, on page 13 the text refers to eqns. (22) and (23), which should read 23 and 24. The term log Vd in the equation on page 62 should read log Va. In the text at the bottom of page 166 PA should read pA for the vapour pressure of pure A. The Table on page 182 states that the sensitivity of the gas density balance is 10^{-10} g s^{-1}, which is between 3—4 orders of magnitude too low. Page 201 gives a reference 16 which should be 76. The reviewer can see little reason for expressing the phase rule (p. 36) other than in the well-known equation $P + F = C + 2$. One statement is made which is incorrect, that the flow through a packed bed is slowest in the middle of the column (p. 123—124).

In general, this is an excellent book, well suited for undergraduate teaching

and it would not be amiss for research workers to consider it with care; it could help them solve their separation problems.

C.F. SIMPSON

Kenneth J. Clevett, *Handbook of Process Stream Analysis*, Ellis Horwood—Halsted Press—Wiley, New York, 1974, xviii + 470 pp., price £ 15.00.

On-line process analysis is of great industrial importance, yet curiously little is to be found on the subject in the normal analytical literature. This text has been written primarily for control, chemical and electrical engineers but contains much useful information for industrial analytical chemists. The author sets out clearly how particular methods have been modified for on-line analysis, and describes in detail a wide variety of the process analyzers which are commercially available. The types of measurement discussed are gas chromatography, viscosity, distillation, flash point, pour point, vapour pressure, u.v., visible and i.r. absorption, titrimetry, density, thermal conductivity, and refractive index. Chapters are also devoted to on-line measurements of oxygen, pH, trace gases, moisture, water quality, gaseous fuel quality, octane number, combustible gases, petroleum quality etc. The final chapter deals with sample-handling systems, and Appendices give analyzer specification data and manufacturers' addresses.

This will be a worthwhile purchase for anyone with problems in the design or application of process analysis. Its perusal would also be a mind-broadening experience for any conventional analytical chemists who can afford to buy it. As about 100 instrument manufacturers have their products "advertised" here, could they not have been asked to contribute to the production costs to ensure a wider availability of a useful and interesting book?

Alan J. Rubin, *Chemistry of Water Supply, Treatment and Distribution*, Ann Arbor Science Publishers, Ann Arbor, Michigan, 1974, vii + 446 pp., price £ 11.95.

Anyone sufficiently unwary to purchase this book on the basis of its title and the publisher's puff would be justified in feeling seriously misled on perusal of its actual content. Suspicions are first aroused by the feverishly ecological tone of the opening paragraphs of the Preface, but it is not until its final paragraphs that the reader realizes that this book is no treatise covering the chemistry of potable waters from source to usable product but merely a collection of about half (18) of the papers presented at a 1973 A.C.S. symposium on the chemistry of water supplies, etc. The topics of the papers, which would have benefited from editorial attention, vary from the nutrient input to a lake,

through discussions of sulfur and arsenic in water supplies, to virus inactivation. There is little of analytical interest.

Radiochemical separation methods — Proceedings of the 7th Radiochemical Conference, Edited by T. Braun and E. Bujdoso, Elsevier Scientific Publishing Company, Amsterdam, 1975, 535 pp., price Dfl. 155.00.

This book contains the Proceedings of the 7th Radiochemical Conference which was held at Marianske Lazne, Chechoslovakia, in April 1973, and has been reprinted from the Journal of Radioanalytical Chemistry. The 52 papers included are printed under the headings: Solvent extraction chemistry, Ion-exchange chemistry, Radiochemical separations,.and Radioanalytical methods. The major topic of the Conference was the problem of the radiochemical methods used in isolation or identification of nuclear reactions products. These Proceedings reflect the main interests of radiochemists from central and eastern Europe.

Recent Analytical Developments in the Petroleum Industry, Edited by D.R. Hodges, Applied Science Publishers, Barking, Essex, 1974 ix + 337 pp., price £ 10.00.

The Institute of Petroleum sponsored a symposium on recent analytical developments in the petroleum industry in London in 1973 and the proceedings appear in this book. The topics of the nineteen papers presented range from applications of raman spectroscopy, x.r.f. and a.a.s., through p.g.c., t.l.c. and g.l.c., to spectrophotometry for phosphate and vanadium traces; discussions of the papers are printed in full. The presentation is workmanlike throughout and the papers reflect the main areas of chemistry which are of interest to analytical chemists in the petroleum industry.

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