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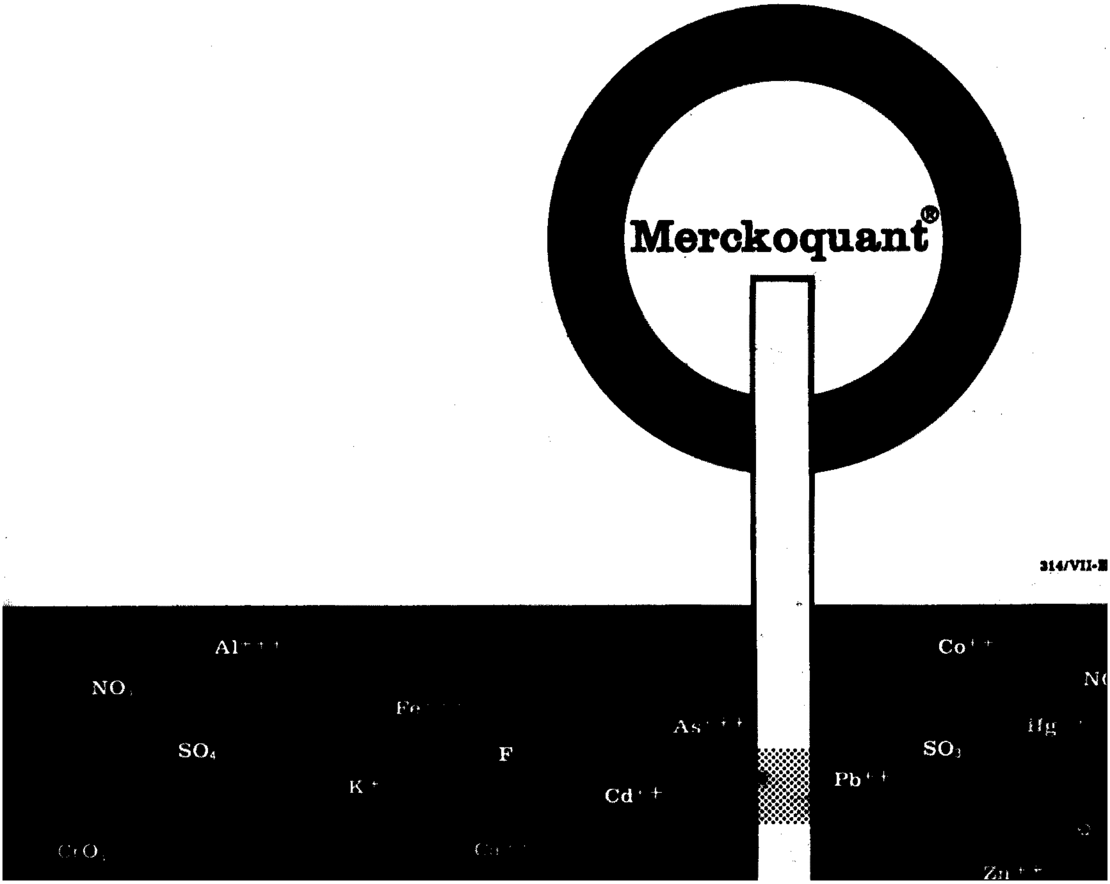
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Activation and decay tables of radioisotopes

by E. BUJDOSÓ, *Research Institute for Non-ferrous Metals, Budapest, Hungary,* I. FEHÉR, *Central Research Institute for Physics, Budapest, Hungary and* G. KARDOS, *UNIVAC, Division of Sperry Rand France, Paris, France.*

1973. 576 pages. Dfl. 100.00 (about US$38.50) ISBN 0-444-99937-X

With the widening use of radioisotopes in science and industry, the calculation of the activity of a sample irradiated by thermal neutrons and the rate of decay has become a routine task in many laboratories. The book greatly facilitates such calculations by means of tables compiled with the aid of a computer.
Activation and decay data are presented including half-lives, gamma-ray energies and intensities of 249 radioisotopes formed by (n,γ) reactions on 173 stable isotopes of 80 elements.
These clear tables will be of great help in activation analysis and in other investigations connected with the production and use of radioisotopes.

Contents:
Introduction. **Explanation of the Tables.** Nuclear data. Activity calculation. Decay calculation. Data of the table on activation by (n,γ) reactions and on the decay of activity. Calculation of the activation by (n,γ) reactions by use of the tables. Calculation of the daughter activity. Data of the table on daughter element formation. Calculation of the daughter activity by the use of the table. Calculation of the expected counting rates. Key to the numerical values. **Examples of how to use the Tables.** Calculation of the disintegration and counting rates of ^{24}Na produced by the irradiation of sodium. Calculation of the activity of ^{131}I produced by the irradiation of tellurium. **References. Activation and Decay Tables. Index to the Target Nuclides. Index to the Radionuclides.**

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DEVELOPMENTS IN SEDIMENTOLOGY, VOL. 15

The Chemistry of Clay Minerals

By CHARLES E. WEAVER, *School of Geophysical Sciences, Georgia Institute of Technology, Atlanta* and LIN D. POLLARD, *Georgia Institute of Technology, Atlanta.*

1973. 222 pages. Dfl. 65.00 (about US $ 25.00) ISBN 0-444-41043-0

Long overdue, this is the first comprehensive compilation and critical evaluation of data on the chemistry of clay minerals. It includes both oxide data and structural formulae for twenty-five varieties of clay minerals, while for the more common minerals, means, standard deviation, range, limits, histograms and correlation coefficients are presented.

The chemical data is evaluated in terms of the interrelation of composition and structure, including a discussion of the effect of ion size and distribution on the octahedral and tetrahedral sheets as well as how these sheets fit together. The relation of chemical composition to mode of origin is discussed for most of the clay minerals and their low-temperature synthesis is briefly reviewed. Also considered are the nature and significance of hydroxy interlayers and cation exchange.

As a graduate-level research reference the book will be very useful to workers in the field of clay mineralogy and chemistry, but students will also find it invaluable as a supplemental textbook.

CONTENTS:

Illite. Glauconite. Celadonite. Smectite. Chlorite. Vermiculite. Mixed-layer clay minerals. Attapulgite and palygorskite. Sepiolite. Kaolinite. Dickite and nacrite. Halloysite. Allophane. Trioctahedral 1:1 clay minerals. Low-temperature synthesis. Relations of composition to structure. References.

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Analytica Chimica Acta, 69 (1974) 1–9

AN ^{227}Ac–Be ISOTOPIC NEUTRON SOURCE FOR APPLICATION IN HIGH-ACCURACY NEUTRON ACTIVATION ANALYSIS

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(Received 27th August 1973)

In previous papers[1,2], it has been shown that neutron activation analysis (n.a.a.) for manganese in pyrolusite and ferromanganese, with the aid of a cylindrical 1-Ci ^{226}Ra–Be neutron source having a total neutron emission of 10^7 n s^{-1}, is comparable to classical wet chemical methods as far as precision and accuracy are concerned. The n.a.a. method, however, is much simpler and faster.

In order to expand the application of the method to other elements, that are easily activated by thermal or fast neutrons, the characteristics of an annular ^{227}Ac–Be source with a total neutron output of 10^8 n s^{-1} were investigated. As an example of its use, the activation analysis for manganese described in the previous paper[1,2] was repeated, and the results were compared with those obtained with the cylindrical source.

THE NEUTRON SOURCE

Description

Actinium-227 is a β, α-emitter with a half-life of 21.77 years and with a chain of relatively short-lived α-emitting daughter isotopes (from ± 2 μs for ^{215}Po to 18 days for ^{227}Th), with an average α-energy of 6.4 MeV. The neutron spectrum[3] of an unmoderated ^{227}Ac–Be source is shown in Fig. 1. As an ^{227}Ac–Be source has the practical advantage of having a much lower ratio of γ-rays to neutrons than a ^{226}Ra–Be source, the necessary lead shielding can be reduced. In order to optimize the flux density, the source has an annular shape closely surrounding the rabbit tube. A schematic representation of the pneumatic transport system, and the moderator consisting of a stainless steel tank filled with water, as well as the shielding with lead bricks and a cadmium foil has already been published[1,2]. The ^{227}Ac–Be source is encapsulated in Monel steel and stainless steel (see Fig. 2: outer diam., 43 mm; inner diam., 31 mm; *h*, 24.4 mm) and has an active zone containing 93 mg of ^{227}Ac, corresponding to an activity of 6.6 Ci (outer diam., 38 mm; inner diam., 36 mm; *h*, 9 mm). It is supported by a polyethylene holder so that the source can be raised or lowered in the surrounding water.

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* Aspirant of the N.F.W.O.

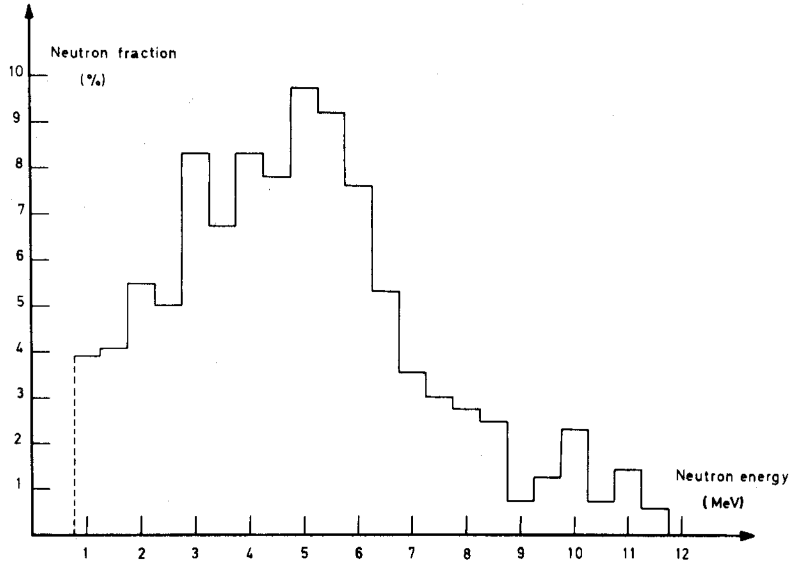

Fig. 1. Neutron spectrum of an ^{227}Ac–Be neutron source (ref. 3).

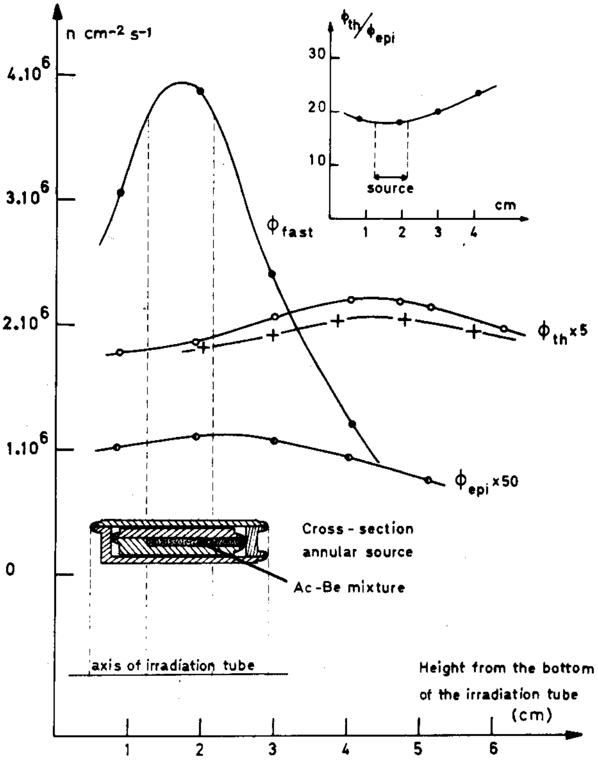

Fig. 2. Axial flux gradients at the irradiation site: (●) Fast flux; (○) thermal flux (×5) with massive polyethylene rod; (+) thermal flux (×5) with hollow polyethylene rod; (◐) epithermal flux (×50).

Flux and flux gradients at the irradiation site

At the time the source was put into operation, the ^{227}Ac had not yet reached complete equilibrium with its several daughter isotopes. The neutron output of the source can therefore be divided into a period in which a slow increase can be observed, until complete equilibrium is reached, followed by a period determined by the simple radioactive decay law, as shown in Fig. 3.

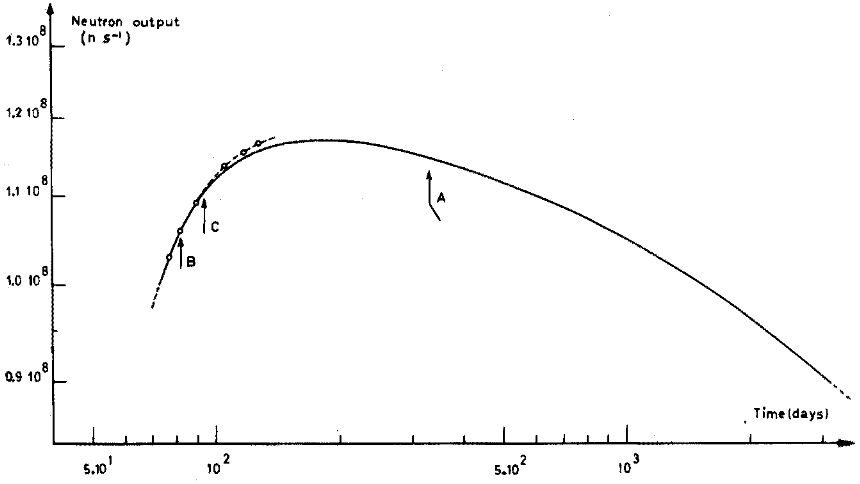

Fig. 3. Growth and decay of the total neutron output of the source. (---) Experimental; (—) calculated. See text for explanation of points A, B and C.

The neutron output after the end of the growth period (point A, Fig. 3) was calculated from a measurement by the manufacturer during the growth period, and the value was found to be $1.147 \cdot 10^8$ n s^{-1}. During the decay period the neutron output can therefore be represented by the following equation:

$$\mathrm{ns}^{-1} = 1.147 \cdot 10^8 \, \mathrm{e}^{-\lambda t} \tag{1}$$

where $\lambda = 8.7145 \cdot 10^{-5}$ (days^{-1}), and t = time in days.

The increase of the flux was regularly monitored over the period of the performance of the experiments presented in this paper and the results are also shown in Fig. 3.

The axial neutron flux gradients for fast, thermal and epithermal neutrons were investigated. A cylindrical sample (h, 6 mm; diam., 19.5 mm) of high-purity silicon and cylindrical pellets (h, 7.4 mm; diam., 20.0 mm) pressed from "carbonyl" iron mixed with graphite, were used to study the fast flux gradient around the source, by means of the reactions $^{28}\mathrm{Si}(n, p)^{28}\mathrm{Al}$ and $^{56}\mathrm{Fe}(n, p)^{56}\mathrm{Mn}$. As expected, the fast neutron flux goes through a sharp maximum at the height of the center of the source as shown in Fig. 2. Cylindrical ferromanganese pellets (h, 7.4 mm; diam., 20.0 mm) were used to determine the thermal neutron flux gradient, which, as shown in Fig. 2, goes through a maximum at about 2.7 cm above the source center. This can be expected, for this layer of water is needed for maximal thermalization of the fast neutrons released by the source.

Massive cylinders of polyethylene were used to vary the height of the pellets in the rabbit. This causes an additional thermalization, as can be seen from the difference in height of the thermal flux maximum when hollow instead of massive polyethylene cylinders are used (Fig. 2). A gain in thermal neutron flux by a factor of 8.9 measured at the top of the flux gradient was obtained with the ^{227}Ac–Be source compared to the ^{226}Ra–Be source.

The epithermal flux and the ratio Φ_{th}/Φ_{epi} were studied by irradiating small pellets of ferromanganese (*h*, 4.5 mm; diam., 12 mm) with and without a cadmium cover. The epithermal flux reaches a maximum at 0.65 cm above the source center (Fig. 2). In Fig. 2, the ratio Φ_{th}/Φ_{epi} is shown as a function of the height of the pellet in the rabbit.

The magnitude of the thermal flux at the top of the thermal flux gradient was determined by irradiating a piece of gold–aluminium alloy and measuring the ^{198}Au activity in standardized conditions on a Ge–Li detector[4]. It was found to be $4.43 \cdot 10^5$ n cm^{-2} s^{-1} (point B, Fig. 3). From this, the epithermal flux at the maximum of its gradient was calculated to be $2.23 \cdot 10^4$ n cm^{-2} s^{-1}. The magnitude of the fast flux at the top of the fast flux gradient was estimated from the data for the neutron flux spectrum of the source[3], from the excitation curve of the reaction ^{56}Fe$(n, p)^{56}$Mn as given by Hughes and Schwartz[5], and from activity measurements of pellets, made with carbonyl iron powder, which were irradiated in the center of the annular source. The value obtained for the fast flux was $3.9 \cdot 10^6$ n cm^{-2} s^{-1} (point C, Fig. 3).

In order to investigate whether changes in the diameter of the pellets could be a critical factor of error in the analysis, the radial flux gradient at the maximum of the axial gradient of the thermal neutrons was studied by irradiating twice three concentric rings of copper and counting the ^{64}Cu activity ($t_{\frac{1}{2}} = 12.8$ h). The results are shown in Fig. 4. A small increase of the thermal neutron flux towards the center of the rabbit may be due to thermalization in the polyethylene cylinder supporting the copper rings.

In conclusion, it can be said that the fast, epithermal and thermal neutron fluxes have their maximal density at three different heights in the irradiation tube. This can be a favourable factor for problems in which two different reactions must be studied simultaneously with different types of neutrons. For example, in

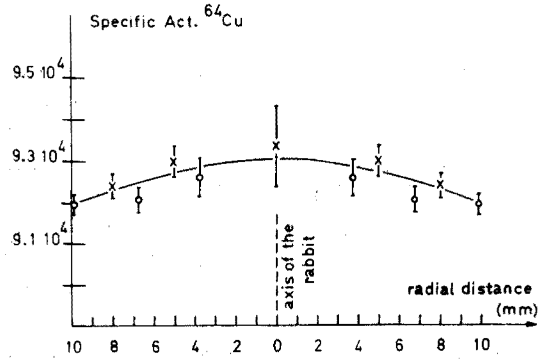

Fig. 4. Radial flux gradient at the irradiation site: (○) 1st irradiation; (×) 2nd irradiation.

the analysis of bauxite for aluminium and silicon, by the reactions $^{27}\mathrm{Al}(n, \gamma)^{28}\mathrm{Al}$ and $^{28}\mathrm{Si}(n, p)^{28}\mathrm{Al}$, one can do a double irradiation at the sites of maximal density of the thermal and fast flux.

DETERMINATION OF MANGANESE IN PYROLUSITE AND FERROMANGANESE

Sample preparation

Previously[1, 2], when the ^{226}Ra–Be neutron source was used, pellets were pressed from a mixture of pyrolusite or ferromanganese with graphite as a binding material. For the experiments in this work, a finely powdered wax* was available of which only a very small amount is needed to obtain pellets with excellent mechanical properties. In order to keep the same pellet dimensions as used in the original work[1, 2], the following mixtures were initially used: 9.84 g of ferromanganese with 0.96 g of wax, and 6.56 g of pyrolusite with 0.65 g of wax. As will be explained below, this method of sample preparation caused errors in the case of pyrolusite, and it was found necessary to make pellets containing only 3 g of pyrolusite and 1.7 g of wax.

Sequence of analysis and iterative calculation procedure

In order to avoid dead-time corrections, the irradiation time had to be shortened. Therefore the time sequence of analysis described previously[1, 2] was changed to 200-s irradiation time, 800-s waiting time and 800-s counting time. This was necessary because in the first place, the thermal neutron flux was about ten times higher than in the case of the ^{226}Ra–Be source, and secondly because of the greater sample weight.

The iterative calculation procedure[1, 2] needed no change except for the slope of the experimental neutron self-shielding calibration curve which had to be recalculated for every new experiment, because it changed with the specific activity of manganese, and this was a function of the increasing neutron flux during the period of the experimental work.

Results and discussion

The experimental neutron self-shielding calibration curves for ferromanganese and pyrolusite were determined separately because of the great difference in composition of the pellets. For ferromanganese, this curve was determined by taking a ferromanganese standard containing 76.51% manganese and diluting it stepwise with carbonyl iron; this permitted the production of pellets of different manganese content with the same total pellet weight. Measurable differences in γ-attenuation were thus avoided. For pyrolusite, the curve was established by taking ores with different compositions and pressing them to pellets which had the same ratio of ore to wax and the same dimensions. The specific activity was plotted against the amount of manganese originally determined with the ^{226}Ra–Be neutron source[1, 2]

It was mentioned above that samples containing 6.5 g of pyrolusite and 0.65 g of wax led to discrepancies when compared to the results obtained with

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Hoechst-Wachs C Pulver.

the previous source[1, 2]. It was suspected that boron was present in some of the samples and this was confirmed by means of emission spectrography. Pyrolusites from Brasil, Katanga, India and South Africa were used for the experiments, but only the South African ones contained an appreciable quantity of boron. Figure 5 shows the abnormal behaviour of the neutron self-shielding calibration curve of a boron-containing ore compared to a normal pyrolusite. If the standard used for the analysis happened to be of South African origin, all results for other ores would be too high. In order to prevent this kind of systematic error, the amount of pyrolusite was restricted to 3 g (containing *ca.* 1.5 g of manganese). To preserve the original dimensions of the pellets, 1.7 g of wax had to be added.

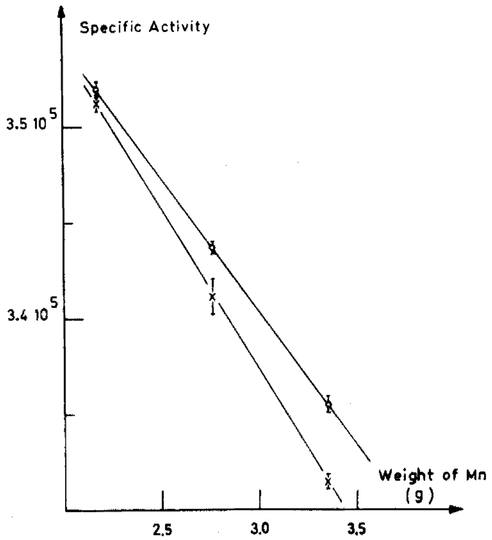

Fig. 5. Neutron self-shielding calibration curve: (○) normal ore; (×) boron-containing ore.

Another source of possible systematic error was discovered during the analysis of ferromanganese. It was traced back to the difference in particle size between samples and standards, which resulted in a difference in neutron shadow effect inside the individual particles; this effect was in addition to the overall neutron shadow effect for the whole sample that can be corrected for by the self-shielding calibration curve. Previously[1, 2], all samples and standards had been milled to particle sizes of less than 50 μm. Two ferromanganese samples from BCS and IRSID with particle sizes between 100 and 250 μm were analysed with a standard containing 76.51% manganese which had a particle size less than 50 μm. The results obtained in this way were too low by *ca.* 2%. After additional grinding and sieving, the results for both samples did agree very well with the wet chemical analysis.

In Table I, all the results are summarized, obtained chemically as well as by n.a.a. with both the ^{226}Ra–Be and the ^{227}Ac–Be neutron source. The chemical methods include the potentiometric titration in presence of pyrophosphate and the oxidation to permanganate by sodium bismuthate. A striking agreement

TABLE I

COMPARISON OF MANGANESE RESULTS IN PYROLUSITE AND FERROMANGANESE OBTAINED BY WET CHEMICAL METHODS, AND BY N.A.A. WITH ^{226}Ra–Be AND ^{227}Ac–Be NEUTRON SOURCES

(All concentration values are expressed as percentages Mn metal)

Sample code	*Chemical analysis*					*N.a.a. (1)* ^{226}*Ra–Be source*[b]	*N.a.a. (2)* ^{227}*Ac–Be source*[b]	*Difference* $NAA_{(1)}-NAA_{(2)}$
	Buyer pyroph.	*Mining Co.*[a]	*Indep. Lab.*[a]	*Univ. of Ghent*				
				pyroph.	*bismuth*			
Pyrolusite								
CKLPY 1	31.02[a]	—	—	—	30.95	30.04±0.06	30.23±0.05	−0.19
GORPY 1	36.76[a]	38.12	37.85	—	—	37.68±0.04	37.70±0.03	−0.02
0770/509	48.43	—	—	48.55	48.35	48.58±0.27	48.79±0.03	−0.21
0670/314	48.46	49.01	—	—	—	48.76±0.24	48.93±0.04[c]	−0.17
1071/402	47.94	48.58	—	—	—	48.93±0.17	48.90±0.22[c]	+0.03
1272/455	47.8	48.8	—	—	—	48.93±0.07	48.75±0.08	+0.18
0571/607	48.56	49.06	—	—	—	49.10±0.21	48.95±0.11	+0.15
0270/301	51.21	—	—	—	—	51.40±0.10	51.36±0.06	+0.04
Ferromanganese								
0170/566	75.84	—	—	—	—	75.45±0.15	75.57±0.05	−0.12
0171/450	77.67	—	—	—	—	77.75±0.22	77.56±0.20	+0.19
0371/070	78.39	—	—	—		78.22±0.22	78.24±0.17	−0.02
IRSID 502/1	—	—	78.3	—			78.51±0.13[c]	—
BCS 280/2	82.6	—	83.3	—	—		83.18±0.03[c]	—

[a] Method of analysis not known.

[b] Standard deviation on the average of three analyses (3 different pellets), taking into account Student's t-factor for 70% confidence level.

[c] One standard deviation on the average of 2 analyses (1 sample with 3 standards each) of the same pellet.

between the results obtained with the two different sources can be observed. This proves that the proposed method of analysis is not sensitive to changes in source design, provided that an experimental neutron self-shielding calibration curve is determined with suitable care. It was confirmed that for pyrolusite the amount of sample and standard used in pressing pellets should be sufficiently small that the possible presence of strong neutron absorbers, *e.g.* boron or cadmium, has a negligible effect on the total neutron absorption of the pellets. For ferromanganese, care should be taken to reduce the particle size sufficiently so that neutron absorption in individual particles becomes vanishingly small.

Thanks are due to the N.F.W.O. for partial financial support, and to Dr. D. De Soete, Dr. M. Pauwels and Mr. N. Henrard (Ir) for providing the pyrolusite and ferromanganese samples. Acknowledgement is also made to Mr. E. George and Mr. L. Dechamps, Société Générale des Minérais, 31 rue du Marais, 1000 Brussels, who were in charge of the source fabrication.

SUMMARY

An annular ^{227}Ac–Be isotopic neutron source, containing 6.6-Ci ^{227}Ac, is described for application in fast and thermal neutron activation analysis, with high accuracy, for major constituents in ores, alloys and industrial concentrates. The characteristics of the neutron output and of the fast, epithermal and thermal flux and flux gradients is described in detail. The determination of manganese in pyrolusite ores and ferro-manganese is compared to results obtained previously with a cylindrical 1-Ci ^{226}Ra–Be source. Two new sources of systematic errors have been discovered.

RÉSUMÉ

Une source isotopique de neutrons ^{227}Ac–Be, contenant 6.6-Ci ^{227}Ac, est décrite pour application a l'analyse par activation neutronique rapide et thermique, de grande précision, pour les constituants principaux des minerais, alliages et concentrés industriels. On décrit en détail les caractéristiques du débit neutronique et du flux rapide, épithermique et thermique. L'analyse du manganèse dans la pyrolusite et le ferro-manganèse est comparée aux résultats obtenus précédemment avec une source cylindrique 1-Ci ^{226}Ra–Be. Deux nouvelles sources d'erreurs systématiques ont été trouvées.

ZUSAMMENFASSUNG

Es wird eine ringförmige ^{227}Ac–Be-Neutronenquelle mit einem Gehalt von 6.6-Ci ^{227}Ac für die Anwendung bei der Aktivierungsanalyse mit schnellen und thermischen Neutronen beschrieben. Mit deren Hilfe werden die Hauptbestandteile in Erzen, Legierungen und industriellen Konzentraten mit hoher Genauigkeit bestimmt. Die Charakteristik der Neutronenemission und des schnellen, epithermischen und thermischen Flusses sowie der Flussgradienten wird ausführlich beschrieben. Die Bestimmung von Mangan in Pyrolusit-Erzen und Ferro-Mangan wird mit Ergebnissen verglichen, die früher mit einer zylindrischen 1-Ci-^{226}Ra–Be-Quelle erhalten worden sind. Zwei neue Ursachen systematischer Fehler wurden entdeckt.

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FLUORIMETRIC ASSAY OF ERGOTAMINE

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Some of the potent pharmacological properties of ergot were recognized at least 2,600 years ago, and the use of ergot in obstetrics dates from mediaeval times[1]. In modern times the ergot alkaloids were the first adrenergic blocking agents discovered, and most aspects of their general pharmacology were elucidated by the classical studies of Dale[2]. According to Wolff[3], the use of the vasoconstrictor property of ergot in the treatment of migraine dates from the early 1880's. Ergotamine (Fig. 1) was the first pharmacologically active ergot alkaloid obtained in a pure crystalline form[4]. It is widely used in treating migraine and migrainous neuralgia.

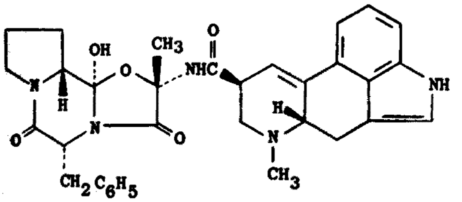


Fig. 1. Structure of ergotamine.

The early colorimetric[5] and spectrophotometric[6] assay procedures were capable of measuring only total alkaloids in the ergot, but more recently the incorporation of some type of chromatographic separation step has enabled the selective assay of specific alkaloids in either ergot extracts or pharmaceutical preparations[7,8]. However, the sensitivity and selectivity of all of the methods currently available are limited by the use of spectrophotometry (u.v. or visible) for the final measurement. This paper describes studies on the fluorescence of ergotamine, and the utilization of this property in a new highly sensitive assay procedure with a potentially increased selectivity.

EXPERIMENTAL

Apparatus

Fluorescence measurements were made with an Aminco-Bowman spectrophotofluorimeter (Model 4-8202) with the standard slit arrangement No. 3 and reflecting mirrors. Measurements of pH were taken on an Orion Model 801 digital pH meter with a Philips combination electrode (Type CA14/02).

A set of Pyrex test tubes and volumetric flasks were used exclusively for these studies, and were specially washed by soaking for 24 h in nitric acid (30%, A.R.), followed by 24 h in a solution of cetyltrimethylammonium bromide (1 mg l^{-1} of distilled water), with copious rinsing in distilled water after each soaking.

Vacuum evaporation was performed on a Buchi Rotovapor R with a water pump.

Reagents

Ergotamine tartrate (Sandoz Australia Pty. Ltd.) was used without further purification. Standard solutions from 0.001 to 5.0 μg ml^{-1} were prepared in 0.01 *M* hydrochloric acid in deionized, fluorescence-free water, by dilutions from a master standard prepared by dissolving 5.0 mg of ergotamine tartrate in 1.0 l of hydrochloric acid (0.01 *M*).

2-Aminopyridine (British Drug Houses, laboratory reagent) was purified by repeated sublimation, and a 10^{-5} *M* solution in sulfuric acid (0.05 *M*) was prepared as a fluorescence standard[9].

All organic solvents were purified by fractional distillation of laboratory reagents, and their fluorescence spectra were checked to ensure absence of contamination.

Glycine–sodium hydroxide buffer, pH 10.0 and 1.0 *M* with respect to glycine, was prepared, and the pH was adjusted with the use of the pH meter.

Solvent extraction studies

The effect of pH on the partition of ergotamine between water and benzene was studied to ascertain the optimal pH for extraction. Several immiscible organic solvents (benzene, ether, chloroform, heptane) were tested in order to determine the least polar solvent which quantitatively removed ergotamine from an aqueous solution at the optimal pH.

Fluorescence studies

The effect of pH between 1 and 14 on the fluorescence intensity of aqueous solutions of ergotamine (1.0 μg ml^{-1}) was studied. The maximal fluorescence intensity of ergotamine in water (pH 10.8) was compared with that obtained in a series of organic solvents, all with the same ergotamine concentration (1 μg ml^{-1}).

The stability of the fluorescent chromophore of ergotamine in hydrochloric acid (0.01 *M*) and ethanol was studied by storing solutions (*ca.* 2 μg ml^{-1}) in stoppered Pyrex vessels on an open laboratory bench at 20–25° for 6 months, reading the fluorescence intensity at regular intervals.

Assay procedure

Pipette 5.0 ml of an aqueous solution containing ergotamine (standard in 0.01 *M* hydrochloric acid, or unknown) into a 30-ml test tube with ground-glass stopper, and add 1.0 ml of glycine buffer and 10.0 ml of benzene. After shaking by hand for 3 min, centrifuge the tubes at 1000 g for 2 min, and pipette 8.0 ml of the benzene layer into a clean tube. Evaporate the benzene to dryness at reduced pressure, and flush the tube with a stream of dry nitrogen for 1–2 min to ensure complete removal of benzene. Add 5.0 ml of ethanol, and shake the tube for 3 min.

Place an aliquot of the ethanol solution in a 10-mm silica cuvette and read the fluorescence intensity with an excitation wavelength of 318 nm and an emission wavelength of 402 nm. As ergotamine is subject to photodecomposition by ultraviolet light, the fluorescence intensity is best read at constant tune (*e.g.* 10 s) after the initial exposure to the exciting light.

The sensitivity of the instrument was always adjusted so that a stock standard solution of 2-aminopyridine (10^{-5} *M* in 0.05 *M* sulphuric acid) gave a predetermined fluorescence intensity.

Concentrations of unknowns were determined by reference to a standard curve. The standard curve was constructed by assaying a series of 10 aqueous standards of ergotamine tartrate at concentrations between 0.1 and 5.0 μg ml^{-1} usually in triplicate, on three separate occasions. The collected data from all determinations were subjected to statistical analysis. The limit of detection of the assay was established by analysing standards between 0.001 and 0.005 μg ml^{-1}, and comparing these with assays of deionized water blanks.

Assay of tablets

Two commercial preparations containing ergotamine tartrate were extracted following the method of the United States Pharmacopeia (17th Ed., p. 236), and aliquots of the extracts were assayed following the above procedure.

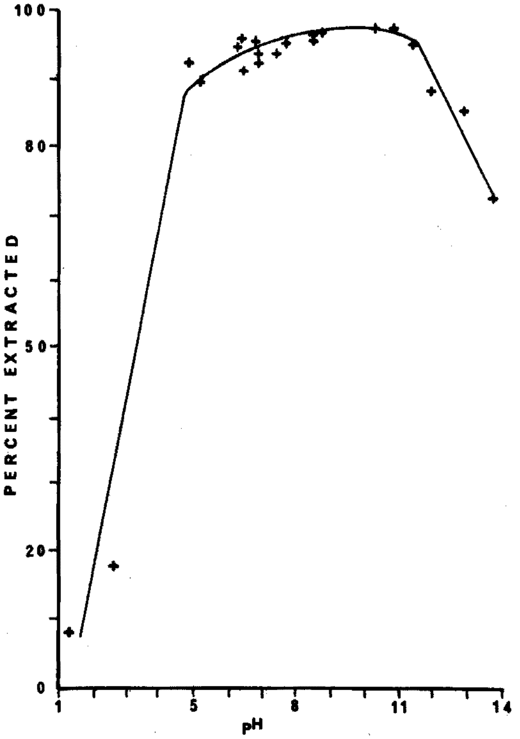
Replicate assays were performed twice on batches of 10 tablets, for each preparation. As one of the preparations contained caffeine citrate (100 mg/tablet), aqueous ergotamine tartrate samples (2 μg ml^{-1}) with and without added caffeine citrate (2 mg ml^{-1}) were assayed in order to detect any interference in the fluorescence intensity of the extracts.

RESULTS AND DISCUSSION

Benzene was the least polar of the solvents tested which gave virtually quantitative extraction of ergotamine from aqueous solutions. As drug metabolites are usually more polar than the parent substance[10], and as it was hoped to apply this assay to biological fluids, benzene appeared to be the solvent of choice, offering the best compromise between completeness and selectivity of extraction.

The effect of pH on the extraction of ergotamine from aqueous solutions into benzene is shown in Fig. 2. Virtually quantitative extraction was obtained in the pH range 8.5–11.0; the maximal percentage extraction was 98.8% at pH 10. Since many of the solutions which one might assay would be acidic extracts (*e.g.* of pharmaceutical preparations), a strong buffer of appropriate pH was chosen for the pH adjustment. Adjustment by other means (*e.g.* titration with sodium hydroxide) is essential if solutions in acids of normality above 0.01 are to be assayed.

The effect of pH on the emission spectrum of ergotamine is shown in Fig. 3. The finding of maximal fluorescence intensity at pH 10.8 is interesting in view of the published method for assaying lysergic acid diethylamide (containing a very similar chromophore) fluorimetrically in acidic solution[11]. No LSD was available to ascertain whether it has maximal fluorescence in acid or alkali. It is also noteworthy that Vining and Taber[12] have stated that the ergot alkaloids do not fluoresce in alkaline solution, presumably because they examined only solutions of high pH



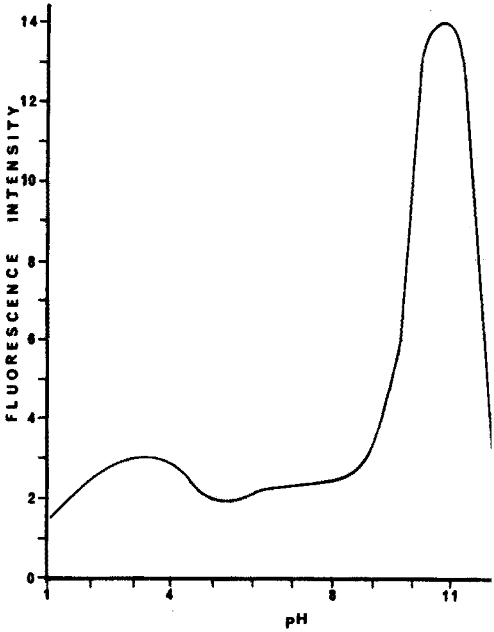


Fig. 2. Effect of pH on extraction of ergotamine from aqueous solution into benzene. Concentration of ergotamine tartrate 1.0 μg ml^{-1}. Relative volume of aqueous phase: benzene, 3:5.

Fig. 3. Effect of pH on the emission spectrum of ergotamine. λ_{ex} 325 nm, λ_{em} 435 nm. Concentration of ergotamine tartrate 1.0 μg ml^{-1}.

(although no actual data were stated). It is probable that the most intensely fluorescent species is an anion; the pK_a for protonation of the neutral molecule[13] is 6.25 which renders it unlikely that the neutral molecule causes the peak in Fig. 3, and a deprotonation in basic solution would not be surprising. That such an ionization may occur is supported by the observation that some of the ergotamine is not extracted by benzene at pH values above 11 (Fig. 2).

The excitation and emission spectra of ergotamine in water, at pH 2.1 and pH 10.8, and in ethanol are shown in Fig. 4. The original intention of measuring fluorescence intensity in water at pH 10.8 was discarded in favour of the simpler course of transferring the extracted ergotamine to alcoholic solution, with a resultant slight gain in sensitivity. However, it is important to remove all traces of benzene completely before addition of ethanol, as further tests confirmed the capacity of benzene to act as a quenching agent[14]. The anticipated hypsochromic shift[14] in moving from aqueous (λ_{ex} 325 nm, λ_{em} 435 nm) to ethanolic solution (λ_{ex} 318 nm, λ_{em} 402 nm) was observed. In fact, the fluorescence was examined in a total of 17 organic solvents and 4 mixtures of these solvents, but while the intensity in other short-chain alcohols was comparable to that in ethanol, only in propane-1,2-diol was any further significant enhancement of fluorescence obtained; this solvent was

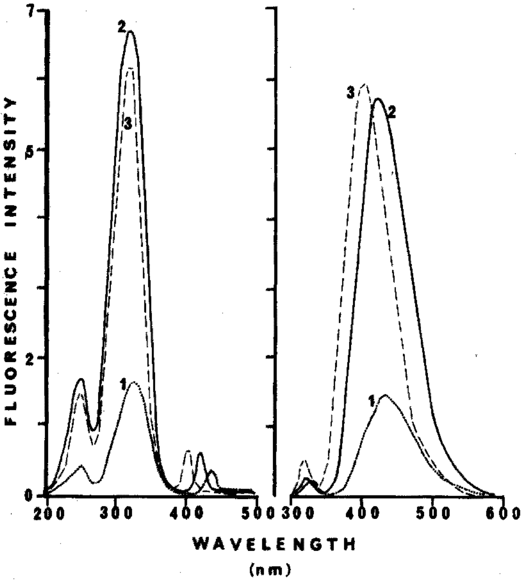


Fig. 4. Excitation spectra (left) of ergotamine in: (1) water at pH 2.1 (λ_{em} 435 nm); (2) water at pH 10.8 (λ_{em} 422 nm; (3) ethanol (λ_{em} 402 nm). Emission spectra (right) of ergotamine in: (1) water at pH 2.1 (λ_{ex} 325 nm); (2) water at 10.8 (λ_{ex} 318 nm); (3) ethanol (λ_{ex} 318 nm).

considered too oily for use in the assay.

A calibration curve was obtained by processing aqueous ergotamine standards. Statistical analysis of the data showed that the points were best fitted by a straight line having the equation

$$R = 5.201\ C + 0.313\ (r^2 = 0.998)$$

where R is the relative fluorescence intensity, C the concentration of ergotamine tartrate, and r^2 the coefficient of determination.

That such a good linear relationship was obtained from data obtained on three separate days was taken as evidence supporting the view that daily working standards are unnecessary. It is, of course, prudent to check the calibration periodically, particularly when new batches of reagents are used. The ability to work without daily standards adds greatly to the convenience of the procedure, but is only possible as a result of the use of 2-aminopyridine as a fluorescence standard[9], which enables compensation for day-to-day variations in the performance of the spectrophotofluorimeter.

Reproducibility of the method was established with replicate assays of a 2.5-μg ml^{-1} standard. Eight such analyses furnished a mean relative fluorescence intensity of 13.82 units, with a standard deviation of 0.27 units.

The limit of detection of the method was assessed as 0.002 μg ml^{-}; a concentration of 0.001 μg ml^{-1} could not be distinguished from the water blank when a 5.0-ml sample was assayed, and showed no peak at λ_{ex} 318 nm and λ_{em} 402 nm.

Studies on the stability of the fluorophore in both 0.01 M hydrochloric acid and ethanol revealed no detectable loss of fluorescence intensity after six weeks while less than 10% was lost after six months. These data are supported by previous work[15,16]

The results of an application of the assay are shown in Table I which summarizes the data obtained from analyses of tablets. Both preparations contained an average of 90% of the labeled content (1.0 mg). Because one of the preparations contained caffeine citrate (100 mg per tablet), the possibility of interference was investigated, but none was demonstrated.

TABLE I

DETERMINATION OF ERGOTAMINE TARTRATE IN PHARMACEUTICAL PREPARATIONS

	Mean % labelled content[a]	*High (% of labelled content)*	*Low (% of labelled content)*	*Standard deviation*
Product A	89.9	93.7	85.7	3.0
Product B	90.0	93.1	86.5	2.4

[a] Mean value of 8 determinations.

The assay possesses potentially higher selectivity than previous colorimetric or spectrophotometric techniques, although related ergot alkaloids (*e.g.* methysergide, λ_{ex} 325 nm, λ_{em} 410 nm in ethanol) may still interfere if present. In this case a chromatographic separation step would become necessary[17–19]

The range of concentrations used for construction of the standard curve was chosen to span the values encountered in the present work. However, it was confirmed that the linear relationship holds down to the limit of detection of the assay (while higher concentrations exceed the capability of the spectrophotofluorimeter).

SUMMARY

Studies on the fluorescence properties of ergotamine in water at various pH values, and in several organic solvents are described. An assay procedure for ergotamine, based on its intense fluorescence in ethanol, is presented. Extraction of ergotamine into benzene from basic aqueous solution is followed by transfer of the extract to ethanol for fluorescence determination. The plot of fluorescence intensity *vs.* concentration is linear up to 5 μg ml^{-1}, and the assay has a limit of detection of 0.002 μg ml^{-1}. Reproducibility data at the 2.5-μg ml^{-1} level are given.

RÉSUMÉ

Une étude est effectuée sur les propriétés de fluorescence de l'ergotamine, dans l'eau, à divers pH, et dans plusieurs solvants organiques. Une méthode est proposée, avec extraction de l'ergotamine dans le benzène, à partir de solutions aqueuses basiques, et transfert de l'extrat dans l'éthanol pour le dosage par fluorescence. La courbe de l'intensité de la fluorescence en fonction de la concentration est linéaire jusqu'à 5 μg ml^{-1}; la limite de détection est de 0.002 μg ml^{-1} On examine la reproductibilité pour 2.5 μg ml^{-1}.

ZUSAMMENFASSUNG

Untersuchungen der Fluoreszenzeigenschaften von Ergotamin in Wasser bei verschiedenen pH-Werten und in verschiedenen organischen Lösungsmitteln werden beschrieben. Ein auf der intensiven Fluoreszenz in Äthanol beruhendes Analysenverfahren für Ergotamin wird vorgeschlagen. Das Ergotamin wird aus basischer wässriger Lösung mit Benzol extrahiert, anschliessend wird der Extrakt für die Fluoreszenzmessung in Äthanol überführt. Die Auftragung der Fluoreszenzintensität gegen die Konzentration ist bis 5 μg ml^{-1} linear, und das Verfahren hat eine Nachweisgrenze von 0.002 μg ml^{-1}. Werte für die Reproduzierbarkeit bei 2.5 μg ml^{-1} werden vorgelegt.

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A COMPARISON OF TWO RAPID METHODS FOR THE ANALYSIS OF COPPER SMELTING SLAGS BY ATOMIC ABSORPTION SPECTROMETRY

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Over the last few years in this Department slag–matte and slag–metal systems in copper reverbatory smelting have been studied with a view to improving the overall recovery in the industrial production of copper from ores and concentrates. Recently, the pyrometallurgical research work has been extended to include the recovery of copper from secondary materials. As a consequence, the number of slag, matte and metal samples presented for analysis has increased considerably.

The conventional methods for the analysis of these slags are long and tedious. It was, therefore, decided to investigate procedures which could be used for the rapid analysis of typical slags, with a minimum loss in accuracy and precision. The chemical analysis of the smelting slags in this laboratory usually involves the determination of silica, alumina, copper, iron and, occasionally, metals such as lead, tin and zinc. The gravimetric determination of silica is time-consuming and can give erroneous results if the several dehydration stages are not carefully carried out.

Recently, several methods have been proposed for the rapid analysis of silicate materials by atomic-absorption spectrometry. The sample dissolution procedures used have been reviewed by Guest[1], and methods for the determination of silica in minerals, ores and electric furnace slags have been reported[2,3].

In this paper, a fusion method with anhydrous lithium metaborate followed by dissolution of the melt in dilute nitric acid[4–8] is compared with a method based on decomposition of the sample by hydrofluoric acid at 110° [9,10]. The decomposition is carried out in a closed vessel to prevent silica losses due to volatilisation. The accuracy and precision of the two methods were compared by carrying out replicate analyses of the British Chemical Standards Basic Slag No. 174/2, Iron Ore Sinter No. 303 and a typical laboratory slag sample. Results for several laboratory slag samples were compared with those obtained previously by classical methods.

EXPERIMENTAL

Apparatus

A Perkin-Elmer model 303 atomic-absorption spectrophotometer was used, with a Boling three-slot burner for the air–acetylene flame, and a standard nitrous oxide burner head for the silica and alumina determinations. The instrumental parameters are listed in Table I.

TABLE I

INSTRUMENTAL PARAMETERS USED

Element	*Wavelength (nm)*	*Oxidant*	*Slit width (mm)*
Si	252	N_2O	0.3
Al	309	N_2O	0.3
Cu	325	Air	1.0
Fe	248	Air	0.3
Zn	214	Air	3.0

For the lithium metaborate fusion technique, graphite crucibles were made from high-purity graphite rod (Cromil and Piercy, Newcastle-upon-Tyne). Some workers[7] have used platinum crucibles for this fusion but, in our experience, dissolution of the melt from a platinum crucible took a considerable time. Graphite crucibles proved to be more convenient to use, although it was necessary to filter particles of graphite from the sample solutions. Crucibles made of platinum–5% gold alloy were also tried but were found to be no more successful than the platinum crucibles. The decomposition vessels used for the hydrofluoric acid dissolution method were of the same pattern as those used by Bernas[9] (Uni-Seal Decomposition Vessels Ltd., P.O. Box 9463, Haifa, Israel).

Reagents

All reagents used were of analytical reagent grade.

Lithium Metaborate Fusion Method

Pre-ignite an empty graphite crucible for 30 min at 950° and allow to cool. Take care not to disturb the powdery inside surface. Mix the slag sample (100–200 mg of 200 mesh) with anhydrous lithium metaborate (1.0 g) and transfer to the pre-ignited graphite crucible. Fuse the mixture in a muffle furnace at 900° for 10 min. Pour the red hot melt into dilute nitric acid (60 ml of 1 *M* concentration) contained in a polypropylene beaker. Stir the solution for about 30 min to aid complete dissolution of the melt. For some slag samples with a high total metal content, it may be necessary to increase the acid concentration of the leaching solution to as much as 20% nitric acid to dissolve the sample completely. Dilute the solution to 100 ml and store in a plastic container. Use this stock sample solution for the determination of silica and alumina and make further dilutions for the determination of copper, iron and zinc.

Prepare calibration standards for silica and alumina by fusing 0.1 g of A.R. grade alumina or silica powder with 1.0 g of anhydrous lithium metaborate in the same manner as that used for the samples. Dilute the resulting solution (1000 μg ml^{-1}) to give the required working range while maintaining the concentrations of lithium metaborate and nitric acid at the same levels as those in the sample solutions. Prepare standard solutions of the other metals to contain the same amount of lithium metaborate and nitric acid as the corresponding sample solutions.

TABLE II

RESULTS OF REPLICATE ANALYSES BY THE HYDROFLUORIC ACID DECOMPOSITION METHOD

Sample	*Mean value*[a] *(%)*	*s*[b]	s_r[c] *(%)*	*Certificate value (%)*
B.C.S. Basic Slag no. 174/2				
SiO_2	11.3	0.159	1.41	11.2
Al_2O_3	0.67	0.028	4.28	0.77
Fe (total)	15.7	0.183	1.16	15.9
B.C.S. Iron Ore Sinter no. 303				
SiO_2	16.6	0.403	2.46	16.5
Al_2O_3	6.50	0.062	0.95	6.81
Fe (total)	36.1	0.477	1.32	36.0
Laboratory Slag no. 596				
Al	10.5	0.333	3.17	10.5[d]
Cu	1.16	0.008	0.70	1.18[d]
Fe	36.5	0.431	1.18	36.4[d]
Zn	2.34	0.036	1.54	2.37[d]

[a] 10 determinations were made for each sample.
[b] s denotes standard deviation.
[c] s_r denotes relative standard deviation.
[d] Results obtained by classical methods.

TABLE III

RESULTS OF REPLICATE ANALYSES BY THE LITHIUM METABORATE FUSION METHOD

Sample	*Mean value*[a] *(%)*	*s*[b]	s_r[c] *(%)*	*Certificate value (%)*
B.C.S. Basic Slag no. 174/2				
SiO_2	11.8	0.125	1.06	11.2
Al_2O_3	0.64	0.024	3.78	0.77
Fe (total)	16.4	0.237	1.44	15.9
B.C.S. Iron Ore Sinter no. 303				
SiO_2	16.4	0.389	2.37	16.5
Al_2O_3	6.95	0.166	2.38	6.81
Fe (total)	36.0	0.14	0.40	36.0
Laboratory Slag no. 596				
Al	11.0	0.418	3.79	10.5[d]
Cu	1.18	0.011	0.91	1.18[d]
Fe	36.2	0.362	1.0	36.4[d]
Zn	2.43	0.060	2.47	2.37[d]

[a–d] See footnotes to Table II.

Hydrofluoric Acid Decomposition Method (ref. 9)

Transfer the prepared sample (100–200 mg of 200 mesh) to a Teflon decomposition vessel. Add aqua regia (0.5–1.0 ml) as a wetting agent followed by hydrofluoric acid (3.0 ml of 48%). Close the vessel, place in an air drying oven at 110° for between 30 and 40 min, and then cool to room temperature. Transfer the decomposed sample to a plastic beaker with the aid of 4–6 ml of distilled water taking care to transfer quantitatively any precipitated metal fluorides which may have formed. Add boric acid (2.8 g) and stir the solution with a Teflon rod to aid dissolution. Dilute to about 40 ml with distilled water when a clear homogeneous sample solution should result. Transfer the solution to a volumetric flask and adjust to volume.

It is recommended that the sample solution should not remain in a glass container for longer than 2 h, and that sample solutions should be stored in plastic containers. Prepare silica calibration standards using the same decomposition technique as that used for the samples. Prepare calibration standards for the non-ferrous metals to contain the same quantity of fluoroboric–boric acid solution as the corresponding sample solutions.

RESULTS AND DISCUSSION

The accuracy and reproducibility of the two methods were obtained by carrying out ten replicate analyses of British Chemical Standards Basic Slag No. 174/2 and Iron Ore Sinter No. 303 and also of a typical copper smelting slag produced in the department. The results are shown in Tables II and III. Both methods exhibit an acceptable degree of accuracy with the exception of the determination of aluminium in Basic Slag 174/2, when both methods gave low results. The hydrofluoric acid decomposition method gave low aluminium results for both British Chemical Standard samples. This may have been caused by incomplete transfer of the fluoride precipitate from the Teflon vessel. The bulk of the precipitate was transferred readily, but a small, finely divided portion was found to adhere firmly to the walls of the Teflon vessel. Application of a Teflon stirring rod and boric acid solution to the walls of the Teflon vessel were found to have little effect. Saturated boric acid solution could not be reheated in the decomposition vessel because of the low capacity (23 ml) of the Bernas type of vessel which was used.

The absorption readings were taken directly from the % absorption meter on the Perkin-Elmer 303, and the precision figures given are a realistic estimate of those that would be obtained in general routine use of the methods.

During the routine analysis of laboratory slag samples by the hydrofluoric acid decomposition method, difficulty was again encountered with a few samples owing to the difficulty of transferring any precipitated fluorides quantitatively. The decomposition was repeated with 125-ml capacity polypropylene bottles as the decomposition vessel. This enabled saturated boric acid solution to be added to the solution resulting from the initial decomposition as recommended by Langmyhr and Paus[10]. After this solution had been reheated to dissolve the precipitated fluorides, a clear solution was formed in most cases. The use of polypropylene bottles gave rise to slightly higher alumina results for some, but not all, samples.

TABLE IV

A COMPARISON OF RESULTS OBTAINED FOR THE ROUTINE ANALYSIS OF SLAG SAMPLES

(Results obtained by: A, classical method; B, lithium metaborate method; C, hydrofluoric acid decomposition method)

Laboratory sample no.	*% Composition*														
	% SiO_2			*% Al_2O_3*			*% Cu*			*% Fe*			*% Zn*		
	A	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*
385	61.3	61.2	62.3	—	—	—	0.04	0.04	0.05	22.2	22.2	22.8	—	—	—
389	61.9	63.1	64.1	—	—	—	0.82	0.81	0.85	21.6	21.1	21.9	—	—	—
393	61.9	61.6	63.4	—	—	—	0.05	0.06	0.06	21.5	21.5	22.0	—	—	—
395	58.8	58.2	59.6	—	—	—	0.10	0.09	0.10	23.7	23.4	23.8	—	—	—
397	52.2	52.3	53.5	—	—	—	0.25	0.20	0.26	24.9	24.5	25.5	—	—	—
399	57.2	57.5	58.3	—	—	—	0.38	0.38	0.36	23.7	23.1	24.2	—	—	—
505	—	—	—	10.2	9.63	9.85	3.50	3.41	3.45	36.5	35.2	35.1	3.74	3.70	3.89
506	—	—	—	10.4	10.9	10.2	2.08	2.03	1.95	37.0	36.0	35.0	3.80	3.78	3.81
509	—	—	—	10.4	10.8	10.5	1.30	1.27	1.28	38.3	37.1	36.6	2.51	2.55	2.57
510	—	—	—	11.9	12.3	11.3	1.28	1.20	1.20	37.5	35.3	36.2	2.47	2.53	2.59
511	—	—	—	11.9	12.4	11.5	1.25	1.21	1.23	37.5	35.4	35.1	2.22	2.26	2.27
596	—	—	—	10.5	10.5	10.4	1.18	1.15	1.16	36.4	36.1	35.9	2.37	2.43	2.39

Laboratory slag sample No. 824 gave a result of 9.25±0.4% Al_2O_3 (mean of 5 determinations) when the Teflon decomposition vessel was used, and a result of 10.1±0.4% Al_2O_3 when a polypropylene bottle was used. However, there was no appreciable difference in the % Al_2O_3 result for B.C.S. Basic slag 174/2 when digested in both types of vessel. A thin film of precipitate could be detected on the sides of the polypropylene bottle after digestion with saturated boric acid solution. Polypropylene bottles are not ideal decomposition vessels because of the difficulty of maintaining a leak-proof seal at 110°. After being used for three digestions, the polypropylene bottles become brittle and have to be rejected.

Sample dissolution problems were only encountered with the lithium metaborate fusion method when samples with a high total metal content were analysed. This was easily overcome by increasing the acid content of the leach solution.

Several samples, chosen at random, were analysed in a routine manner by the lithium metaborate fusion and the hydrofluoric acid decomposition methods. The results obtained are shown in Table IV. It can be seen that the results compare well with those obtained previously by classical methods, particularly in the case of the silica determination where the atomic-absorption methods offer a major "analysis time" advantage over the gravimetric method previously used.

Conclusions

Both the lithium metaborate fusion and the hydrofluoric acid decomposition methods provide rapid alternative means for determining silica in slag samples. Analyses for metals such as copper, iron and zinc can be carried out by making dilutions of the original sample solution. If the hydrofluoric acid decomposition method is to be used, the larger volume type of decomposition vessel, *e.g.* that marketed by Perkin-Elmer which has a capacity of 110 ml is preferred in order to facilitate the dissolution of precipitated fluorides. However, in certain cases, it may be necessary to use the fusion method to ensure complete dissolution of the sample. The lithium metaborate fusion method was found to be more convenient to use for most samples with the exception of those with a high total metal content.

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SUMMARY

A fusion method with anhydrous lithium metaborate is compared with a hydrofluoric acid decomposition method for the analysis of copper smelting slags. Atomic-absorption spectrometry is used to determine silica, alumina, copper, zinc and iron in the resulting sample solutions. The accuracy and precision of both methods are found to be similar. The fusion method is more convenient for general routine use.

RÉSUMÉ

Une méthode de fusion avec métaborate de lithium anhydre est comparée avec une méthode de décomposition à l'acide fluorhydrique pour l'analyse du cuivre dans des scories. La spectrométrie par absorption atomique est utilisée pour le dosage de la silice, de l'alumine, du cuivre, du zinc et du fer dans les solutions obtenues. L'exactitude et la précision de ces deux méthodes sont similaires. La méthode par fusion convient mieux pour le travail de routine.

ZUSAMMENFASSUNG

Eine Methode des Schmelzaufschlusses mit wasserfreiem Lithiummetaborat und eine Methode der Zersetzung mit Fluorwasserstoffsäure für die Analyse kupferhaltiger Schlacken werden miteinander verglichen. Siliciumdioxid, Aluminiumoxid, Kupfer, Zink und Eisen werden in den resultierenden Probelösungen durch Atomabsorptionsspectrometrie bestimmt. Die Genauigkeit und die Reproduzierbarkeit beider Methoden sind ähnlich. Die Methode des Schmelzaufschlusses ist für allgemeine Routinezwecke bequemer.

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THE SEPARATION OF MERCURY FROM SEA WATER BY ADSORPTION COLLOID FLOTATION AND ANALYSIS BY FLAMELESS ATOMIC ABSORPTION

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The discovery of significant levels of mercury, a heavy metal toxic pollutant, in ocean-feeding fish and other marine organisms, and the realization[1] that dissolved ionic mercury in the oceans plays a fundamental role in the food chains, has stimulated efforts in the direction of improving the analytical methodology of mercury in natural waters.

Hosohara *et al.*[2], Leatherland and Burton[3], and Leatherland and McCartney[4] have resorted to spectrophotometric procedures by measuring the absorbance of the dithizone–mercury complex. Despite its high sensitivity, the dithizone method may not be suitable for serial routine analysis.

In an early study, Stock and Cucuel[5] coprecipitated mercury in sea water with copper(II) sulfide, then electrodeposited it on a copper wire, distilled into a capillary, and evaluated the mercury by measuring the size of the mercury globule. As pointed out by Manning[6], deposition methods require lengthy sample preparation times and cannot be compared favorably with current conventional flameless atomic absorption techniques. Non-flame methods appear to be uniquely suited to the determination of mercury and are now most commonly employed for this purpose.

Flameless atomic absorption can be used directly with natural waters without need of prior preconcentration provided that the mercury concentration is 0.2 μg l^{-1} or higher[7,8]. For waters containing less than 0.2 μg l^{-1} it is desirable to use a procedure which couples preconcentration with a method of determination such as flameless atomic absorption which is rapid, possesses high sensitivity and can be used, if possible, with small sample volumes.

Data on the concentration of mercury in sea water which is present normally in trace quantities are limited to comparatively few studies. Weiss *et al.*[9,10] in an analysis of water from the pier at the Scripps Institution of Oceanography at La Jolla, California reported 83.9±9 ng kg^{-1} and in a similar study of the waters off the coast of Mexico found a range of 22–173 ng of mercury kg^{-1} at a site 60 km from the coast and 12–27 ng kg^{-1} 150 km from shore. The mercury was preconcentrated on copper(II) sulfide, filtered with a millipore filter, and determined by neutron activation. The time required for the analyses was about one week.

Igoshin and Bugusevich[8], in their analysis of sea water, employed 500-ml samples in which the mercury was preconcentrated by coprecipitation with cadmium sulfide at the methyl orange end-point (pH 3.2–4.1). The mercury-enriched cadmium sulfide was filtered on a millipore filter and dissolved in aqua regia, and aliquots were analyzed by flameless atomic absorption. The sensitivity claimed was 0.02 $\mu g\ l^{-1}$. We have not been able to duplicate this work, recover such small amounts of mercury, and confirm the reported sensitivity. The failure was found to be due to the dependence of the recovery of mercury on pH. At a pH of 1.0 a sensitivity of 0.02 $\mu g\ l^{-1}$ was attained but at the methyl orange end-point the recoveries were low and erratic.

Recently, Topping and Pirie[11] reported mercury concentrations ranging from 8–55 ng l^{-1} for North Sea waters. Their ingenious preconcentration method consisted of reducing the mercury in a 4-l sample by the addition of tin(II) chloride and bubbling the reduced mercury from the sample into a test tube containing a potassium permanganate–sulfuric acid mixture. The solution was analyzed by flameless atomic absorption. The authors reported an enhanced sensitivity of 0.002 $\mu g\ l^{-1}$ for this method which can be used at sea. The large sample volume (4 l) used is a handicap.

In a recent series of papers Kim and Zeitlin[12–14] have shown that trace metals such as copper, zinc, molybdenum, and uranium, can be separated from sea water by an adsorption colloid flotation process which is based on a collector–surfactant–gas system. This technique eliminates filtration, is rapid (the separation requires 2–3 min) and readily adaptable as a preconcentration step before analysis by atomic absorption or spectrophotometric analysis, and the recoveries of model trace metals studied are excellent. Zinc and copper which exist, in part at least, as positively charged species were separated by the use of a positively charged iron(III) hydroxide collector at a basic pH, a negatively charged surfactant and air; whereas molybdenum and uranium present as anionic species are separable by negatively charged iron(III) hydroxide at an acid pH with a positively charged surfactant. In each case the trace species coprecipitated on the collector is floated to the surface by the surfactant and air as a foam from which it is easily removed and analyzed. Since ionic mercury is presumably present[15] in sea water as $HgCl_3^-$ and $HgCl_4^{2-}$, an investigation was initiated in order to determine whether coprecipitated dissolved ionic mercury could be separated from the sea water by adsorption colloid flotation, and determined by flameless atomic absorption.

EXPERIMENTAL

Apparatus and equipment

Atomic absorption measurements were made with a Perkin Elmer 303 atomic absorption spectrophotometer equipped with a mercury hollow-cathode lamp. The absorption cell constructed from ground glass tubing equipped with quartz windows, and associated items, were essentially similar to those described by Hatch and Ott[16] The flotation unit used was identical to that described by Kim and Zeitlin[12].

The following instrument settings were used: lamp current, 5 mA; wavelength 253.7 nm; slit setting, 3; recorder scale, 10; noise suppression, 4; speed 0.5 in. min^{-1}.

Standards and reagents

All chemicals were of analytical grade. Aqueous reagents were prepared in distilled deionized water.

Standard mercury stock solution, 0.100%. Dissolve 0.1354 g of mercury(II) chloride in 100 ml of 0.5 *M* sulfuric acid. Standards are prepared by appropriate dilution of stock solution.

Reagent purification. Since scale 10.0 was used, it was necessary to prepare reagents with low blank values. Two key reagents which possess high amounts of mercury are hydrochloric acid and tin(II) chloride. These may be purified by the method of Omang[17]. Before analysis, both the hydrochloric acid solution containing a small amount of tin(II) chloride and the tin(II) chloride solution were placed in separate BOD bottles, an air stream was passed through each for 5 min. and the mercury containing air stream was exhausted in a hood.

Owing to the relatively high oxidation potential of the nitric acid, which is used in the preparation of aqua regia, the above method of purification cannot be used, because the addition of tin(II) to nitric acid will not result in the reduction of mercury(II) to elemental mercury. The nitric acid was purified by distillation on a Vigreux column.

Stability of standards. Omang[17] used 1 ml of 9 *M* sulfuric acid and 0.5 ml of 2% potassium permanganate per 100 ml in order to preserve the standards. According to this worker, the stability of a 0.1 p.p.m. mercury solution as measured by a mercury-203 tracer remained constant for one week. Coyne and Collins[18], in a study of the stability of mercury solutions, concluded that acid potassium permangante is not a preservation technique which is commonly used or recommended by environmental testing laboratories. Notwithstanding, we have found that standard solutions containing 0.01, 0.1, and 1.0 p.p.m. of mercury treated with acid potassium permanganate as suggested by Omang have maintained their stability for a period of six months. The stability was assessed by the pipetting of measured quantities of mercury into the flameless atomic absorption apparatus which reproducibly resulted in a given and unchanged peak height within acceptable instrumental fluctuations for six months.

Sample treatment

Igoshin and Bogusevich[8] and Omang[17] have used a 24-h digestion period with permanganate–sulfuric acid to break down organic mercury before analysis. Kopp *et al.*[7] have reported that this treatment is not effective and cite the incomplete oxidation of phenylmercury(II) acetate and methylmercury(II) chloride. Incomplete oxidation should yield low results since not all the mercury would exist in the form of mercury(II) and not undergo reduction to the elemental state on addition of tin(II) chloride. In our work, sea-water samples were treated with permanganate–sulfuric acid for 24 h at room temperature before work-up and analysis.

Samples

Sea-water samples were obtained from a tide pool located on the tip of Black Point on the southern shore of the island of Oahu in the Hawaiian Island Chain. The water in the tide pool was being constantly replenished by breaking surf. On the day of sampling the winds in this area were typical north-east trades. These winds are cross-wind at Black Point with a slight offshore direction. The sewage outfall for the city of Honolulu is located downwind of Black Point and, when the trades blow, is not expected to pollute the waters of this area. Sea-water samples were also taken at various depths at a station located off the Waianae coast on the western shore of Oahu. Standards containing inorganic and organic mercury were supplied from the Federal Water Quality Administration.

Procedures

Inorganic mercury. Since inorganic mercury is in the form of mercury(II) ions which are readily coprecipitated by cadmium sulfide, samples need only to be acidified with 10 ml of concentrated hydrochloric acid. Analyses should be carried out within 24 h. Into the acidified sea water, pipette 5.5 ml of a cadmium sulfate solution (containing 9.64 g of $3\ CdSO_4 \cdot 8\ H_2O$ per 250 ml) and 10 ml of a freshly prepared solution of sodium sulfide (containing 3.91 g of sodium sulfide nonahydrate per 250 ml). If necessary, readjust the pH to 1.0. A blank is prepared with sea water from which all mercury had been previously removed by coprecipitation on cadmium sulfide and subsequent flotation. After several minutes, separate the samples one at a time by the flotation method. Pour the sample into a 500-ml flotation cell and adjust the gas flow (nitrogen) to 10 ± 2 ml min^{-1}. Inject the surfactant (2 ml of a solution of 0.05 g of octadecyltrimethylammonium chloride in 100 ml of ethanol) through the rubber serum and adjust the level of water in the cell to just below 1 mm of the level of the froth drain by allowing water to enter the cell from the reservoir. A complete separation is obtained within 1–2 min. Time of separation can be shortened or lengthened by increasing or decreasing the amount of surfactant or the nitrogen flow rate. Excessive flow rates should not be used, because they may result in a loss of mercury through breaking bubbles at the surface of the cell similar to the ejection of salts from the surface layer of the ocean into the marine atmosphere.

Remove the yellow froth from the top of the cell into a 150-ml beaker with a Teflon spatula. The amount of entrained water carried along in the removal process varies from 15 to 20 ml depending upon the care exercised by the analyst. Dissolve the froth in 10 ml of freshly prepared aqua regia. Slight heating may be necessary. No loss of mercury has been observed up to 50°. Elemental sulfur will form upon addition of the aqua regia and may be mistaken for undissolved cadmium sulfide; 10 ml of aqua regia, however, is sufficient to dissolve all cadmium sulfide. Dilute the solution to volume in a 50-ml volumetric flask.

Pipette a 5.0-ml aliquot from the volumetric flask directly into a 50-ml flask adapted for the flameless atomic absorption apparatus. Add 20 ml of tap water and 2 ml of a freshly prepared 5% (w/v) tin(II) chloride solution, and connect the flask to the rest of the apparatus. Set the pump to speed 4 or 5 and record the absorption peak within 1 min, after which the mercury vapor is flushed from the system into a hood ventilator.

Before the sample solution, which is now mercury-free, is discarded, pipette carefully measured amounts of a standard solution into the flask, *i.e.* 0.1 ml of 0.01 p.p.m. standard gives 0.001 μg Hg. The concentration range of standards used was 0.001–0.010 μg. The addition of such small volumes to the sample does not cause a change in peak height. Connect the flask to the apparatus and record the absorbance of each standard. (If this scheme of running a sample followed by a standard run in the sample matrix succeeded by another sample, etc., is used, it is possible to carry out duplicate analyses of 5 samples in the concentration range of 0.02–0.10 μg l^{-1} in about 1 h.) Convert the measured peak values for the samples and standards to absorbance values. Since any mercury present in the reagents is ejected from the system before the standards are pipetted into the flask, the absorbance value of the blank is only subtracted from the sample absorbance values, not the absorbance values of the standards.

Organic mercury. Pipette 10 ml of 0.5 *M* sulfuric acid and 5 ml of 2% potassium permanganate into each 500-ml sea-water sample, and allow to stand for 24 h[8,17]. Then add 2–3 drops of 10% (w/v) hydroxyammonium chloride solution to reduce permanganate. After the sample has become clear, pipette in the cadmium sulfate and sodium sulfide solutions and complete the analysis, as described above for inorganic mercury. The method employing persulfate as an oxidant in addition to permanganate[7] as suggested by the Federal Water Quality Administration (F.W.Q.A.) was not used, because high results were obtained with F.W.Q.A. standards containing organic mercurials.

RESULTS AND DISCUSSION

As evidenced by the recovery of added inorganic mercury and analysis of F.W.Q.A. standards (Tables I and II), adsorption colloid flotation coupled with flameless atomic absorption is effective for the preconcentration and analysis of inorganic mercury. Analysis of standards yielded a linear working plot in conformity with Beer's law. In the preconcentration step involving cadmium sulfide, the pH of 1.0 is crucial, because use of the methyl orange end-point suggested by Igoshin and Bugusevich resulted in low values (Table III). The separation by flotation is rapid and can be applied routinely. With some practice it was possible to carry out 25 separations in about 2 h.

TABLE I

RECOVERY DATA FOR 0.01 μg OF INORGANIC MERCURY ADDED TO 0.5 l OF FILTERED SEA WATER

Sample	*Absorbance*[a]	*μg Hg/0.5 l*
A	0.0089	0.013
B	0.0089	0.013
C	0.0089	0.013
D	0.0089	0.013
E	0.0112	0.017
F	0.0112	0.017

$\bar{x} = 0.014$ μg l^{-1}
s = 0.002 μg 0.5 l^{-1}[b]
$s_r = 14.3\%$[c]
Range = 0.004 μg

[a] Corrected for absorbance of blank = 0.0088.
[b] s for 1 l sea water would be 0.004 μg l^{-1}, twice the value for 0.5 l.
[c] s_r = relative standard deviation.

TABLE II

ANALYSIS OF F.W.Q.A. INORGANIC MERCURY STANDARDS IN SEA WATER

Standard	*Absorbance*[a]	*μg Hg l⁻¹ expected*	*μg Hg l⁻¹ found*
1	0.1495[b] (scale 10.0)	0.34	0.34
2	0.0556[c] (scale 10.0)	4.2	4.2

[a] Corrected for blank.
[b] Average of three samples having absorbance values of 0.1516, 0.1516, and 0.1452.
[c] Average of three samples having absorbance values of 0.0556, 0.0556, and 0.0554.

TABLE III

RECOVERY FROM SAMPLES TO WHICH INORGANIC MERCURY HAD BEEN ADDED[a]

Sample	*Absorbance of sample*	*Absorbance of standards*	*% Recovery*
1 (0.05 μg)	0.2441	0.2924	83.5
2 (0.04 μg)	0.1938	0.2218	87.4
3 (0.03 μg)	0.1427	0.1549	92.1
4 (0.02 μg)	0.0862	0.0969	88.9

[a] Coprecipitation was achieved using cadmium sulfide at the methyl orange end-point. % recovery data are based on the absorbance of mercury standards.

The recovery of our standards containing inorganic mercury and those of F.W.Q.A. was virtually quantitative (Tables I and II). In reproducibility and sensitivity studies, 500-ml sea-water samples were spiked with 0.01 μg of inorganic mercury and together with a sea-water blank were analyzed for inorganic mercury. The results given in Table I indicate a mercury concentration of 0.014 ± 0.002 μg per 500 ml.

Sea-water samples collected at Black Point, Oahu, on analysis for inorganic mercury showed a range of 0.038–0.078 ± 0.020 μg l^{-1}. The Black Point water when analyzed for total mercury gave concentrations close to those for inorganic mercury indicating absence of measurable organic mercury.

Sea-water samples collected off the Waianae coast at various depths were analyzed for total mercury. The data generally showed a decrease in mercury with depth. The mercury concentrations above 200 m ranged from 0.109 to 0.224 μg l^{-1}, while the deeper waters ranged from 0.012 to 0.091 μg l^{-1}. Further increase in mercury in the deepest water mass (below 1000 m) was noted but poor precision in the analysis precludes definite conclusions.

In two separate papers, Hosohara *et al.*[2] and Hosohara[19] report inorganic mercury concentrations ranging from 0.08 to 0.15 μg l^{-1} in surface waters and increasing to 0.15–0.27 μg l^{-1} at depths to 3000 m, thus showing a definite increase with depth. Hosohara explained the low mercury concentrations in the surface waters in terms of adsorptive uptake by plankton. Beneath the photic zone, inorganic mercury would be expected to increase.

Our data for total mercury reveal an opposite trend since a maximum concentration was found in the surface zone which may be attributed to the combination of mercury dissolved in sea water plus mercury removed from plankton by the permanganate–sulfuric acid pretreatment and released to the sea water. Beneath the photic zone the mercury levels are lower, possibly because of absence of planktonic species at these depths. This interpretation, however, is a tentative one and before more definite conclusions may be drawn, a detailed and systematic program of analysis for mercury should be undertaken with emphasis on the distribution of the organic and inorganic forms in sea water and increase in precision.

We would like to express our gratitude to the crew of the research vessel TERITU for their assistance and to the Federal Water Quality Administration for supplying standards.

SUMMARY

Adsorption colloid flotation has been found capable of separating ionic mercury from sea water quantitatively at levels as low as 0.02 μg l^{-1} with use of a cadmium sulfide collector and octadecyltrimethylammonium chloride as the surfactant. The mercury in 25 samples can be separated in 2 h. Following the separation the mercury was analyzed by flameless atomic absorption. Recovery of mercury from 0.5 l samples spiked with 0.010 μg of inorganic mercury gave 0.014 ± 0.002 μg/0.5 l. Black Point, Oahu near-shore sea water was found to contain mercury in the range 0.038–0.078 μg l^{-1} with no measurable organic mercury fraction. Sea-water samples collected at an open ocean station analyzed for total mercury revealed the highest mercury concentrations above 200 meters. Mercury concentrations in general showed a decreasing trend with increase in depth.

RÉSUMÉ

La flottation par adsorption colloïdale permet de séparer quantitativement le mercure ionique dans l'eau de mer, jusqu'à une limite inférieure de 0.02 μg l^{-1}· on utilise le sulfure de cadmium comme collecteur et le chlorure d'octadécyltriméthylammonium comme surfactant. On peut effectuer cette séparation du mercure dans 25 échantillons en 2 heures. Le mercure est ensuite dosé par absorption atomique sans flamme. En général les concentrations en mercure ont tendance à diminuer lorsque le profondeur de la mer augmente. Des échantillons d'eau préléves dans l'océan présentent les teneurs les plus élevées en mercure au-dessus de 200 mètres de profondeur.

ZUSAMMENFASSUNG

Für die quantitative Abtrennung ionischen Quecksilbers von Meerwasser bei so niedrigen Gehalten wie 0.02 μg l^{-1} eignet sich die Adsorptions-Kolloid-Flotation unter Verwendung von Cadmiumsulfid als Sammler und Octadecyltrimethylammoniumchlorid als Schäumer. Das Quecksilber in 25 Proben kann in

2 h abgetrennt werden. Nach der Abtrennung wurde das Quecksilber durch flammenlose Atomabsorption bestimmt. In Proben von 0.5 l, die mit je 0.010 μg anorganischen Quecksilbers versetzt worden waren, wurden Quecksilbermengen von 0.014±0.002 μg/0.5 l wiedergefunden. Küstennahes Meerwasser (Black Point, Oahu) enthielt Quecksilber im Bereich 0.038–0.078 μg l^{-1} ohne messbaren Anteil organischen Quecksilbers. Im offenen Ozean gesammelte Meerwasserproben zeigten die höchsten Konzentrationen an Gesamt-Quecksilber oberhalb 200 m. Die Quecksilberkonzentrationen nahmen im allgemeinen mit zunehender Tiefe ab.

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THE DETERMINATION OF COPPER IN SEA WATER BY ATOMIC ABSORPTION SPECTROMETRY WITH A GRAPHITE ATOMIZER AFTER ELUTION FROM CHITOSAN

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Chitosan is a polymer obtained from marine food canning wastes. As many applications of chitosan are foreseen, a production plant has recently been put in operation[1]. The chelating ability of chitosan has been demonstrated and it was found suitable for metal ion collection in the prevention and monitoring of inland and sea-water pollution[2].

Among transition metal ions, copper can be easily collected from sea water by chitosan. The chemical forms of copper in sea water have recently been studied by polarography, and it was concluded[3] that copper is mainly present in sea water as Cu^{2+}, $CuCl^{+}$ and $[Cu(HCO_3)\cdot 2OH]^{-}$. However, little information is available on organic complexes in sea water, and on colloidal forms of copper; most of the analytical methods for the determination of copper in sea water include the addition of acids in order to destroy any organic complexes[4].

Pulse polarography has recently been applied[5–7] for the determination of copper and some other metals. Atomic fluorescence has been coupled to solvent extraction[8], and neutron activation analysis to co-crystallization[9].

A study of the significance of certain physical and chemical forms of copper, manganese and zinc in waters from the Gulf of Mexico suggested that a major portion of these elements exists in the divalent ionic form or in weakly-bound complexes. However, a significant difference was observed between the concentrations determined with and without oxidation with peroxydisulphuric acid, and this indicates that a substantial amount of the metals might exist as organic complexes in sea water[10]. In this study[10], copper in sea water was determined by extraction with diethyldithiocarbamate in chloroform, and neutron activation analysis; the extraction results were compared to results obtained after co-precipitation with iron(III) hydroxide, and it was shown that the co-precipitation method yielded lower results. Copper in surface waters from the Gulf of Mexico was found[10] to occur in the range 0.36–4.20 $\mu g\ l^{-1}$.

Studies on the isotope exchange of ^{54}Mn, ^{60}Co and ^{65}Zn showed that spikes do not go into equilibrium with the natural forms of these elements in sea water for many hours[11]. Research on model systems seems to indicate that amino acids can play a role in the complexation of metal ions[12].

Copper can also be extracted from sea water with ammonium pyrrolidinedithiocarbamate in ethyl acetate. The organic solution was sprayed into a flame for atomic absorption spectrometry: the copper concentration in Belfast Lough was found[13] to be 1.85 μg l^{-1}.

A method based on the spectrometric determination of copper in sea water with 2,9-dimethyl-1,10-phenanthroline has also been developed[14]; it was claimed that the determination was possible on sea water with no preliminary separation. The average particulate copper content was 1.13 ± 0.09 μg l^{-1} and the average soluble copper was 6.10 ± 0.86 μg l^{-1} in Florida waters.

Copper has also been determined in the Italian seas[15]. Its concentration in the Adriatic sea was found to be 6.10 μg l^{-1} in the upper 50 m, while below 200 m it increased to 6.48 μg l^{-1}; in the Ligurian sea the average concentration of copper was 4.8 μg l^{-1}, ranging between 1.0 and 12.7 μg l^{-1} in surface waters. These results were obtained by dithizone extraction and spectrophotometry[16,17].

The separation of copper from sea water has been easily achieved with a synthetic chelating polymer, Dowex A-100 (also known as Dowex A-1 or Chelex) in conjunction with atomic absorption spectrometry on the nitric acid eluates[20–22] The copper concentrations in the deep waters of the tropical Atlantic Ocean were in the range 0.4–12.3 μg l^{-1} with a mean value of 1.0 μg l^{-1}.

For the present research, chitosan was selected, as it chelates copper much more effectively than the other soluble chelating agents so far used for the extraction of copper from sea water, and it is even superior to the Dowex A-100 resin because of its negligible sensitivity to magnesium salts and absence of swelling.

EXPERIMENTAL

Columns

Chitosan was supplied by the Food, Chemical & Research Laboratories (4900 Ninth Ave. N.W., Seattle, Wash., U.S.A.). The chitosan columns were 30 × 3 mm and were filled with 100 mg of 100–200 mesh chitosan.

For the phenanthroline procedure, a preliminary washing was done with 30 ml of a 1% solution of 1,10-phenanthroline, ethanol and water. For the alternative sulphuric acid procedure, a preliminary washing was done with 30 ml of 1 *M* sulphuric acid, water, 20 ml of 0.1 *M* sodium hydroxide solution and water, repeated four times. These treatments allowed removal of traces of copper from the polymer.

Through these columns 1 l of sea water was passed, and then, after rinsing with 15 ml of distilled water, copper was eluted with four 5-ml fractions of the phenanthroline or sulphuric acid solutions, respectively.

The Dowex A-100 columns were 60 × 12 mm and were washed with 2 *M* nitric acid; copper was eluted with six 5-ml fractions of 2 *M* nitric acid.

Analyses carried out on the polymers showed that traces of copper remained after the preliminary washings, and it seems that they cannot be removed by any treatment.

Instrumentation

A Perkin–Elmer 305 atomic-absorption spectrometer equipped with a hot graphite atomizer HGA-70, and a Perkin–Elmer deuterium arc background corrector was used with a Hitachi Perkin–Elmer 56 recorder. The solutions under examination (20 μl) were introduced with an Eppendorf pipette. The background correction is not strictly necessary for routine determinations but it was used for the assessment of the method, in order to ensure that the readings were not altered by smokes.

The HGA-70 programme was as follows: programme 7 with 20-s drying, 20-s charring and 10-s atomization; voltage 9 V.

Sea water

The surface sea-water samples were collected a few km offshore of Capo Conero, Ancona, Italy, and filtered on Millipore 0.45 μm membranes on a Teflon support.

RESULTS AND DISCUSSION

The data presented in Table I show that chitosan is the most suitable

TABLE I

PERCENTAGE COLLECTION OF ELEMENTS ON CHITOSAN AND CELLULOSES

(200 mg of polymer with 50 ml of 0.44 m*M* solutions at initial pH 2.5 and 6.0. Data for 0.01 *M* EDTA solutions are also included)

pH		*Time (h)*	*Ni*	*Cu*	*Zn*
Chitosan					
2.5	alone	1	28	2	12
		12	60	94	46
	EDTA	1	1	2	11
6.0	alone	1	72	86	71
		12	95	100	89
	EDTA	1	15	0	0
Diethylaminoethylcellulose					
2.5	alone	1	4	3	0
		12	30	31	10
	EDTA	1	10	0	17
6.0	alone	1	27	81	86
		12	26	86	50
	EDTA	1	14	0	0
p-Aminobenzylcellulose					
2.5	alone	1	0	0	0
		12	0	0	0
	EDTA	1	0	0	0
6.0	alone	1	0	12	16
		12	2	15	20
	EDTA	1	10	0	0

polymer, among those with the anhydroglucose skeleton, for the collection of copper; this is probably due to its higher nitrogen content in the form of free amino groups.

An eluent for copper should fulfil several requirements: it should be sufficiently free of copper to yield a very small background signal on atomic absorption analysis as a solution at the desired concentration; it should be effective enough to take the copper out of chitosan in a narrow chromatographic band; it should be thermally destroyed during the "charring" step and should not attack or degrade the polymer.

The data for several complexing agents in ethanol are reported in Table II. 1,10-Phenanthroline as a 1% solution was tested and found quite suitable for elution when applied at 50° [23]. The disodium salt of EDTA can elute copper even more easily as can ethylenediamine, but they are not suitable because 0.1 *M* EDTA contains so much copper that it prevents the determination, while ethylenediamine slightly attacks the polymer. Dimethylglyoxime is also a good eluting agent, but its copper content prevents its use; it should be noted that dimethylglyoxime does not elute nickel completely.

TABLE II

DETERMINATION OF COPPER AND NICKEL IN ELUATES FROM CHITOSAN COLUMNS

(Reference solutions: 0.2 p.p.m. Cu = 100 abs. u.; 0.4 p.p.m. Ni = 100 abs. u. Unless otherwise mentioned, elution was done at 20°)

Eluent	*Reading (abs. %)*	*Copper elution yield (%)*	*Reading (abs. %)*	*Nickel elution yield (%)*
Ethanol	0	0	0	0
Methanol	3	0	2	0
8-Quinolinol (0.1%)	3	0	15	0
Bipyridine (0.1%)	0	0	12	0
Dimethylglyoxime (1%)	15	100	2	partial
1,10-Phenanthroline (0.1%)[a]	0	partial	0	partial
1,10-Phenanthroline (1%)[a]	0	100	0	80
Sulphuric acid (1*M*)	0	100	0	100

[a] Elution at 50°.

Aliquots (50 ml) of a 3% sodium chloride solution with 1 μg of copper added were passed through columns containing 0.5 g of chitosan. The elution was performed with four 5-ml portions of 1,10-phenanthroline solution at 50°. Repeated analyses indicated a yield between 91 and 100%, averaging 96%. Actual readings for the 0.2-p.p.m. reference solution and the four elution fractions are shown in Fig. 1.

For the sea-water analyses, smaller columns containing 100 mg of chitosan only were used, as their capacity was expected to be large enough for 1-l samples. The linearity between the volume of sea water and the readings was verified.

In practice, sea-water samples of 500 ml are large enough for routine determinations. The readings for such conditions were similar to those reported in Fig. 1.

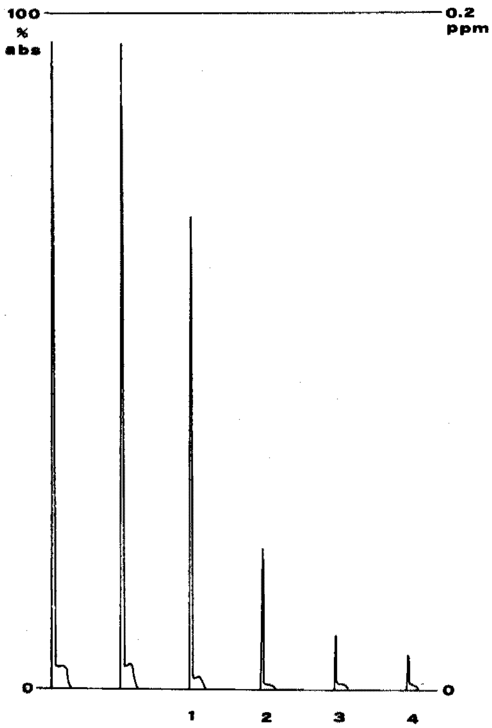

Fig. 1. Readings for copper on 0.2-p.p.m. solution and on four 5-ml fractions of a 1% solution of 1,10-phenanthroline after elution of copper from a chitosan column at 50°. The recordings refer to the 10-s hot graphite atomization period with deuterium background correction.

When 1-l fractions of a single sea-water sample were analysed by collection of copper on chitosan and elution with 1,10-phenanthroline solution, the reproducibility was good. For 10 separate determinations, the average value was found to be 3.39 μg Cu l^{-1} with a standard deviation of ±0.25 μg Cu l^{-1} Of course the results refer to certain chemical forms of copper only.

As copper in sea water occurs also in other complex forms which may escape fixation on the polymer because of their stability, some measurements were made on the sea water after treatment with persulphate by the procedure of Slowey and Hood[10]; this oxidative treatment destroys the organic matter which complexes part of the copper present in sea water, and thus makes it available for subsequent interaction with the chelating polymer. When this procedure was applied to the above sea-water sample, the average value of 10 determinations was 6.06 μg Cu l^{-1}, the standard deviation being 0.56 μg Cu l^{-1}. Copper could also be eluted from a chitosan column with 1 *M* sulphuric acid at room temperature. Preliminary investigations under the conditions described above gave an overall yield of 100%. A comparison of the methods is presented in Table III, which includes also the method of Riley and Taylor[20].

It should be emphasized that Dowex A-100 collects only a part of the copper present in untreated sea water; the average result obtained in this work was 1.2 μg Cu l^{-1}, while for persulphate-treated sea water, the average result was

TABLE III

COMPARISON OF METHODS FOR THE DETERMINATION OF COPPER IN SEA WATER

	Method of Riley and Taylor (ref. 20)	*Proposed methods*	
Polymer	Dowex A-100, 50–100 mesh	Chitosan, 100–200 mesh	Chitosan 100–200 mesh
Column size (mm)	60 × 12	15 × 3	15 × 3
Sea-water vol. (ml)	1000	1000	1000
Washing (ml)	250	15	15
Eluent	30 ml of 2M HNO_3	20 ml of 1% 1,10-phenanthroline	20 ml of 1 M H_2SO_4
Elution temp.	room	50°	room
Instrumental technique	Atomic absorption spectrometry[a]	Hot graphite atomic absorption spectrometry	

After evaporation of the eluate to dryness and dissolution in nitric acid–acetone.

6.00 μg Cu l^{-1}. The reagent solutions were copper-free and blank analyses indicated no copper contamination. The results obtained with chitosan correspond to those obtained with Dowex A-100 only when persulphate-treated sea water is used.

CONCLUSIONS

The natural chelating polymer chitosan collects about one-half of the copper naturally occurring in untreated Millipore-filtered sea-water. The synthetic chelating resin Dowex A-100 collects about one-fifth of the copper present in the same water. The total quantity of copper can be collected, after oxidative destruction of its organic complexes, by both polymers.

Attention should be paid to this finding, as the literature relevant to Dowex A-100 gives the impression that this resin can collect the total copper present in the sea water as such, whereas in practice it collects only a minor part of the total. In fact, a demonstration that Dowex A-100 collects copper from sea water with a yield of 100% has never been given, but it has been implied that such a yield was obtained. Therefore, the determination of copper by means of chitosan or Dowex A-100 should be carried out on persulphate-treated water.

The elution of copper from chitosan can be performed with 1,10-phenanthroline; other organic chelating agents tried do not give complete elution of copper, because of the very strong fixation of copper on chitosan. Another approach to elution is the protonation of the amino groups of chitosan, with complete release of the chelated copper.

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SUMMARY

Copper in sea water was determined by passing 1 l of persulphate-pretreated sea water through a 30 × 3 mm chitosan column, and eluting with 20 ml of a 1% solution of 1,10-phenanthroline, or with 20 ml of 1 *M* sulphuric acid. Copper was determined in the eluates by hot graphite atomic absorption spectrometry. The result for Adriatic sea water (Ancona, Italy) was 6.06 ± 0.56 μg Cu l^{-1}. Diethylaminoethylcellulose, *p*-aminobenzylcellulose and Dowex A-100 were also tested; Dowex A-100 collects only a minor part of the copper present in sea water.

RÉSUMÉ

Le dosage du cuivre dans l'eau de mer a été effectué après passage d'un litre d'eau traitée au persulfate sur colonne de chitosane de 30 × 3 mm, et élution avec 20 ml de solution à 1% de 1,10-phénanthroline ou avec 20 ml d'acide sulfurique. La technique analytique est basée sur la spectrométrie d'absorption atomique, avec atomisation sur graphite et correction du fond a l'aide du compensateur au deutérium. Le résultat obtenu avec l'eau de l'Adriatique (prélevée à Ancone, Italie) est de 6.06 ± 0.56 μg Cu l^{-1}, en accord avec les résultats d'autres auteurs. Des essais ont été également effectués sur diéthylaminoéthylcellulose, *p*-aminobenzylcellulose et sur résine Dowex A-100 (connue aussi comme A-1 ou Chelex); on a constaté que cette dernière ne peut fixer qu'une portion limitée du cuivre présent dans l'eau de mer.

ZUSAMMENFASSUNG

Kupfer in Meerwasser wurde bestimmt, indem 1 l des mit Persulfat vorbehandelten Meerwassers durch eine Chitosan-Säule von 30 × 3 mm gegeben und der von der Säule zurückgehaltene Anteil mit 20 ml 1%-iger 1,10-Phenanthrolin-Lösung oder mit 20 ml 1 *M* Schwefelsäure eluiert wurde. Das Kupfer wurde in den Eluaten mittels Atomabsorptionsspektrometrie unter Verwendung heissen Graphits bestimmt. Das Ergebnis bei adriatischem Meerwasser (Ancona, Italien) war 6.06 ± 0.56 μg Cu l^{-1}. Diäthylaminoäthylcellulose, *p*-Aminobenzylcellulose und Dowex A-100 wurden ebenfalls geprüft; Dowex A-100 hält nur einen kleineren Teil des in Meerwasser vorhandenen Kupfers zurück.

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A PRECISE METHOD FOR THE GENERATION OF STANDARD METAL SALT PARTICULATES

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A method has been developed for the production of standard dusts. The dusts are suitable for calibration and verification of methods to be used for the analysis of hazardous airborne particulates. They are also suitable as references for dust stain index measurements.

Health hazards caused by air pollutants can be classified under two broad categories, namely, acute toxins and cumulative toxins. Acute toxins are generally gaseous materials such as hydrogen sulfide, sulfur dioxide, ozone and oxides of nitrogen. They are readily obtainable in pure form and standard atmospheres of the respective pollutants can be produced either through various dilution techniques or by the use of permeation tubes. Cumulative and additive toxins consist mainly of particulate species and include most of the carcinogens and suspected carcinogens. Studies of particulate pollutants have always suffered because of the difficulty involved in the production of standard samples. A standard "Arizona Road Dust" is commercially available for reference as a standard dust stain index. Several devices for dispersing dusts have been described[1], but they tend to be expensive, cumbersome to use and unreliable. A method for preparing standard metal-containing dusts for use as a reference material is, therefore, badly needed by scientists working in the fields of industrial hygiene, toxicology and air pollution.

A simple method has now been developed which permits the production of standard dusts. These dusts can be made up to contain any desired concentration of any given metal oxide or any combination of metal oxides. Any desired ratio of concentrations of different pollutant species can be produced in nanogram to milligram ranges. The standard particulate samples may be collected by means of sequential tape samplers. The collected particulates are obtained in such form that they are readily processed by standard analytical techniques such as the ring oven method, atomic absorption spectrometry, emission spectrometry or neutron activation.

The metal particulates encountered in the ambient atmospheres are generally formed as a result of some incineration or combustion process involving these metals. Logically, therefore, if materials containing the metals of interest are burned under controlled conditions, one should be able to generate metal particulates at a desired mass rate.

DESIGN OF DUST GENERATOR

A dust generator has been designed which consists of an atomizer burner of the type used in commercial flame photometers. The burner is mounted at the base of a miniature Pyrex glass stack into which is inserted a sampling probe made of Pyrex glass. The tip of the sampling probe is ground to a sharp edge to minimize the deflection of particulates. Tygon tubing is used to connect the sampling probe to a tape sampler, a wet test meter and a vacuum pump. A schematic diagram of the generator is given in Fig. 1.

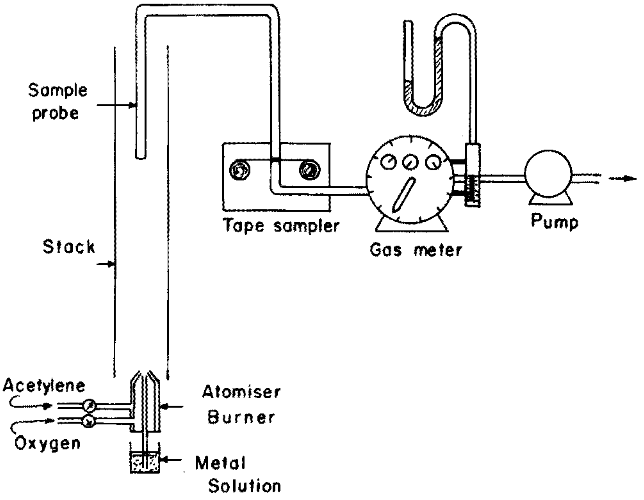


Fig. 1. Standard dust generator.

The atomizer burner is operated with acetylene as the fuel and oxygen as the oxidant. A dilute solution (10–100 p.p.m.) of the desired metal or mixture of metal salts is aspirated into the flame at a steady rate. When the metal solution, in the form of a fine mist, comes into contact with the flame, the solvent is evaporated and the metal salt residue disintegrates and is atomized. A major portion of the metal population in the flame is present as neutral ground-state atoms. A very small portion of the metal atoms are energized to higher excited states and a few atoms are even ionized, depending on the metal and its ionization potential. However, when these metal atoms are discharged from the flame to relatively cooler environments, because of the natural reactivity of the neutral atoms they react with the excess oxygen to form their respective oxides. The oxides thus produced collide with one another and agglomerate to form particulates which generally have an average size range of 0.1 to 10 μm. The system equilibrates within a few minutes after the burner is lit and aspiration is begun. The flow rates of oxygen and acetylene can be controlled very precisely, which in turn controls the aspiration rate of the solution and the formation rate of the particulates. The particulates thus produced move up the stack at a constant velocity once the steady state is reached.

The flue velocity can be determined with a standard pitot tube and a differential pressure manometer gauge. Depending on the flue velocity, the dimensions of the stack and the sampling probe, and the location and temperature of the sampling site, the conditions for isokinetic sampling can be established for the production of representative reproducible samples.

EXPERIMENTAL

Apparatus and Materials

Atomizer-Burner. Beckman Flame photometer burner assembly together with an oxygen and acetylene regulator.

Pyrex Stack. Length 60 in., i.d. 3.75 in.

Pyrex Sampling Probe. I.d. 5/16 in. with tip ground to a sharp edge.

Tape Sampler. Model 23000, Gelman Instrument Company, Ann Arbor, Michigan.

Wet-Test Meter. E. H. Sargent and Company, Chicago, Illinois.

Filter Tapes. Whatman No. 41, 1-in. width.

Tygon Tubing. I.d. 0.25 in.

Reagents

Stock metal solutions containing 100 μg ml^{-1} were used.

Mixed Ligand Extractant. 0.1 g of dithizone, 0.75 g of 8-quinolinol and 20 ml of acetyacetone were dissolved and made up to 100 ml with ethyl propionate.

Procedure

The atomizer burner was operated by feeding oxygen at 10 pounds in.$^{-2}$ and acetylene at 0.5 pounds in.$^{-2}$. This gave an aspiration rate of 3 ml min^{-1} for aqueous solutions. The sampling probe was located in the center of the stack, 4 ft. above the top of the burner. The sampling rate was fixed at 0.5 cf min^{-1}. The temperature at the sampling site was constant at 114° once the steady state had been attained. The above experimental conditions were maintained throughout the studies described below.

OBSERVATIONS AND DISCUSSION

The feasibility of generation of metal oxide particulates was first studied by aspirating a 100 μg ml^{-1} solution of lead into the flame. Samples were collected on Whatman 41 filter tape for 5, 10 and 15 min. A blank run was made by aspirating deionized water under the same conditions as those used with the lead solution. It is interesting to note that by aspirating water alone, a distinct dust stain was obtained, whereas a sample collected without aspiration and only the flame burning did not produce any detectable stain. This indicated that without aspiration, the fuel was completely oxidized to carbon dioxide and water. The dust obtained when only water was aspirated was probably due to the incomplete combustion of acetylene caused by the lowering of the flame temperature.

The amount of lead oxide particulates collected was determined by digesting the sample spots with nitric acid, filtering, making to a volume of 10 ml, and aspirating the solution into an air–acetylene flame of an atomic absorption spectrometer (Perkin–Elmer Model 403). The mass of lead present in each spot was determined by reference to a standard calibration curve. The results are shown in Table I.

TABLE I

LEAD CONCENTRATION AS A FUNCTION OF TIME

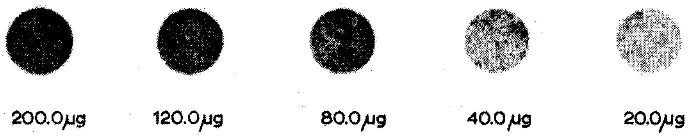
Sampling time (min)	*Lead found (μg)*
5	25.0
10	50.0
15	74.0

TABLE II

GENERATION OF MIXED METAL PARTICULATES

Sampling time (min)	*Metals found (μg)*		
	Cu	*Ni*	*Co*
5	25.0	24.0	24.0
7	37.0	36.5	36.4
10	55.0	54.0	52.0

Further, a solution containing a mixture of copper, nickel and cobalt ions, each at a concentration of 100 μg ml^{-1}, was aspirated into the atomizer-burner under the same conditions as above. Samples were collected for 5, 7, and 10 min, and the amount of each metal was determined as described above. The results are given in Table II.



To study the reproducibility of the production of particulates, a 10 μg ml^{-1} zinc solution was aspirated under the same conditions as above, and 32 samples were collected consecutively, each for 6 min. Ten samples were taken at random and the amount of zinc was determined by the mixed ligand extraction method[2]. The sample spots were cut from the tape and placed in 150-ml beakers, and 2 ml of 15% (w/v) ammonium acetate solution were added followed by 10 ml of mixed ligand. The samples were allowed to sit for 15 min with occasional agitation. The contents of the beaker were transferred to 25-ml test tubes, and water was added to raise the level of organic layer to the mouth of the test tube. The organic phase was aspirated into an air–acetylene flame of an atomic absorption spectrometer, and the absorbances were recorded. A calibration curve was

TABLE III

REPRODUCIBILITY OF ZINC PARTICULATE SAMPLES

Sample	*μg of Zn*	*Sample*	*μg of Zn*
Zn-1	3.5	Zn-20	3.6
Zn-2	3.5.	Zn-23	3.1
Zn-9	3.3	Zn-24	3.7
Zn-10	3.5	Zn-30	3.3
Zn-17	3.4	Zn-31	3.3
	Mean value in μg of Zn, 3.4±0.1		

prepared by extracting known amounts of standard zinc as described above and measuring the absorbances. The results are shown in Table III.

To study the effect of concentration of the metal solution aspirated on the amount of particulates collected, zinc solutions having concentrations of 10, 20, 40, 60, 80 and 100 μg ml^{-1} were aspirated and dust samples were collected for 10 min. The amount of zinc in the spots was determined by the mixed ligand method. The results showed an essentially linear relationship between the concentration of the aspirated zinc solution and the total amount of zinc collected in 10 min. The amounts collected were 5, 10, 19, 30, 38 and 48 μg respectively for the above 6 solutions.

CONCLUSION

A simple, stable and versatile dust generator has been designed. The generator may be used to produce a single metal oxide species, or a mixture of metals may be converted to their respective oxides to produce a complex dust having any desired inter-metal ratio. The dust generated simulates the metal oxide dusts present in the ambient atmosphere both in composition and size. All of the preliminary studies of the particle size of the dust generated indicate that they fall in the size range of 0.1 μm to 5 μm, which includes the size range of respirable dusts.

As mentioned above, the metal oxides collected are always accompanied by a certain amount of soot. Since both the amount of soot and the amount of metal oxides collected can be very precisely controlled, the use of this technique can be visualized for standardizing and calibrating the dust stain index. Preliminary studies with metal solutions in solvents other than water, indicate that metal oxides can be collected without the accompanying soot. Studies are in progress for the determination and the control of the particle sizes of the dust generated. The particulates generated are most probably in the form of oxides, because of the high temperature of the flame and ready availability of oxygen. By using flames of lower temperature, it may be possible to generate particulates in different atmospheres such as hydrogen sulfide, chlorine or bromine, to produce desired species of the metals. Further development of this technique should have potential in toxicological, industrial hygiene and air pollution studies.

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SUMMARY

A convenient precise method for producing standard dusts has been developed. Metal-containing particulate samples are generated by aspirating solutions containing metals of interest into an atomizer-burner employing an oxygen–acetylene flame. The particulates thus generated are collected by means of a miniature stack by conventional isokinetic sampling techniques. The particulate samples are deposited on filter tapes and are suitable for calibrating trace analytical methods. Particulate samples containing one or more metals, in any desired ratios of their concentrations, can be generated. Initial studies of particulate sizes indicate that the size range generated is in the respirable range, *i.e.*, 0.1–5 μm.

RÉSUMÉ

Une méthode précise est proposée pour la préparation d'étalons de poussières. On procède par aspiration de solutions renfermant les métaux à doser dans un buileur-atomiseur à flamme oxygène–acétylène. Les particules sont recueillies selon les techniques conventionnelles d'échantillonnage isocinétique. Les échantillons, déposés sur filtre, sont utilisés comme étalons pour l'analyse de traces. Les dimensions des particules doivent correspondre aux grandeurs respirables, soit 0.1 à 5 μm.

ZUSAMMENFASSUNG

Es wurde eine bequeme genaue Methode für die Herstellung von Standard-Stäuben entwickelt. Aus metallhaltigen Teilchen bestehende Proben werden erhalten, indem Lösungen des betreffenden Metalls in einen Atomisierungsbrenner unter Verwendung einer Sauerstoff–Acetylen–Flamme gesaugt werden. Die so erzeugten Teilchen werden mittels eines kleinen Schachtes nach konventionellen isokinetischen Verfahren der Probenahme gesammelt. Die Teilchen der Proben werden auf Filterstreifen abgeschieden und eignen sich für die Eichung spurenanalytischer Methoden. Es können teilchenhaltige Proben hergestellt werden, die ein oder mehrere Metalle in jedem gewünschten Konzentrationsverhältnis enthalten. Erste Untersuchungen der Teilchengrössen weisen darauf hin, dass diese in dem atemfähigen Bereich, d.h. 0.1–5 μm, liegen.

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THE ATOMIZATION OF METAL CHELATES IN CHLORINATED ORGANIC SOLVENTS IN FLAME SPECTROMETRY

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The role of organic reagents and solvents in flame spectrometry has been recognized for a long time, and their use is interesting from the analytical point of view[1–4]. Enhancement of the absorption or emission of light produces an increased sensitivity of measurement, and extraction of metal chelates can be used to concentrate a particular element and separate it from interfering elements.

The effects on spraying organic compounds and solvents into the flame have been explained on the basis of the physical properties of the solvents[5–7], changes in the flame temperature[8,9], reduction properties caused by higher concentrations of carbon[9,10] and, particularly, the different mechanisms of atomization of metal chelates when compared with organic compounds[11,12].

As yet, no available theory can explain the experimental facts entirely, for it is very difficult to establish the actual states of elements in flames; the phenomena in the flame interfere, their kinetics are little known, and it is difficult to speak about chemical equilibria. It is practically impossible to observe the individual phenomena influencing the atomization without partial interference from others. This report details some results of a study of the influence of organic solvents, with special emphasis on chlorinated hydrocarbons, on the atomization of metal-organic compounds.

EXPERIMENTAL

Apparatus

A Jarrell-Ash absorption and emission flame spectrometer with a 0.5-m Ebert monochromator, curved slits and a photomultiplier (Hamamatsu R 213), was used to measure the light absorption.

Atomization was achieved with a turbulent hydrogen–air flame; the flow of hydrogen was 10 l min^{-1} and that of air 7 l min^{-1}. The original Jarrell Ash "Hetco" total-consumption burner was used.

Westinghouse hollow-cathode lamps for copper and magnesium served as the spectral source; they were fed by a direct current of 10 mA for copper and 5 mA for magnesium. The absorption lines Cu 327.7 nm and Mg 285.2 nm were measured. Light was interrupted by means of a mechanical chopper at 9000 cycles min^{-1}.

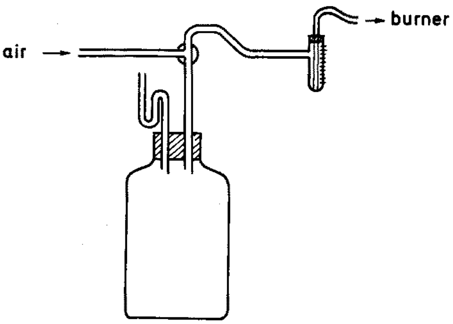

Fig. 1. The arrangement for regulating the flow rate of the solution.

The arrangement used to regulate the flow rate of the solution through the burner is shown in Fig. 1. It is composed of a large glass vessel, a manometer, a glass stopper, and a measuring tube containing the solution to be sprayed into the flame. The flow rate of the solution was regulated by changing the overpressure in the graduated tube and measured by observing the level of the solution in the tube.

Reagents

All the reagents were of analytical grade. The standard copper and magnesium solutions were prepared from N.B.S. organometallic substances. For the copper solution, bis (1-phenyl-1,3-butanedione)-copper(II) was used. It was dissolved, as recommended by the producer, so that the final concentration was 5 μg Cu ml^{-1} The magnesium solution was prepared by dissolving magnesium cyclohexanebutyrate (2 μg Mg ml^{-1}). Copper tetramethylene- and diethyldithiocarbamate, cupferrate and oxinate were prepared by precipitation from aqueous solutions[13]; they were then dissolved in an appropriate solvent.

Procedure

The standard solutions of copper and magnesium were measured and added to the organic solvent, a mixture of benzene and carbon tetrachloride. The prepared solution was then sprayed into the flame. Absorption was measured at different flow rates and heights in the flame.

RESULTS AND DISCUSSION

In a series of preliminary experiments, solutions of copper tetramethylenedithiocarbamate (TMDTC), diethyldithiocarbamate (DDTC), cupferrate and oxinate were sprayed into the flame. The results obtained (Fig. 2) show that the organic ligands used do not greatly affect the extent of absorption, which proves that the composition of the organic part of a metal chelate influences the physical and thermal properties of the solution and flame only to a small extent. This is understandable, for the quantities of the metal chelate in the solution are very small. The role of the organic ligands in metal-organic complexes appears primarily in the different mechanism of atomization when compared with inorganic compounds and their aqueous solutions; it depends very little on the type of ligand.

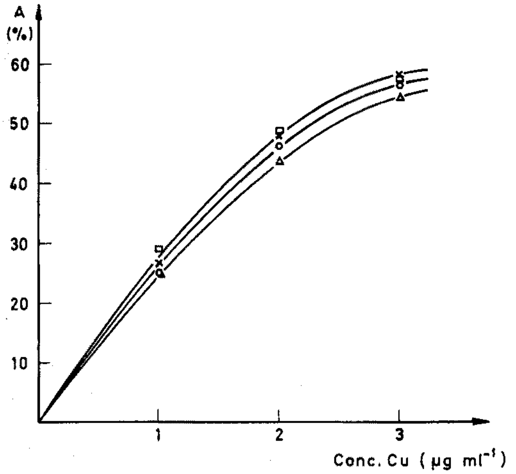

Fig. 2. The influence of organic ligands on the absorption of copper. Solvent, ethyl acetate. (×) TMDTC; (□) DDTC; (○) cupferrate; (△) oxine.

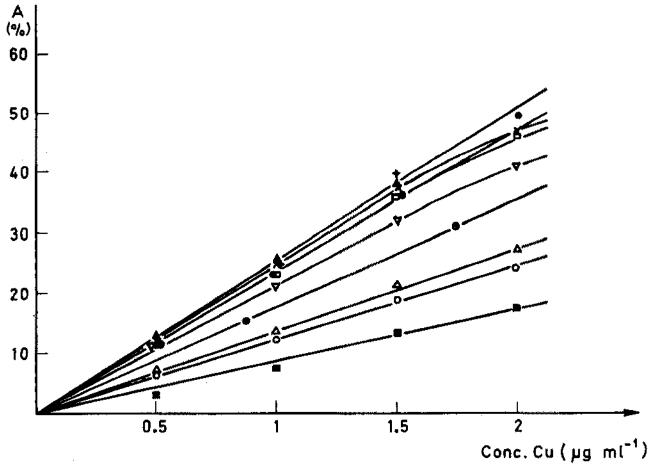

Fig. 3. The influence of organic solvents on the absorption of copper as its TMDTC chelate. (+) Ethyl acetate; (▲) acetylacetone; (×) benzene; (□) xylene; (●) MIBK; (V) amyl acetate; (⊙) water; (△) chloroform; (○) carbon tetrachloride; (■) cyclohexanone.

The situation is quite different when the same metal chelate dissolved in different organic solvents is sprayed into the flame. Figure 3 shows the absorbance *vs.* concentration curves obtained when copper tetramethylenedithiocarbamate, dissolved in different solvents, was sprayed. The absorption of the solutions of copper-TMDTC in ethyl acetate, acetylacetone, benzene, xylene, methyl isobutyl ketone and amyl acetate, when compared with that of aqueous solutions of copper(II) ions, is higher, whereas chlorinated hydrocarbons as solvents cause lower absorption. Organic solvents can therefore be divided, according to their behaviour in the flame, into two groups. The first is constituted by aliphatic and aromatic hydrocarbons, and the second by chlorinated hydrocarbons. Cyclohexanone may be

TABLE I

PROPERTIES OF SOME ORGANIC SOLVENTS

Solvent	*Mol. wt.*	*Density*	*B.p. (°)*	*Heat of combustion (kcal mol^{-1})*	*Viscosity at 20° (cp)*	*Surface tension at 20° (dynes cm^{-1})*
Benzene, C_6H_6[a]	78.11	0.879	80.1^{760}	782.3	0.65	28.9
Toluene, $C_6H_5CH_3$[a]	92.13	0.867	110.6	934.2	0.59	28.5
Cyclohexanone, $C_6H_{10}O$[a–c]	98.14	0.998	155.7		$2.30^{17.3°}$	
Chlorobenzene, C_6H_5Cl[a–c]	112.56	1.106	132	735.8	0.80	33.2
o-Xylene, $C_6H_4(CH_3)_2$[a]	106.16	0.897	144	1091.7	0.81	30.1
1,3-Dichlorobenzene, $C_6H_4Cl_2$[a–c]	147.01	1.288	172	671.8	1.39	$41.6^{-22°}$ $28.6^{+90.7°}$
Ethyl acetate, $CH_3COOC_2H_5$[a]	88.11	0.901	77.1	536.9	0.46	23.9
Amyl acetate, $CH_3COOC_5H_{11}$[a,c]	130.18	0.874	124–4.5	1042.5	0.90	24.7
Acetylacetone, $CH_3COCH_2COCH_3$[a,c]	100.11	0.972	139^{746}	615.9		31.2
Methyl isobutyl ketone, $(CH_3)_2CHCH_2COCH_3$[a]	100.16	0.801	116.9		0.58	22.7
Chloroform, $CHCl_3$[a]	119.38	1.492	61.2	89.2	0.58	27.1
Carbon tetrachloride, CCl_4[a]	153.82	1.594	76.8^{760}	37.3	0.97	27.0
Water[a]	18.02	1.00	100		1.00	72.8

[a] See ref. 14. [b] See ref. 15. [c] See ref. 16.

excluded from this comparison although it could represent a less interesting group of solvents of high viscosity. In Table I, some properties of the organic solvents used are summarized.

A comparison of the observed sensitivities with the viscosities of individual solvents might give a simplified explanation; the effects shown in Fig. 3 might be ascribed to the different viscosities and the resultant flow rates of solvents when sprayed into the flame, or to the possible influence of these solvents on the temperature of the flame. In order to exclude the effects of viscosity and flow rate on the extent of absorption, the apparatus was modified so that it was possible to observe or control the flow rate of the solvent through the capillary of the burner.

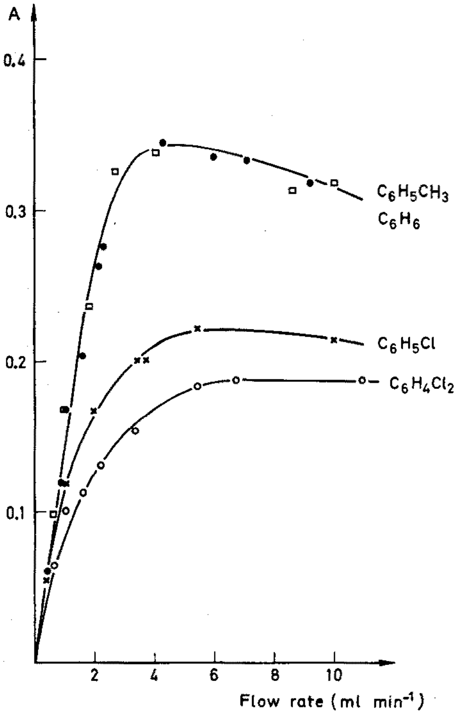

Fig. 4. The influence of organic solvents on the absorption of magnesium (2 μg ml^{-1}) at controlled flow rates of solution.

Figure 4 gives the absorbance *versus* flow rate curves for solutions of magnesium (magnesium cyclohexanebutyrate) which were obtained by controlling the flow rate of four solvents. It can be seen that the absorbance is exactly the same at every flow rate for benzene and toluene, but that it is considerably lower and also different for chlorinated hydrocarbons. On the basis of these results, it was concluded that chlorine in the chlorinated organic solvents has a special role in atomization. Further experiments were therefore directed towards a study of the atomization of metal-organic compounds dissolved in chlorinated hydrocarbons, related to the amount of chlorine.

Model solutions were prepared so that different quantities of benzene and carbon tetrachloride were mixed together and copper bis(1-phenyl-1,3-butanedione) or magnesium cyclohexanebutyrate were then dissolved in them.

Figure 5(a) shows the dependence of the absorption for copper (5 μg Cu ml^{-1}) on the flow rate of such solutions. The absorption was measured at two flame heights, 2 and 5 cm above the top of the burner. Figure 5(b) shows the corresponding curves for magnesium. The curves demonstrate that the light absorption depends on the quantity of chlorine in the solvent. The influence of chlorine is even more evident on Fig. 6(a), which shows the curves for the absorption of copper (5 μg Cu ml^{-1}) in relation to the quantity of chlorine in the solvent for the flow rates 2.5 and 4 ml min^{-1}, and for flame heights of 2 and 5 cm. The increased quantity of chlorine in the solvent at first reduces the absorption but after the absorption has reached a minimum, it increases again, reaching the starting value at 4.0 g-mol chlorine. These differences are even better expressed when the observation height is increased to 5 cm. The regularities are the same for the flow of 2.5 and 4 ml min^{-1}.

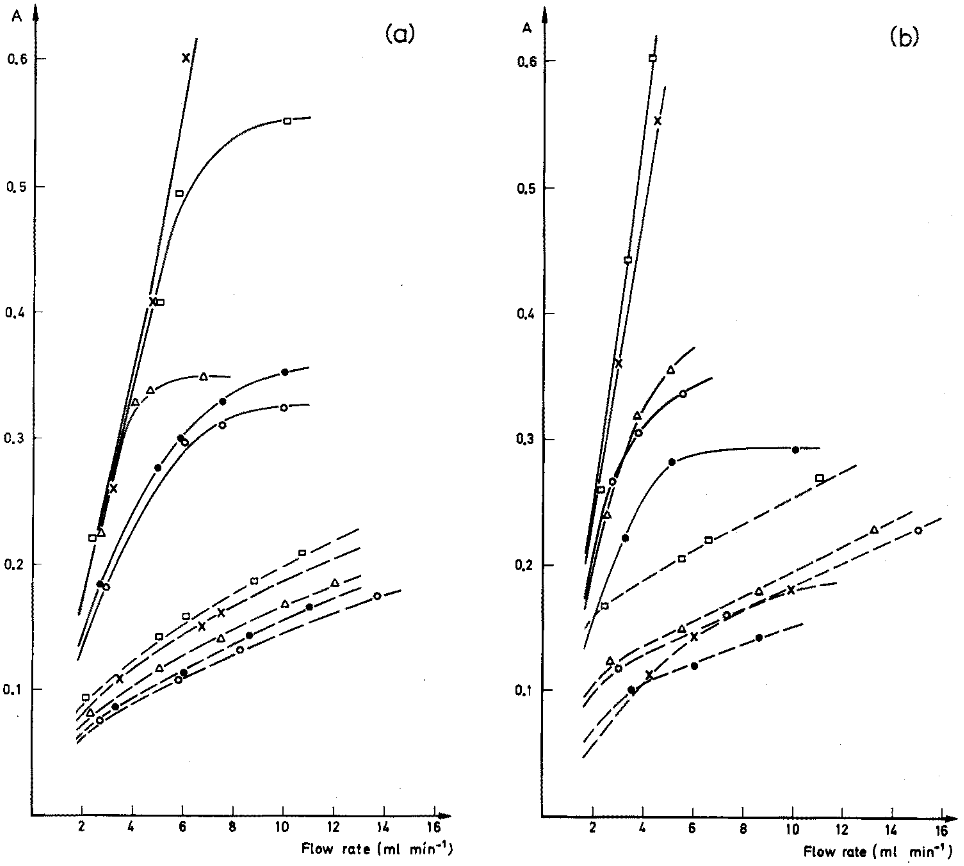

Fig. 5. The absorption signal for copper at 5 μg ml^{-1} (a) and magnesium at 2 μg ml^{-1} (b) at different flow rates of solution in dependence on the amount of chlorine. (×) 0 *n* (C_6H_6); (●) 1.0 *n* (C_6H_6–CCl_4); 2.0 *n* (C_6H_6–CCl_4); (△) 3.0 *n* (C_6H_6–CCl_4); (□) 4.0 *n* (CCl_4), where *n* = g mol Cl/100 ml. (——) *h* = 5 cm; (---) *h* = 2 cm.

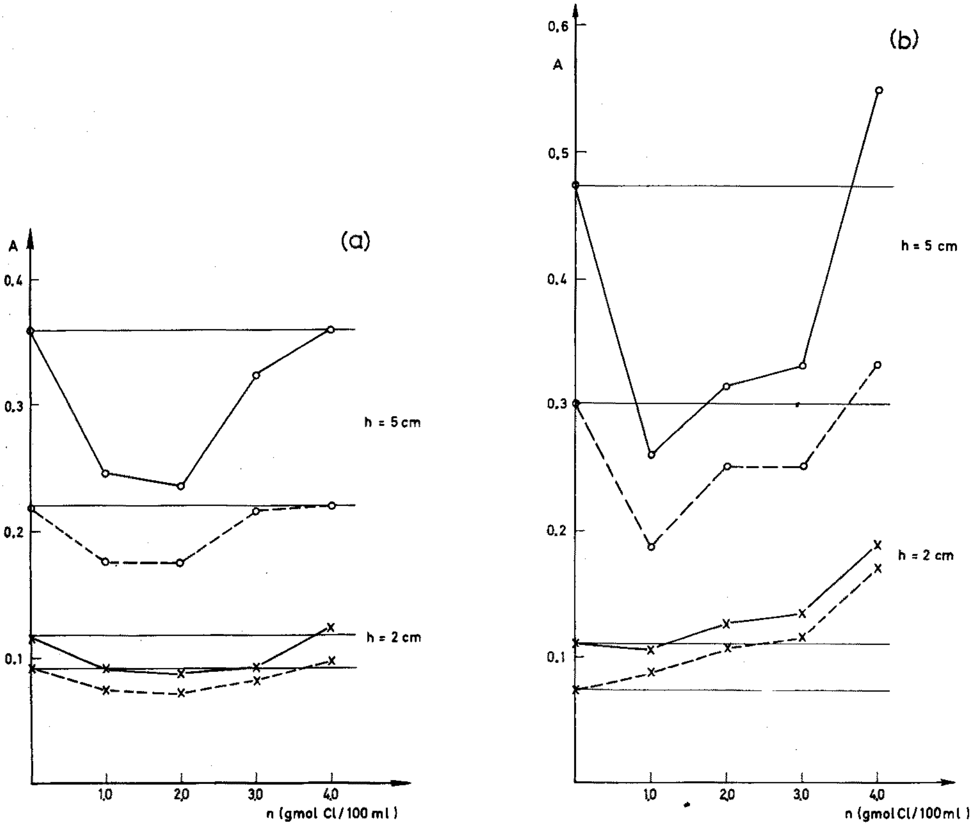

Fig. 6. The absorption signals for copper at 5 $\mu g\ ml^{-1}$ (a) and magnesium at 2 $\mu g\ ml^{-1}$ (b) in relation to the amount of chlorine in the organic solvent. Observation height 2 and 5 cm above the burner tip.

Similar regularities were also found in the atomization of magnesium solutions for an observation height of 5 cm; at the lower height of 2 cm, the absorption increased as the quantity of chlorine in the solvent increased (Fig. 6b).

Figure 7 shows the absorption for copper and magnesium solutions as a function of the height of observation in the flame. The results obtained show that, in the flame, chlorine and its compounds play an important role in the atomization of metal chelates which have been dissolved in such solvents. The influence of chlorine can be easily observed for copper and magnesium, although there are certain differences in the behaviour of these two elements, the effects being more clearly expressed for magnesium. The differences in absorption, depending on the quantity of chlorine, are greater at greater observation heights, *i.e.* when the species has been in the flame longer. All these facts show that increased quantities of chlorine or its compounds shift the chemical equilibria in the flame towards the formation of more volatile compounds. The differences between copper and magnesium simply confirm this, for it is characteristic of magnesium that it forms undissociated compounds in the flame, and the influence of chlorine is therefore greater than for copper. The initial decrease in the absorp-

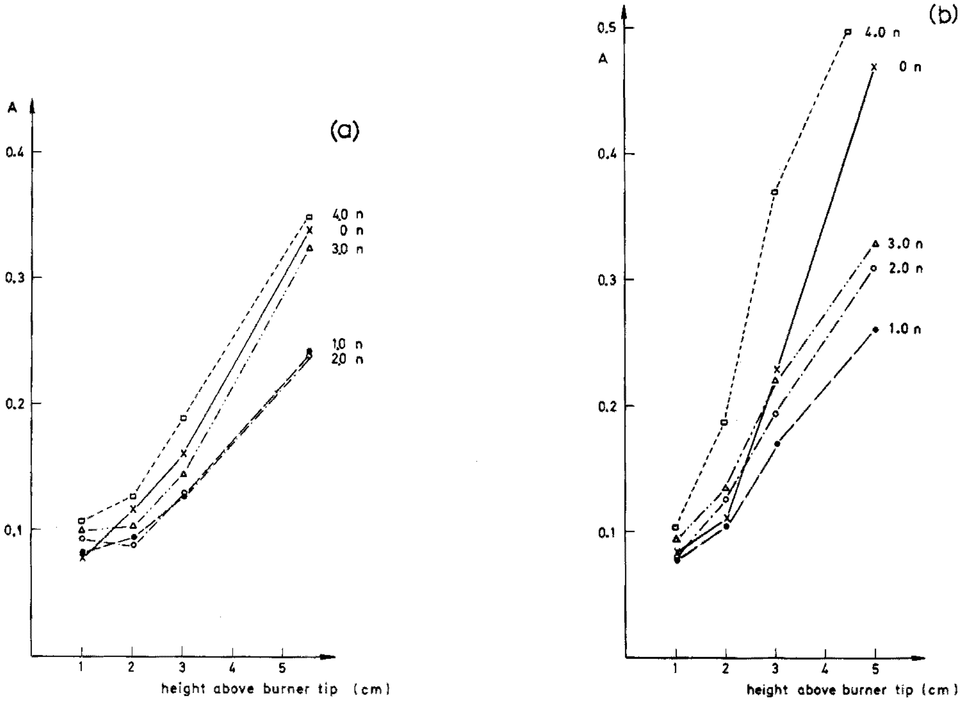

Fig. 7. The height profiles for organic solutions of copper at 5 μg ml^{-1} (a) and magnesium aT 2 μg ml^{-1} (b). Flow rate, 4 ml min^{-1}; Oil, 0, 1, 2, 3 and 4 g/mol of chlorine in 100 ml of solvent (n=g mol Cl/100 ml).

tion at lower concentrations of chlorine could be explained by the cooling of the flame, and consequently the lower degree of atomization of the individual compounds or elements.

Quantitative explanations of these phenomena are not simple. Too little is known about the distribution and size of the particles, and the forms in which copper and magnesium appear in the flame are not well defined. It would also be necessary to take into account the geometry of the flame and to measure its temperature. However, these observations are very interesting, for no similar data have appeared in the literature, and the phenomena deserve further study.

SUMMARY

A study of the influence of organic solvents, particularly chlorinated hydrocarbons, on the atomization of metal-organic compounds is described. The results obtained show that chlorine and its compounds play in the flame an important role in the atomization of metal chelates. Increased amounts of chlorine in the solvent first reduce the absorption, which reaches a minimum, but then increases again, reaching the starting value at a defined amount of chlorine.

RÉSUMÉ

Une étude est effectuée sur l'influence de solvants organiques, en particulier les hydrocarbures chlorés, sur l'atomisation de composés organo–metalliques. Les résultats obtenus montrent que la chlore et ses composés jouent un rôle important dans la flamme, lors de l'atomisation des chélates métalliques. Des quantités croissantes de chlore dans le solvent réduisent l'absorption, atteignant un minimum, puis ensuite l'augmentent à nouveau, pour atteindre la valeur de départ, pour une quantité déterminée de chlore.

ZUSAMMENFASSUNG

Der Einfluss der organischen Lösungsmitteln auf die Atomisierung der metallorganischen Verbindungen wurde in der Studie verfasst. Besondere Aufmerksamkeit ist den chlorierten Kohlenwasserstoffen gewidmet worden. Die erzielten Resultate ergeben, dass Chlor bzw. seine Verbindungen in der Flamme eine wichtige Rolle bei der Atomisierung der Metallchelate spielen. Durch eine zunehmende Chlormenge in dem Lösungsmittel wurde die Absorption zuerst vermindert, nach einem Minimum vergrössert sich die Absorption und erreicht wieder den Anfangswert bei einem gewissen Chlorgehalt.

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A STUDY OF THE TUNGSTEN–HYDROQUINONE COLOR

SPECTROPHOTOMETRIC DETERMINATION OF TUNGSTEN IN TUNGSTEN STEELS

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The fact that tungsten gives a red color with hydroquinone in concentrated sulfuric acid seems to have been first noted by Defacgz[1]. Heyne[2] applied the method to the determination of tungsten by evaporating an alkaline solution of the tungsten to dryness, dissolving the salts by heating with 0.5 ml of sulfuric acid, adding 1 ml of 10% hydroquinone solution in sulfuric acid and comparing the color visually with standards. Heyne also stated that iron, titanium, molybdenum, and rhenium interfered by giving colors. Bogatzki[3] found that to overcome the effect of water in hindering the development of the color 1 g of hydroquinone should be present for every g of water. He stated that the interference from iron and molybdenum could be overcome by reducing these elements with tin(II) chloride. To prevent the reduction of the tungsten by the tin(II) chloride and the precipitation of tin salts, he recommended that some phosphoric acid be present. He noted (without giving specific figures) that small amounts of phosphoric acid did not interfere but that large amounts hindered the color development. Bogatzki made a study of the effect of hydrochloric acid and found that 0.32 g of hydrogen chloride caused the wavelength of maximal absorbance to shift from about 460 to 490 nm; he advocated that the color be read at about 570 nm to eliminate interference from extraneous organic matter and nitrate. Bogatzki described a method for tungsten in steels that involved dissolution of the sample in sulfuric and phosphoric acids, oxidation of the iron with nitric acid, addition of tin(II) chloride, dilution to 100 ml, and treatment of a 2-ml aliquot with 20 ml of 10% hydroquinone solution. Klinger *et al.*[4] reported that Bogatzki's method was satisfactory only for steels containing more than 1% tungsten.

Johnson[5] determined tungsten in tungsten steels by dissolving the sample n a mixture of hydrochloric, nitric, sulfuric, and phosphoric acids, evaporation to fumes, addition of tin(II) chloride, dilution to 100 ml, and treatment of a 4-ml aliquot with 40 ml of 5% hydroquinone solution. Ikenberry *et al.*[6] determined tungsten and niobium simultaneously in stainless steels by hydrolyzing the tungsten and niobium with sulfurous acid, fusing with hydrogen sulfate, diluting to 100 ml with ammonium oxalate solution, treating a 5-ml aliquot with a mixture of sulfuric, phosphoric, and nitric acids, evaporation to fumes, addition of tin(II) chloride, dilution to 100 ml with 6% hydroquinone solution, and measurement of the tungsten color at 460 nm and the niobium color at 525 nm.

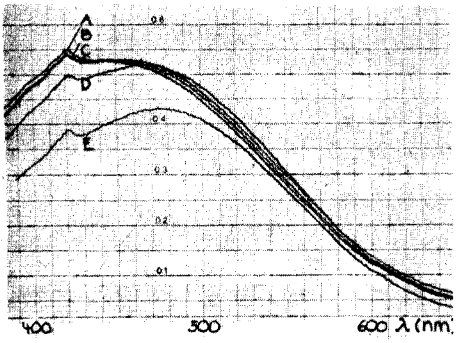
Bricker and Waterbury[7] determined tungsten in uranium–tantalum–tungsten alloys by dissolution of the sample in sulfuric, nitric, and hydrofluoric acids, dilution to a known volume, evaporation of an aliquot to fumes of sulfuric acid, addition of 3 ml of hydroquinone solution (5.5%), and dilution to 10 ml with sulfuric acid. These investigators found that metals could be characterized in the following three categories insofar as interfering with the tungsten–hydroquinone color in a sulfuric acid medium (without phosphoric acid or tin(II) chloride) was concerned: metals that gave a color with hydroquinone (molybdenum, titanium, and tantalum); metals that interfered by reason of their natural color (nickel, copper, cobalt, vanadium, ruthenium, chromium(VI), and manganese(VII)); metals that gave precipitates (iron(II and III), tin, manganese(II), mercury, chromium(III and VI), cerium(III), arsenic, and zinc). Insofar as quantitative interference was concerned, Bricker and Waterbury found that iron, molybdenum, titanium, vanadium and ruthenium interfered when the ratio of these metals to tungten was as low as 1:1; arsenic, cerium(III), nickel and chromium(III and VI) interfered when the ratio exceeded 5:1; copper, tin, and manganese(VII) interfered when the ratio exceeded 10:1; tantalum, manganese(II), mercury, zinc, and cobalt interfered when the ratio exceeded 30:1.

The purpose of the present paper is to clarify certain aspects of the tungsten–hydroquinone color, particularly the effect of water, phosphoric acid, tin(II) chloride, and hydrochloric acid on the development of the color. Another purpose was to study the effect of tin(II) chloride in eliminating the interference from iron, molybdenum, vanadium, and titanium, and to investigate the application of the method to the determination of tungsten in steel.

STUDY OF THE TUNGSTEN–HYDROQUINONE COLOR

For the study of the effect of phosphoric acid, water, tin(II) chloride, and hydrochloric acid, aliquots of standard sodium tungstate solution No 2 (see Experimental) containing 0.5 mg of tungsten were transferred to 50-ml beakers, 5 ml of sulfuric acid were added, and the solutions were evaporated on the hot plate just to fumes of sulfur trioxide. The solutions were allowed to cool and different amounts of phosphoric acid, water, tin(II) chloride, and hydrochloric acid were added. Hydroquinone solution (30 ml of 8% in sulfuric acid) was added and the solutions were diluted to 50 ml in volumetric flasks with sulfuric acid. The absorbance spectra (with distilled water in the reference cell) obtained are shown in Figs. 1–6. The absorbance spectra of the blank under various conditions are shown in Fig. 7. Absorbance spectra for the study of the interferences from iron(III), molybdenum(VI), vanadium(V), and titanium(IV) are shown in Figs. 8–11, respectively.

The above spectra show that the wavelength of maximal absorbance for the tungsten–hydroquinone color in the sulfuric acid medium (without phosphoric acid) occurred at about 450 nm. Up to 2 ml of phosphoric acid (per 50 ml) did not change this wavelength significantly. More phosphoric acid (5 ml) caused the wavelength of maximal absorbance to shift (with 10 ml of phosphoric acid the shift was about +25 nm). The presence of phosphoric acid in the range 1–5 ml caused a slight change in absorbance (increase or decrease), depending on whether tin(II) chloride or hydrochloric acid was present and on the wavelength; 10 ml of phos-



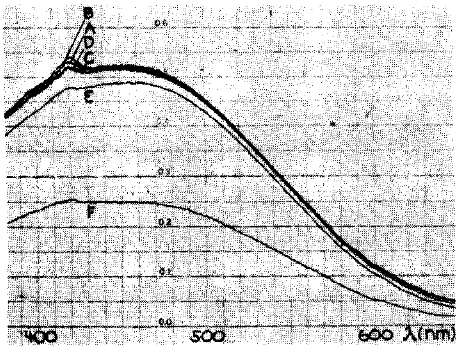
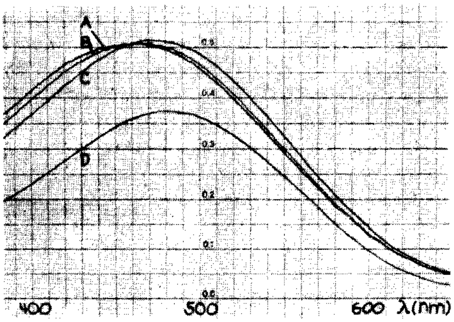


Fig. 1. Effect of H_3PO_4 (0.5 mg W and 2 ml H_2O per 50 ml). (A) 0, (B) 1, (C) 2, (D) 5 and (E) 10 ml H_3PO_4.

Fig. 2. Effect of H_2O (0.5 mg W and 2 ml H_3PO_4 per 50 ml). (A) 0, (B) 1, (C) 2, (D) 3, (E) 5 and (F) 10 ml H_2O.



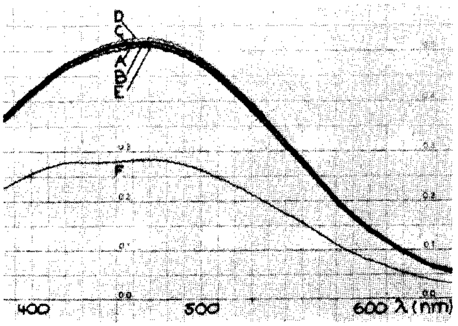
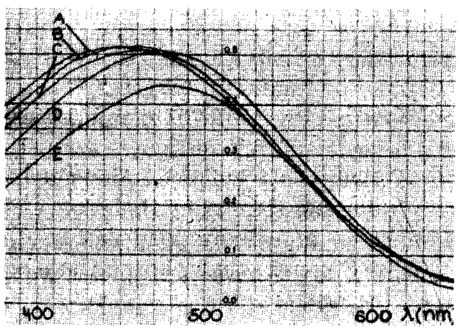


Fig. 3. Effect of H_3PO_4 (0.5 mg W, 2 ml H_2O, and 5 drops 50% $SnCl_2$ per 50 ml). (A) 1, (B) 2, (C) 5 and (D) 10 ml H_3PO_4.

Fig. 4. Effect of H_2O (0.5 mg W, 2 ml H_3PO_4, and 5 drops 50% $SnCl_2$ per 50 ml). (A) 0, (B) 1, (C) 2, (D) 3, (E) 5 and (F) 10 ml H_2O.



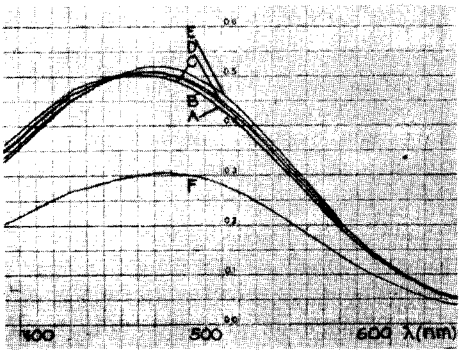


Fig. 5. Effect of H_3PO_4 (0.5 mg W, 2 ml H_2O and 5 drops (1+3) HCl per 50 ml). (A) 0, (B) 1, (C) 2, (D) 5 and (E) 10 ml H_3PO_4.

Fig. 6. Effect of H_2O (0.5 mg W, 2 ml H_3PO_4, and 5 drops (1+3) HCl per 50 ml). (A) 0, (B) 1, (C) 2, (D) 3, (E) 5 and (F) 10 ml H_2O.

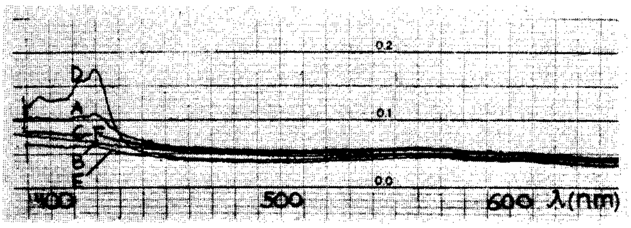


Fig. 7. Blank. (A) 2 ml H_2O and 3 ml H_3PO_4 per 50 ml; (B) 2 ml H_2O, 3 ml H_3PO_4, and 5 drops 50% $SnCl_2$ per 50 ml; (C) 2 ml H_2O, 3 ml H_3PO_4, and 5 drops (1+3) HCl per 50 ml; (D) 2 ml H_2O per 50 ml; (E) 2 ml H_2O and 5 drops 50% $SnCl_2$ per 50 ml; (F) 2 ml H_2O and 5 drops (1+3) HCl per 50 ml.

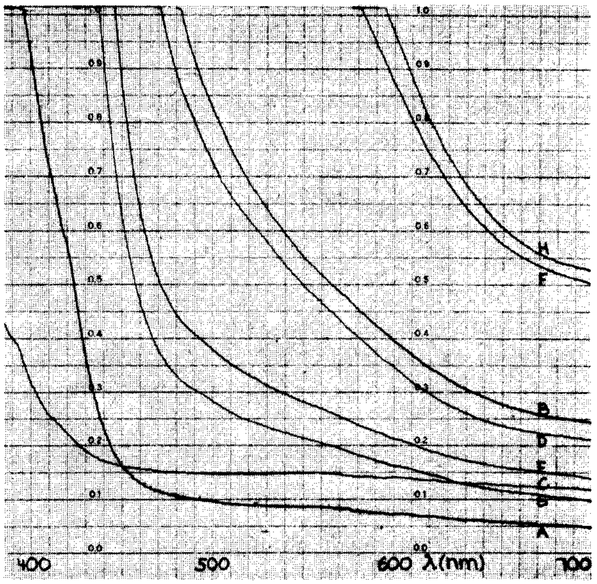


Fig. 8. Interference of iron. (A) 20 mg Fe, 2 ml H_2O, and 5 drops 50% $SnCl_2$ per 50 ml; (B) 20 mg Fe and 2 ml H_2O per 50 ml; (C) 20 mg Fe, 2 ml H_2O, 3 ml H_3PO_4, and 5 drops 50% $SnCl_2$ per 50 ml; (D) 20 mg Fe, 2 ml H_2O, and 3 ml H_3PO_4 per 50 ml; (E) 50 mg Fe, 2 ml H_2O, and 5 drops 50% $SnCl_2$ per 50 ml; (F) 50 mg Fe and 2 ml H_2O per 50 ml; (G) 50 mg Fe, 2 ml H_2O, 3 ml H_3PO_4, and 5 drops 50% $SnCl_2$ per 50 ml; (H) 50 mg Fe, 2 ml H_2O, and 3 ml H_3PO_4 per 50 ml.

phoric acid caused a marked decrease in absorbance at the wavelength of maximal absorbance.

This experimental work, with phosphoric acid, bears out the statement by Bogatzki[3] that phosphoric acid prevents the precipitation of tin salts (this precipitation prevented the testing of a sample containing no phosphoric acid in Fig. 3). The work did not corroborate the observation of Bogatzki that phosphoric acid is necessary to prevent reduction of tungsten by the tin(II) chloride.

Water in amounts of up to 10 ml had no effect on the wavelength of maximal absorbance; 1–5 ml of water caused a slight change in absorbance (increase or decrease), depending on the amount of water and whether tin(II) chloride or hydrochloric acid was present, whereas 10 ml of water causes a marked decrease in absorbance.

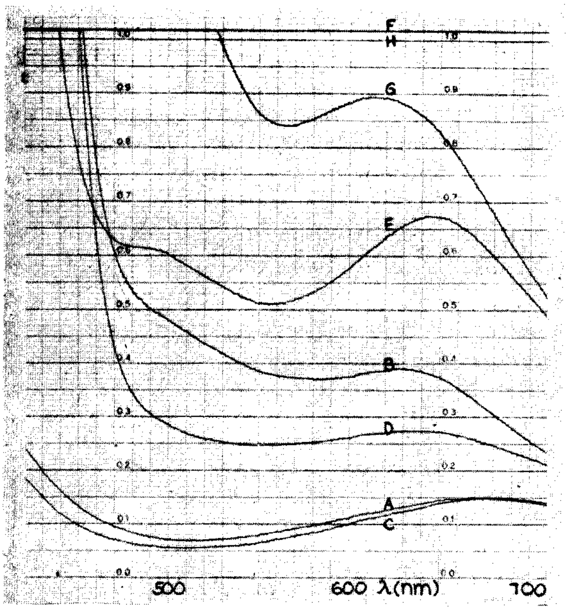


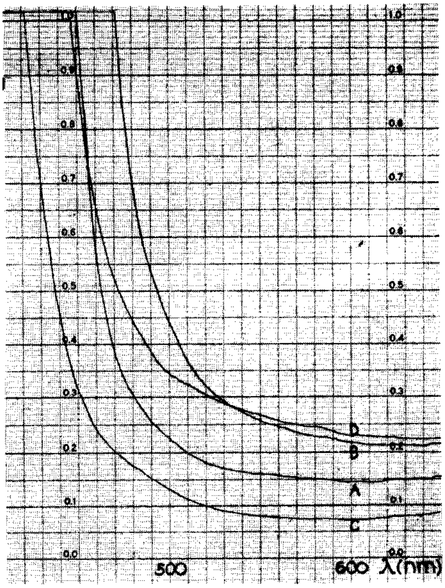
Fig. 9. Interference of molybdenum. (A) 20 mg Mo, 2 ml H_2O, and 5 drops 50% $SnCl_2$ per 50 ml; (B) 20 mg Mo and 2 ml H_2O; (C) 20 mg Mo, 2 ml H_2O, 3 ml H_3PO_4, and 5 drops 50% $SnCl_2$ per 50 ml; (D) 20 mg Mo, 2 ml H_2O, and 3 ml H_3PO_4 per 50 ml; (E) 50 mg Mo, 2 ml H_2O, and 5 drops 50% $SnCl_2$ per 50 ml; (F) 50 mg Mo and 2 ml H_2O per 50 ml; (G) 50 mg Mo, 2 ml H_2O, 3 ml H_3PO_4, and 5 drops 50% $SnCl_2$ per 50 ml; (H) 50 mg Mo, 2 ml H_2O, and 3 ml H_3PO_4 per 50 ml.

It is recommended that some water (2.0 ml) be present since it was found at times that the solution was not clear in the complete absence of water.

Five drops (0.25 ml) of 50% tin(II) chloride or 5 drops of (1+3) hydrochloric acid caused the wavelength of maximal absorbance to increase (about 15 nm). The presence of tin(II) chloride or hydrochloric acid did not affect the absorbance significantly.

Five drops of 50% tin(II) chloride or 5 drops of (1+3) hydrochloric acid eliminated a small peak that occurred at about 420 nm (this peak is apparently due to an impurity in the hydroquinone).

Tin(II) chloride markedly reduced the interference from iron, molybdenum, and vanadium, but it had no effect on the interference from titanium. The effect of phosphoric acid on the interference from iron and molybdenum when the phosphoric acid was added with the tin(II) chloride seemed to depend somewhat on the amount of iron and molybdenum present (no experiments were conducted on varying amounts of vanadium). Phosphoric acid increased the interference from 20 mg of iron, decreased the interference from 20 mg of molybdenum, and decreased the interference from 20 mg of vanadium; it decreased the interference from 50 mg of iron and increased the interference from 50 mg of molybdenum. Experiments with a Beckman Model B spectrophotometer (in place of the Cary spectrophotometer) confirmed that the behavior of phosphoric acid on the interference of iron and molybdenum in the presence of tin(II) chloride was dependent on the amount of iron and molybdenum present.



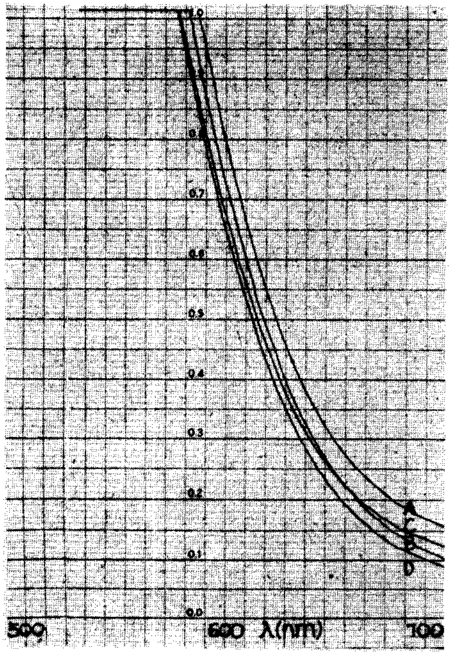


Fig. 10. Interference of vanadium. (A) 20 mg V, 2 ml H_2O, and 5 drops 50% $SnCl_2$ per 50 ml; (B) 20 mg V and 2 ml H_2O per 50 ml; (C) 20 mg V, 2 ml H_2O, 3 ml H_3PO_4, and 5 drops 50% $SnCl_2$ per 50 ml; (D) 20 mg V, 2 ml H_2O, and 3 ml H_3PO_4 per 50 ml.

Fig. 11. Interference of titanium. (A) 1 mg Ti, 2 ml H_2O, and 5 drops 50% $SnCl_2$ per 50 ml; (B) 1 mg Ti and 2 ml H_2O per 50 ml; (C) 1 mg Ti, 2 ml H_2O, 3 ml H_3PO_4, and 5 drops 50% $SnCl_2$ per 50 ml; (D) 1 mg Ti, 2 ml H_2O, and 3 ml H_3PO_4 per 50 ml.

After considering all aspects of the problem of interference of iron, molybdenum, and vanadium, it was concluded that it would be advantageous to use 5 drops of 50% tin(II) chloride and 2.0 ml of phosphoric acid, and to measure the color at 530 nm.

The effect of concentration of hydroquinone solution was investigated for the recommended method. In this study, tungsten solutions containing 0.5 mg of tungsten were fumed with 5 ml of sulfuric acid and then 2.0 ml of phosphoric acid, and 2.0 ml of water were added. Various amounts of hydroquinone solution (8%) were then added, the volumes were brought up to 50 ml in volumetric flasks with sulfuric acid, and the color was measured at 530 nm. The results showed that full color development was achieved with 20–40 ml of hydroquinone solution. The use of 30 ml is recommended.

The tungsten–hydroquinone color developed immediately; however, the solution should stand for 5 min or more so that air bubbles can dissipate. The color is stable for several hours.

Considerable effort was made to apply the method to the direct determination of tungsten in steels. However, the attempts failed because of the complexity of interference from the many alloying elements (iron, molybdenum, vanadium, chromium, etc.) present in tungsten steels. It was necessary, therefore, to separate the tungsten from the bulk of the interfering elements by the use of cinchonine. The precipitated tungsten was then filtered off, ignited at 600°, and dissolved by boiling with sodium hydroxide solution. It has been established that tungstic oxide is soluble in sodium hydroxide solution[8, 9]. It was necessary to treat the tungstic oxide with hydrofluoric acid to eliminate silica, which would otherwise coat the tungstic oxide and prevent its complete dissolution by the sodium hydroxide. Some experiments were conducted on digesting the precipitated tungstic acid (and filter paper) with sodium hydroxide or ammonia solutions and filtration, but the recoveries were low.

Unfortunately, the limitations of the cinchonine separation technique were such that it was not reliable for small amounts of tungsten (less than 0.2%), as judged by experiments with synthetic samples prepared by adding portions of a NBS standard tungsten steel to high-purity iron.

EXPERIMENTAL

Apparatus and reagents

A Cary Model 15 spectrophotometer and a Beckman Model B spectrophotometer were used.

Sulfuric acid (s.g. 1.84), phosphoric acid (85%), hydrochloric acid (s.g. 1.14), and hydrofluoric acid (48%) were used.

Standard tungsten solution No. 1 (1.0 mg W ml^{-1}): dissolve 1.7940 g of sodium tungstate dihydrate in water and dilute to 1 l in a volumetric flask.

Standard tungsten solution No. 2 (0.10 mg W ml^{-1}): pipet 50 ml of standard tungsten solution No. 1 into a 500-ml volumetric flask and dilute to the mark.

Tin(II) chloride solution (50%): dissolve 20 g of tin(II) chloride dihydrate in 10 ml of hydrochloric acid by warming on the hot plate. Cool and add 30 ml of water.

Hydroquinone solution (8%): dissolve 16 g of hydroquinone (Eastman-Kodak) in 200 ml of concentrated sulfuric acid and allow to stand for 1–2 h with occasional stirring. Prepare fresh daily.

Cinchonine solution: dissolve 62.5 g of cinchonine in 55 ml of hydrochloric acid (1 + 1).

Cinchonine wash solution: dilute 30 ml of cinchonine solution to 1 l with water.

Recommended method for the determination of tungsten in tungsten steels

Preparation of calibration curve. Transfer 2.0, 4.0, 6.0, 8.0, and 10.0 ml of standard tungsten solution No. 2 to 50-ml beakers. Add 5 ml of sulfuric acid and evaporate just to fumes of sulfur trioxide. Run a reagent blank simultaneously. Allow to cool. Add 2.0 ml of phosphoric acid, 2.0 ml of water, and 5 drops of tin(II) chloride solution (50%), and cool to room temperature. Transfer 30 ml of hydroquinone solution (8%) to a graduated cylinder previously rinsed with sulfuric acid, add about 20 ml of this hydroquinone solution to the beaker, decant into a 50-ml volumetric flask previously rinsed with sulfuric acid, and rinse the beaker with the remaining 10 ml of the hydroquinone solution (in small portions). Cool to room temperature, dilute to the mark with sulfuric acid, and mix. Measure the absorbance at 530 nm in 5–60 min against the reagent blank. Plot absorbance against mg of tungsten per 50 ml.

Procedure. Transfer the following size of sample to a 400-ml beaker: 0.2–1% W, 5 g; 1–5% W, 2 g; above 5% W, 0.5 g. Add 50 ml of hydrochloric acid and warm gently until the sample is dissolved (black particles may remain). Add 10 ml of nitric acid (1 + 1) and digest at about 100° until the precipitate is yellow and no black particles remain. Dilute to about 150 ml with hot water, add 5 ml of cinchonine solution and a little paper pulp, and digest at about 95° for 1–2 h. Allow samples containing less than 2% tungsten to stand overnight at room temperature. Filter through a Whatman No. 42 filter paper and transfer and wash with hot cinchonine wash solution. Place the filter paper and precipitate into a platinum crucible (about 35-ml capacity). Char carefully and ignite at 600° until the paper is completely burned off. Add 1 ml of sulfuric acid (1 + 1) and 3 ml of hydrofluoric acid, evaporate to dryness, and ignite at 600° for 30 min. Add 20 ml of sodium hydroxide solution (10%) to the crucible, warm on the hot plate until the tungstic oxide has dissolved, and wash the solution into the original 400-ml beaker. Cover with a watch glass and boil for several min. Add 150 ml of water and again boil for several min. Cool and dilute to 500 or 1000 ml in a volumetric flask. Pipet an aliquot of 5–25 ml (preferably containing 0.4–0.9 mg of tungsten) into a 50-ml beaker. Add 5 ml of sulfuric acid, evaporate to fumes of sulfur trioxide and develop the color as described

TABLE I

TUNGSTEN IN N.B.S. TUNGSTEN STEELS BY THE HYDROQUINONE METHOD

Sample	*Content (%)*		*Tungsten found (%)*	s_r
50a	18.25 W, 0.009 Mo, 0.97 V,		18.12	
	3,52 Cr, 0.660 C, 0.289 Mn,		18.12	
	0.020 P, 0.007 S, 0.48 Si,		18.00	
	0.047 Cu, 0.045 Ni, 0.042 As,		18.40	
	0.025 Sn			
		Average	18.21	0.18
132	6.29 W, 7.07 Mo, 1.64 V,		6.20	
	4.11 Cr, 0.803 C, 0.252 Mn,		6.40	
	0.027 P, 0.004 S, 0.339 Si,		6.40	
	0.149 Cu		6.20	
		Average	6.32	0.11
134	1.82 W, 8.70 Mo, 1.14 V,		1.83	
	3.73 Cr, 0.81 C, 0.15 Mn,		2.00	
	0.015 P, 0.005 S, 0.32 Si,		2.00	
	0.11 Cu, 0.08 Ni		1.83	
		Average	1.90	0.09

for the preparation of a calibration curve. Convert the reading to mg of tungsten by referring to the calibration curve and calculate the percentage of tungsten.

RESULTS

The results obtained for National Bureau of Standard steels by the proposed method are shown in Table I. The accuracy of the method was satisfactory.

This work was performed under an Army Materials Testing Technology Project (AMS Code 53960.OM.6350).

SUMMARY

The red color produced by tungsten and hydroquinone in a concentrated sulfuric acid medium was studied. Various factors affecting the color were examined including the effects of phosphoric acid, water, hydrochloric acid, tin(II) chloride, and wavelength of measurement. The interferences from iron, molybdenum, vanadium, and titanium were studied. A method is described for the determination of tungsten in tungsten steels that involves precipitation of the tungsten with cinchonine, ignition to tungstic oxide, dissolution of the tungstic oxide in sodium hydroxide, and development of the tungsten hydroquinone color on an aliquot of the solution.

RÉSUMÉ

Une recherche est effectuée sur la coloration rouge produite par le tungstène et l'hydroquinone en milieu acide sulfurique. Divers facteurs affectant la coloration sont examinés: influence de l'acide phosphorique, de l'eau, de l'acide chlorhydrique, du chlorure d'étain(II) et de la longeur d'onde choisie pour les mesures. On examine également les interférences du fer, du molybdène, du vanadium et du titane. Une méthode est décrite pour le dosage du tungstène dans des aciers au tungstène, avec précipitation au moyen de cinchonine, calcination en oxyde tungstique, dissolution de cet oxyde dans l'hydroxyde de sodium et formation de la coloration tungstène–hydroquinone sur une partie aliquote de la solution.

ZUSAMMENFASSUNG

Die durch Wolfram und Hydrochinon in konzentrierter Schwefelsäure hervorgerufene Rotfärbung wurde untersucht. Verschiedene die Färbung beeinflussende Faktoren wie der Einfluss von Phosphorsäure, Wasser, Salzsäure, Zinn(II)-chlorid und Wellenlänge der Messung wurden geprüft. Die Störungen durch Eisen, Molybdän, Vanadin und Titan wurden untersucht. Eine Methode für die Bestimmung von Wolfram in Wolframstählen wird beschrieben. Sie beruht auf der Fällung des Wolframs mit Cinchonin, dem Verglühen zu Wolframoxid, Auflösen des Wolframoxids in Natronlauge und der Entwicklung der Wolfram-Hydrochinon-Färbung in einem aliquoten Anteil der Lösung.

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SPECTROPOLARIMETRIC DETERMINATION OF COPPER(II), NICKEL(II) AND IRON(III) IONS WITH N-CARBOXYMETHYLPYRROLIDINE-2-CARBOXYLIC ACID

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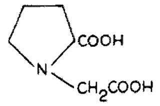
The polarimetric titration of metal ions is a relatively new technique in analytical chemistry, and several papers concerning this subject have been published in recent years[1–8]. The necessary conditions for such titrations with an optically active titrant are as follows: first, the stability constant of the complex formed must be high enough to allow the determination of the metal ion under the experimental conditions (concentration of the metal and titrant, pH, etc.); secondly, the molar rotations of the ligand and of the complex must be different enough to permit a good appreciation of the end-point. This last condition affects the selection of the proper wavelength at which to carry out the titration; another consideration is that regions of high absorbance must be avoided, because this could decrease the sensitivity of the detection.

In a previous paper from this laboratory[9], it was shown that copper(II) ion can be titrated polarimetrically with L(–)proline; this suggested an investigation of the behaviour of derivatives of L(–)proline, such as N-carboxymethylpyrrolidine-2-carboxylic acid(I), the chelating ability of which should be better than that of the parent compound.

EXPERIMENTAL



Reagents

N-carboxymethylpyrrolidine-2-carboxylic acid (CMPCA) was prepared by reaction of proline and chloroacetic acid in the presence of an excess of sodium hydrogencarbonate; the product obtained was isolated and purified as described by Korman and Clarke[10]. The high purity of the compound made it possible to prepare standard solutions by weighing; these were stable for at least two months.

Copper(II) nitrate, nickel(II) nitrate and iron(III) perchlorate (Carlo Erba, reagent grade) were used without further purification; their solutions were standardized against EDTA.

Nitric acid, potassium hydroxide, potassium nitrate and sodium perchlorate were Carlo Erba reagent-grade chemicals.

An acetic acid–sodium acetate buffer was used at pH 5.0; a buffer containing glycine and nitric acid was selected for measurements at pH 3.0, and an ammonia–ammonium nitrate buffer was used for pH 10.0.

All solutions were prepared with twice-distilled water.

Apparatus

A Beckman 1019 potentiometer equipped with glass and calomel electrodes was used for pH titrations; the titrant was added with a microsyringe buret. Polarimetric measurements were carried out with a Perkin-Elmer 141 MC spectropolarimeter equipped with a 5-ml quartz flow-through cell which had an optical path of 10 cm; the cell was connected with a titration vessel of the type described by Palma and Pearson[4], modified in order to permit thermostatting of solutions. All measurements were carried out at 25° and unit ionic strength (KNO_3 or $NaClO_4$).

ESTABLISHMENT OF ESSENTIAL CONSTANTS

Determination of the acidity constants of CMPCA

The values of these acidity constants were computed from the results of pH titrations of solutions of CMPCA in the concentration range $9.10 \cdot 10^{-4}$–$7.68 \cdot 10^{-3}$ *M*. A typical titration plot is given in Fig. 1. From the data obtained, the values of the degree of formation at different pH were calculated, and these were used for computation of the constants. The resulting values, given as negative logarithms at 25° and unit ionic strength (KNO_3), are as follows:

$pK_1 = 1.47$; $pK_2 = 2.32$; $pK_3 = 10.14$

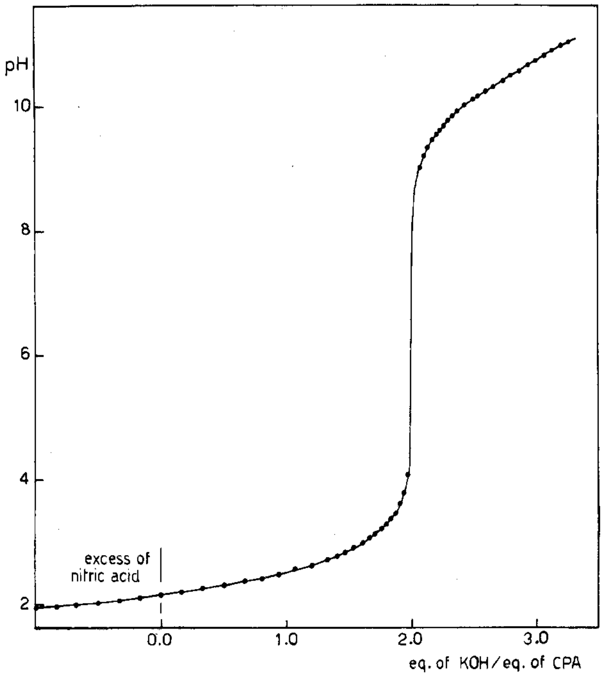


Fig. 1. Titration curve for a $5.98 \cdot 10^{-3}$ *M* solution of CMPCA.

Theoretical titration plots were calculated from these values of the constants, and the agreement with the experimental points proved to be quite good.

Evaluation of the stability constants of the complexes formed by CMPCA with various metal ions

In order to estimate the stability constants of the complexes of CMPCA with some metal ions, pH titrations were made; in the solutions the concentration of the metal ion ranged from $1.33 \cdot 10^{-3}$ *M* to $7.00 \cdot 10^{-3}$ *M* and the ligand-to-metal ratio from 1:1 to 3:1. The metal ions chosen for this study were copper(II), nickel(II), zinc(II), cobalt(II) and cadmium(II). From the potentiometric data, the curves of the formation degrees were drawn; it was found that the experimental points concerning solutions with different ratios of ligand to metal ion did not lie on the same curve but were somewhat scattered. This behaviour can probably be attributed to the formation of mixed or polynuclear complexes, in addition to ML and ML_2. However, as it was not the purpose of this work to establish the exact values of the stability constants of all the complexes formed, but only to obtain a rough estimate of the unprotonated complexes in order to ascertain the feasibility of the compleximetric titrations, the order of magnitude of the formation constants of ML and ML_2 was calculated. The values obtained are given in Table I.

TABLE I

ESTIMATED VALUES OF THE LOGARITHMS OF THE FORMATION CONSTANTS OF METAL COMPLEXES OF CMPCA

Metal ion	*Log β_1*	*Log β_2*
Copper(II)	11	18
Nickel(II)	9	17
Zinc(II)	8	14
Cobalt(II)	8	14
Cadmium(II)	7	12

Determination of the specific rotation of CMPCA

The optical rotatory dispersion (o.r.d.) curve of the ligand at a fixed pH value, and the variation of the specific rotation of the free acid as a function of pH at a fixed wavelength, were determined. The measurements concerned samples with concentration ranging from 0.036 to 1.000 g per 100 ml of solution. The results are given in Figs. 2 and 3. As can be seen from Fig. 2, the value of the specific rotation of CMPCA at constant wavelength is constant in a wide range of pH. Figure 3 shows the o.r.d. curve for solutions whose acidity is enclosed in this range. The value of $[\alpha]_D^{25}$ obtained for CMPCA is $-57.7°$ for solutions with pH values ranging from 2.0 to 6.0.

Figure 4 collects, as functions of wavelength, the values of the molar rotation of the ligand in acidic solutions, as well as those of the 1:1 complexes formed with copper(II), nickel(II) and iron(III) ions; Cu(II)–CMPCA and Ni(II)–CMPCA solutions were buffered at pH 5.0 whereas the Fe(III)–CMPCA system was buffered at pH 3.0. The curves suggest that iron(III) should be titrated at 404.7 nm,

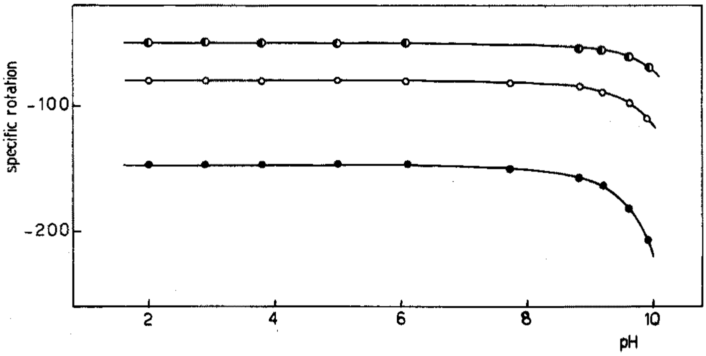


Fig. 2. Variation of the specific rotation of CMPCA as a function of pH. λ: (◐), 620.0, (○), 500.0 and (●), 380.0 nm.

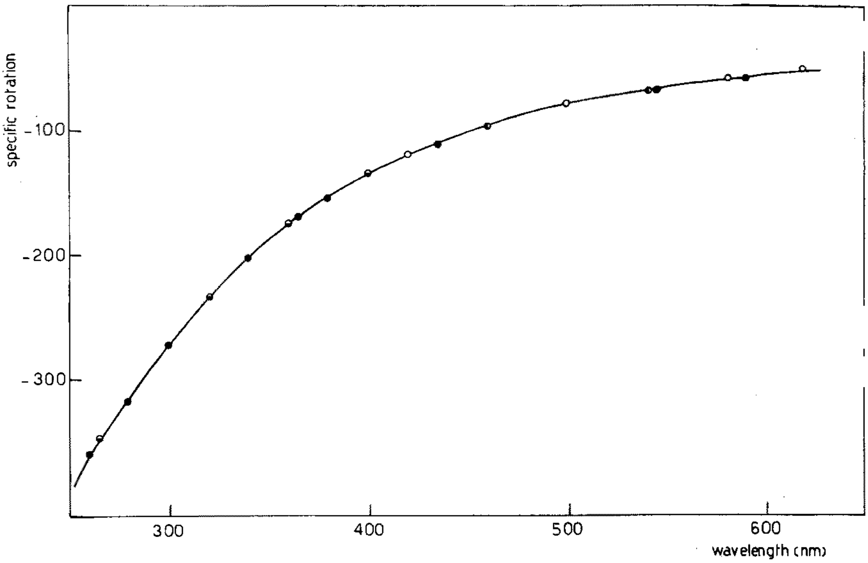


Fig. 3. Variation of the specific rotation of CMPCA as a function of wavelength. pH: (○), 2.0; (●), 2.2; (◐), 3.8; (◒), 5.0.

nickel(II) at 255.0 nm, and copper(II) at different wavelengths in the range 300.0–350.0 nm.

RESULTS OF THE SPECTROPOLARIMETRIC TITRATIONS

The technique of the titrations has been described elsewhere[9]. The solutions of the titrant used had concentrations ranging from $2.00 \cdot 10^{-2}$ *M* to $1.16 \cdot 10^{-1}$ *M*.

Determination of copper(II)

Copper(II) ion was titrated at pH 5.0 rather than at pH 10.0, in order to obtain in the resulting plot straight lines forming a small angle. In fact, the molar rotation of the 1:1 copper(II)–ligand complex in the available wavelength range

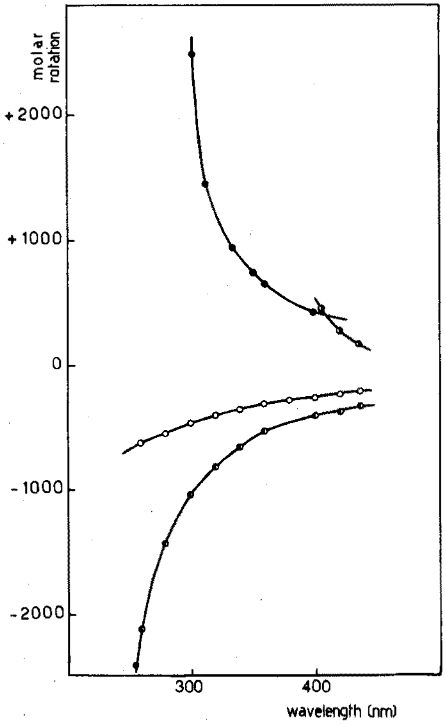


Fig. 4. Variation of the molar rotations of CMPCA and its 1:1 complexes as a function of wavelength. (○), CMPCA at pH values ranging between 2.0 and 6.0; (●), copper(II)–CMPCA complex at pH 5.0; (◑), iron(III)–CMPCA complex at pH 3.0; (◐), nickel(II)–CMPCA complex at pH 5.0.

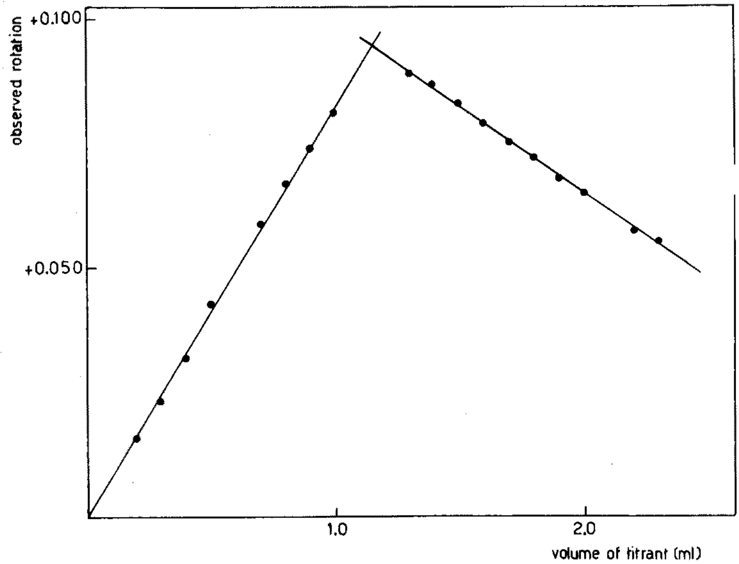


Fig. 5. Spectropolarimetric titration of copper(II) nitrate ($1.00 \cdot 10^{-3}$ *M*) with CMPCA ($5.21 \cdot 10^{-2}$ *M*) at pH 5.0 and 334.0 nm.

is negative at pH 10.0 and positive at pH 5.0; as the molar rotation of the titrant is always negative under the same conditions, the best results are obtained by working at pH 5.0. The solutions to be titrated contained copper(II) ion in the concentration range $1.93 \cdot 10^{-4}$–$3.00 \cdot 10^{-3}$ M. Figure 5 shows a typical titration plot, and Table II gives some of the results obtained.

TABLE II

RESULTS OF SPECTROPOLARIMETRIC TITRATIONS OF COPPER(II)

Wavelength (nm)	*Copper(II) (mg)*		*Deviation*	
	Taken	*Found*	*(mg)*	*(%)*
350.0	11.440	11.390	−0.050	−0.44
334.1	7.625	7.621	−0.004	−0.07
334.1	3.812	3.821	+0.009	+0.26
312.6	1.855	1.839	−0.016	−0.81
302.2	0.737	0.745	+0.008	+1.09

Determination of nickel(II)

All titrations were carried out at pH 5.0 and 255.0 nm; the reasons that suggested the selection of pH were similar to those considered for the determination of copper(II). The nickel(II) was determined in solutions with concentrations varying from $3.52 \cdot 10^{-4}$ M to $1.41 \cdot 10^{-3}$ M. Some results are given in Table III, and Fig. 6 shows a typical titration plot.

TABLE III

RESULTS OF SPECTROPOLARIMETRIC TITRATIONS OF NICKEL(II) ION AT 255.0 nm

Nickel(II) (mg)		*Deviation*	
Taken	*Found*	*(mg)*	*(%)*
5.783	5.800	+0.017	+0.29
4.337	4.304	−0.033	−0.76
1.445	1.433	−0.012	−0.83

Determination of iron(III)

The solutions, in which the concentration of iron(III) ranged between $9.23 \cdot 10^{-4}$ M and $4.07 \cdot 10^{-3}$ M, were studied at pH 3.0 and at 404.7 nm. As can be seen from Fig. 7, which shows a typical plot, two break-points are obtained, probably because of the formation of two complexes in which the metal-to-ligand ratios are 1:1 and 1:2, respectively. The detection of the first end-point is very easy and leads to good results; the values obtained from the second one are also satisfactory if the solutions are not too dilute, and they can give support to the data obtained from the first break. Table IV gives the results of some titrations.

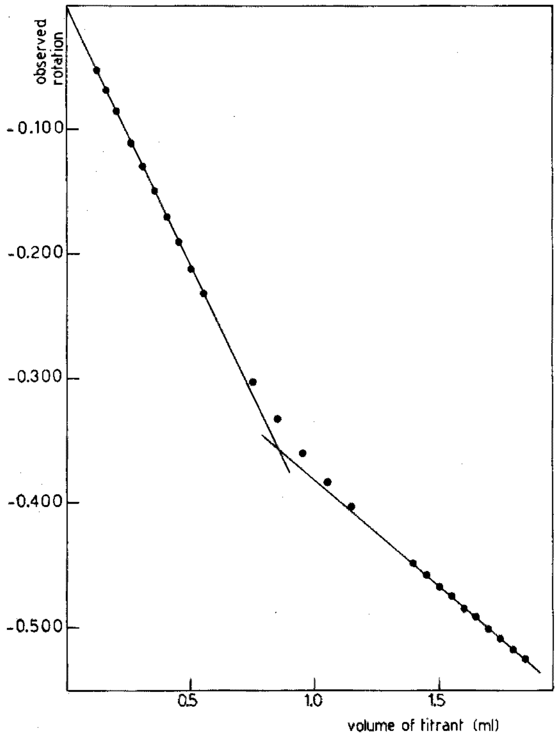


Fig. 6. Spectropolarimetric titration of nickel(II) nitrate ($1.41 \cdot 10^{-3}$ M) with CMPCA ($1.16 \cdot 10^{-1}$ M) at pH 5.0 and 255.0 nm.

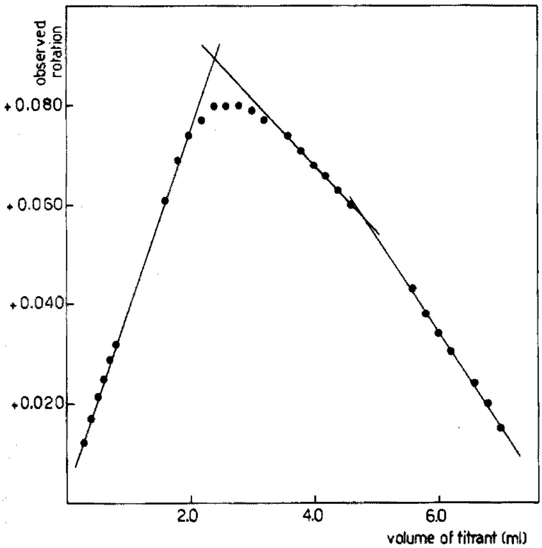


Fig. 7. Spectropolarimetric titration of iron(III) perchlorate ($1.98 \cdot 10^{-3}$ M) with CMPCA ($5.79 \cdot 10^{-2}$ M) at pH 3.0 and 404.7 nm.

TABLE IV

RESULTS OF SPECTROPOLARIMETRIC TITRATIONS OF IRON(III) ION AT 404.7 nm

Iron(II) (mg)		*Deviation*		*Break-point considered*
Taken	*Found*	*(mg)*	*(%)*	
15.914	15.997	+0.081	+0.52	First
15.914	16.078	+0.164	+1.03	Second
11.458	11.519	+0.061	+0.53	First
7.746	7.736	−0.010	−0.13	First
7.746	7.728	−0.018	−0.26	Second
3.608	3.625	+0.017	+0.47	First

Other metal ions

Titrations of other metal ions, such as cobalt(II) and zinc(II), were tested, but the results were unsatisfactory. It follows from the estimated values of the constants given in Table I that the conditional constants are too low for the titration of these ions at pH 5.0; at pH 10.0, in the presence of ammonia buffer, the displacement reaction of ammonia from zinc complexes by means of CMPCA occurs only partially. In addition, the molar rotations of the ligand and of the complexes are probably too close together.

SUMMARY

N-Carboxymethyl pyrrolidine-2-carboxylic acid (CMPCA) is suggested as an optically active titrant. The values of the acidity constants of the ligand were determined and the order of magnitude of the stability constants of the complexes formed by CMPCA with some metal ions was evaluated. In order to determine the best conditions for the spectropolarimetric titrations, the dependence of the molar rotation of CMPCA and its complexes on wavelength and pH was examined. The spectropolarimetric titrations of copper(II), nickel(II) and iron(III) ions were carried out successfully.

RÉSUMÉ

L'acide 2-carboxy,1-pyrrolidinacétique (CMPCA) est proposé comme titrant optiquement actif. On a déterminé les valeurs des constantes d'acidité du ligand et evalué l'ordre de grandeur des constantes de stabilité des complexes formés entre le CMPCA et quelques ions métalliques. Afin de déterminer les conditions optima des analyses spectropolarimétriques on a examiné le pouvoir rotatoire molaire de CMPCA et de ses complexes en fonction de la longueur d'onde et du pH. Les titrages spectropolarimétriques des ions cuivre(II), nickel(II) et fer(III) ont été effectués avec succès.

ZUSAMMENFASSUNG

N-Carboxymethylpyrrolidin-2-carboxylsäure (CMPCA) wird als ein optisch aktiver Titrant vorgeschlagen. Die Säurekonstanten des Liganden wurden bestimmt, und die Grössenordnung der Stabilitätskonstanten der von CMPCA mit einigen Metallionen gebildeten Komplexe wurde geschätzt. Im Hinblick auf die besten Bedingungen für die spektropolarimetrischen Titrationen wurde die Abhängigkeit der molaren Drehung von CMPCA und dessen Komplexen von der Wellenlänge und vom pH-Wert untersucht. Spektropolarimetrische Titrationen von Kupfer(II), Nickel(II) und Eisen(III) wurden mit Erfolg ausgeführt.

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CHELATE VON β-DICARBONYLVERBINDUNGEN UND IHREN DERIVATEN

TEIL XXXIV*. DIE VERWENDUNG VON THIODIBENZOYLMETHAN ZUR EXTRAKTIONSPHOTOMETRISCHEN BESTIMMUNG VON THALLIUM- UND QUECKSILBERSPUREN

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Über Möglichkeiten zur extraktionsphotometrischen Metallbestimmung mit Thiodibenzoylmethan wurde bereits mehrfach berichtet[2–6]

Ausführliche Untersuchungen haben gezeigt, dass die Metallextraktion mit diesem Reagens aus stärker saurer Lösung vorgenommen werden kann, als dies beim sauerstoffanalogen Dibenzoylmethan gefunden wurde[2]. Die Extraktion in stark saurem Milieu erfordert jedoch in der Regel Schüttelzeiten, die analytisch kaum vertretbar sind. In Fig. 1 wird diese Tatsache am Beispiel der Extraktion von Kupfer mit Thiodibenzoylmethan demonstriert. Demnach lassen sich erst im alkalischen Bereich Extraktionsbedingungen einstellen, die die Extraktion der Metallchelate in weniger als 3 Min gewährleisten.

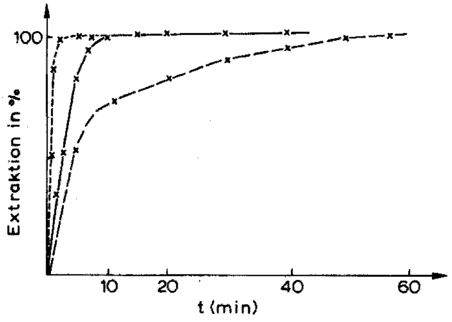

Fig. 1. Zeitabhängigkeit der Kupferextraktion mit Thiodibenzoylmethan bei verschiedenen pH-Werten. (– – –) pH = 2.55; (——) pH = 4.75; (------) pH > 8.

Eine Ausnahme bildet hierbei die Quecksilberverbindung des Thiodibenzoylmethans, die sowohl aus 6 *M* Salpetersäure als auch aus 6 *M* Kalilauge in kurzer Zeit mit Lösungen von Thiodibenzoylmethan in Benzol quantitativ extrahiert werden kann.

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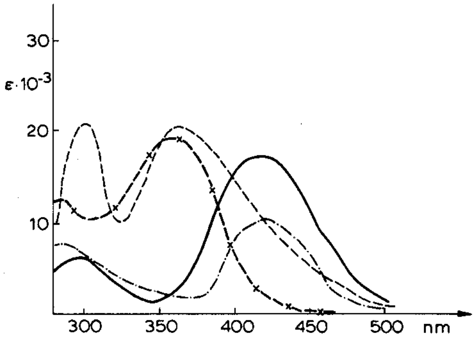
* Teil XXXIII: zie Lit. 1.

Auch das Absorptionsspektrum der Quecksilberverbindung unterscheidet sich deutlich von denen der anderen Metallchelate. Während bei den meisten Metallchelaten des Thiodibenzoylmethans intensive Innerligandbanden nahe 400 nm anzutreffen sind, weist das Absorptionsspektrum der Quecksilberverbindung bei 360 nm ein Maximum auf und ähnelt damit weitgehend dem Spektrum des Disulfids von Thiodibenzoylmethan (Fig. 2). Diese hypsochrome Verschiebung des Absorptionsmaximums ist auf abnehmende Elektronendelokalisation zurückzuführen und entspricht einer Schwächung der Chelatstruktur im Quecksilberkomplex. Im I.R.-Spektrum deutet die Lage der Carbonylbande (1622 cm^{-1})[7] ebenfalls auf Besonderheiten der Quecksilberverbindung hin und legt den Schluss nahe, dass die Carbonylgruppe nur schwach am Metall koordiniert sein kann.



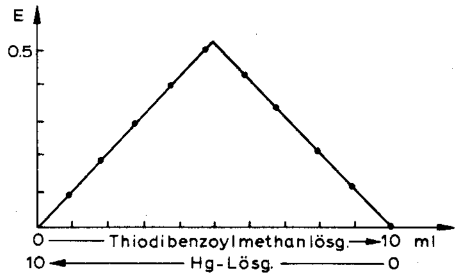


Fig. 2. Absorptionsspektren des Thiodibenzoylmethans (——) und seines Disulfides (–×–×–), sowie der Komplexe mit Thallium(I) (·–·–·–) und Quecksilber(II) (----).

Fig. 3. Jobsche Kurve für den Quecksilberkomplex mit Thiodibenzoylmethan.

Für die Komplexzusammensetzung der festen Substanz wurde ein Verhältnis Metall zu Ligand von 1:2 ermittelt. In Lösung (Benzol) konnte jedoch ein Verhältnis von 1:1 festgestellt werden (Fig. 3), in der gleichen Form werden Quecksilber(II)-ionen auch mit Thiodibenzoylmethan extrahiert. Die hier angeführten Besonderheiten der Quecksilberverbindung im Vergleich zu anderen Metallchelaten des Thiodibenzoylmethans lassen den Schluss zu, dass mercaptidartige Bindungsverhältnisse vorliegen müssen. Die von Musso *et al.*[8] bei den Quecksilberverbindungen des Acetylacetons und des Dipivaloylmethans nachgewiesene Quecksilber–Kohlenstoff-Bindung an der mittelständigen CH_2-Gruppe des entsprechenden Diketons kann bei der Quecksilberverbindung des Thiodibenzoylmethans mit Sicherheit ausgeschlossen werden. ^{13}C-NMR-Untersuchungen am Quecksilberkomplex des Thiodibenzoylmethans lassen deutlich die (=CH)-Gruppierung am mittelständigen C-Atom erkennen, wie sie in gleicher Weise auch bei den anderen Chelaten des Thiodibenzoylmethans gefunden wurde[9].

Für eine analytische Anwendung ist auch der Thallium(I)-Komplex des Thiodibenzoylmethans gut geeignet. Die Untersuchung der Komplexzusammensetzung in Lösung ergab ein Verhältnis Metall zu Ligand von 1:1. Der Vorteil der erarbeiteten Methode ist darin zu sehen, dass hier die bei vielen anderen photometrischen Verfahren zur Thalliumbestimmung erforderliche Oxydation von

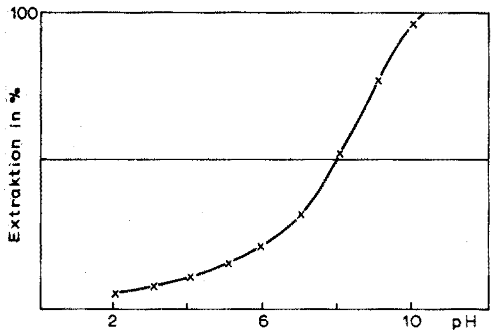


Fig. 4. pH-Abhängigkeit der Thalliumextraktion mit Thiodibenzoylmethan.

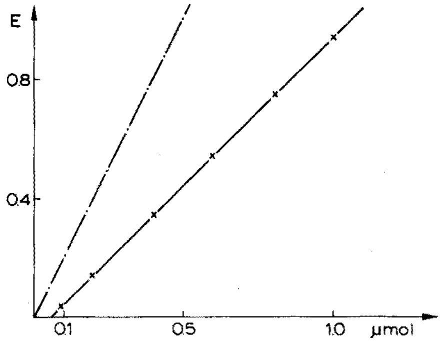


Fig. 5. Eichgeraden für die extraktionsphotometrische Bestimmung von Quecksilber (·—·—·—), und Thallium (–×–×–) mit Thiodibenzoylmethan.

Thallium(I) zu Thallium(III) entfällt. Somit können Extraktion und photometrische Bestimmung in einem Arbeitsgang schnell durchgeführt werden.

Die Abhängigkeit der Extraktion des Thalliumchelates vom pH-Wert der wässrigen Phase wird in Fig. 4 wiedergegeben. Die Extraktion des Thalliums ist ab pH 11 maximal.

Bei der extraktionsphotometrischen Metallbestimmung mit Thiodibenzoylmethan wirkt sich überschüssiger Ligand durch die starke Eigenfärbung störend aus. Die Reextraktion von überschüssigem Liganden gelingt durch Behandeln der organischen Phase mit 3 *M* Kalilauge. Während die Quecksilberverbindung des Thiodibenzoylmethans durch Alkalien nicht angegriffen wird, werden geringe Mengen des gebildeten Thalliumchelates zersetzt. Bei genauer Dosierung des zur Reextraktion des überschüssigen Liganden erforderlichen Laugenzusatzes ist der erwähnte Nachteil jedoch analytisch vertretbar, zumal er als "systematischer Fehler" in der Eichmessung berücksichtigt wird.

Die extraktionsphotometrischen Bestimmungen von Quecksilber und Thallium mit Thiodibenzoylmethan lassen sich beim Einsatz von geeigneten Maskeirungs-

TABELLE I

EINFLUSS VON FREMDMETALLEN AUF DIE PHOTOMETRISCHE QUECKSILBERBESTIMMUNG MIT THIODIBENZOYLMETHAN

Metall	*Maskierungsmittel*	*Keine Störung*
Fe, Mn	EDTA	1:10000
Zn, Pd	EDTA	1:10000
Sn, Bi	EDTA	1:10000
In, Sb	EDTA	1:10000
Pb, Tl	EDTA	1:10000
Ni, Ga	EDTA	1: 500
Pb, Ag	EDTA, Thiosulfat	1: 100
Cd, Co	EDTA, Thiosulfat	1: 100
Cu	EDTA, Thiosulfat	1: 10

TABELLE II

EINFLUSS VON FREMDMETALLEN AUF DIE PHOTOMETRISCHE BESTIMMUNG VON THALLIUM MIT THIODIBENZOYLMETHAN

Metall	*Keine Störung*
Alkali- und Erdalkalimetalle, B, Ga, In, Al, Cr, Fe, Mn	1:10000
Cu, Ni, Co, Zn, Pb, Pd, Hg	1:1000
As, Sb, Bi, Sn, Ag, Cl^-, Br^-, I^-, SO_4^{2-}, NO_3^-, $C_2O_4^{2-}$	1:1000
SCN^-, F^-, EDTA, S^{2-}, Thioglykolsäure	1:1

mitteln hochselektiv gestalten und sind mit geringem Arbeits- und Zeitaufwand leicht durchführbar. Den Einfluss von Fremdmetallen auf die Bestimmungen geben die Tabellen I und II wieder.

EXPERIMENTELLER TEIL

Geräte und Chemikalien

Zur Durchführung der photometrischen Messungen stand das Spektralkolorimeter Spekol (VEB Carl Zeiss–Jena) mit Zusatzverstärker ZV zur Verfügung. Die pH-Messungen erfolgten mit dem pH-Meter OP 204 mit Einstab-Glaselektrode (Fa. Radelkis, Budapest).

Als Metallstandardlösungen kamen 10^{-4} *M* Lösungen von Thallium(I)-nitrat und Quecksilber(II)-nitrat zum Einsatz.

Thiodibenzoylmethan (Schmp. 83–84°) wurde nach den Angaben der Literatur[10] hergestellt und in Form einer $3 \cdot 10^{-3}$ *M* Lösung in Benzol verwendet. Bei der Untersuchung des Einflusses von Begleitelementen auf die extraktionsphotometrische Metallbestimmung wurden entsprechende Mengen 0.1 *M* Lösungen der p.a.-Salze mit einer Mikrokolbenbürette zugesetzt.

Zur Untersuchung der pH-Abhängigkeit der Extraktion wurden Pufferlösungen nach Schwabe[11] von pH 2 bis pH 12 verwendet. Bei allen Versuchen kam bidestilliertes Wasser zum Einsatz.

Testung der Verfahren

Die analytische Testung und die statistische Auswertung der extraktionsphotometrischen Bestimmungen erfolgten im wesentlichen nach der von Gottschalk[12] vorgeschlagenen Methode.

Quecksilberbestimmung

Der Arbeitsbereich für die Quecksilberbestimmung betrug 0.05–0.5 μmol Quecksilber. Die Testung des Verfahrens wurde jeweils mit 0.5, 1, 2, 3, 4 und 5 ml Quecksilberstandardlösung durchgeführt.

Zur Analyse wird die Probelösung mit 5 ml 0.1 *M* EDTA-Lösung, 10 ml 6 *M*

Salpetersäure und 10 ml Reagenslösung 3 Min geschüttelt. Die wässrige Phase wird verworfen und die organische Phase zweimal mit je 20 ml 3 *M* Kalilauge 1 Min geschüttelt. Nach dem Zentrifugieren misst man die Extinktion in 1 cm-Küvetten bei 360 nm gegen eine analog behandelte Blindlösung.

Thalliumbestimmung

Als günstiger Arbeitsbereich erwiesen sich 0.1–1.0 μmol Thallium. Die Testung des Verfahrens wurde mit 1, 2, 4, 6, 8 und 10 ml Thalliumstandardlösung durchgeführt.

Zur Analyse versetzt man die Probelösung in der angegebenen Reihenfolge mit 3 ml 1 *M* Hydroxylammoniumchlorid-Lösung, 10 ml 3 *M* Kalilauge, die 1 *M* an Cyanid sein muss, und 10 ml Reagenslösung. Nach 5 Min Schüttelzeit wird die organische Phase zweimal mit je 20 ml 3 *M* Kalilauge 1 Min geschüttelt. Die Extinktion der organischen Phase wird bei 415 nm gegen eine analog behandelte Blindlösung in 1 cm-Küvetten gemessen. Konstante Extraktionsbedingungen sind einzuhalten.

Die Ergebnisse der Eichmessungen sind in Fig. 5 dargestellt. Bei der Quecksilberbestimmung ist das Lineargesetz erfüllt. Der Gehalt einer Probe in μmol Quecksilber errechnet sich nach folgender Beziehung: $b=[\omega]\cdot E$.

Für die Thalliumbestimmung wurde das Vorliegen des erweiterten Lineargesetzes ermittelt. Dabei kann der Gehalt einer unbestimmten Probe leicht nach folgender Gleichung berechnet werden: $b=[\omega]\cdot E+C_{\mathrm{A}}$.

TABELLE III

KENNGRÖSSEN ZUR PHOTOMETRISCHEN BESTIMMUNG VON THALLIUM UND QUECKSILBER MIT THIODIBENZOYLMETHAN

	Hg	*Tl*
Verfahrenskonstante (ω) (μmol)	0.48	1.0
Prakt. Extinktionskoeffizient (ε) (cm^2 μmol^{-1})	20.6	10.0
C_{A} (μmol)		+0.06
Standardabweichung S_{K} (μmol cm^{-3})	±0.00035	±0.003
Varianzen $V(b_0\text{–}b_{\mathrm{u}})$ (Rel.%)	±0.7–7.0	±0.3–3.0
Fehlerbereiche $T(S=99\%)$	±0.0009	±0.008
$T(S=99.9\%)$ (μmol cm^{-3})	±0.0013	±0.011

Wichtige Kenngrössen der Verfahren sind in Tabelle III zusammengefasst.

ZUSAMMENFASSUNG

Thiodibenzoylmethan ist zur extraktionsphotometrischen Spurenanalyse von Quecksilber (0.05 bis 0.5 μmol) und Thallium (0.1 bis 1.0 μmol) gut geeignet. Beide Verfahren lassen sich bei Einsatz geeigneter Maskierungsmittel hochselektiv gestalten und sind mit geringem Arbeits- und Zeitaufwand leicht durchführbar. Der Einfluss zahlreicher Frendmetalle wie Ga, In, Pb, Sn, As, Sb, Bi, Cd, Zn, Pd, Ag, Cu, Ni, Co, Fe, Mn, Cr, Al, Alkali- und Erdalkalimetalle, sowie der Anionen Fluorid, Chlorid, Bromid, Jodid, Thiocyanat, Sulfid, Sulfat, Nitrat, EDTA und Thioglykolsäure wurde untersucht.

SUMMARY

Thiodibenzoylmethane is a very suitable ligand for the extraction and spectrophotometric determination of traces of mercury (0.05–0.5 μmol) and thallium (0.1–1.0 μmol). Both methods are rapid and can be made highly selective by means of masking reagents. The influence of numerous other metals, *e.g.* Ga, In, Pb, Sn, As, Sb, Bi, Cd, Zn, Pd, Ag, Cu, Ni, Co, Fe, Mn, Cr, alkali and alkaline earth metals, and also of anions as fluoride, chloride, bromide, iodide, thiocyanate, sulphide, sulphate, nitrate, EDTA and thioglycolic acid was examined.

RÉSUMÉ

Le thiodibenzoylméthane est un ligand qui convient très bien pour l'extraction et le dosage spectrophotométrique de traces de mercure (0.05–0.5 μmol) et de thallium (0.1–1.0 μmol). Ces deux méthodes sont rapides et peuvent être rendues très sélectives par addition de réactifs de masquage. On a examiné l'influence de nombreux autres métaux: Ga, In, Pb, Sn, As, Sb, Bi, Cd, Zn, Pd, Ag, Cu, Ni, Co, Fe, Mn, Cr, alcalins et alcalino-terreux, et également celle des anions tels que fluorure, chlorure, bromure, iodure, thiocyanate, sulfure, sulfate, nitrate, EDTA et acide thioglycolique.

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PLASTICIZED OPEN-CELL POLYURETHANE FOAM AS A UNIVERSAL MATRIX FOR ORGANIC REAGENTS IN TRACE ELEMENT PRECONCENTRATION

PART I. COLLECTION OF SILVER TRACES ON DITHIZONE FOAM

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The use of chelating agents in liquid–liquid extraction for the preconcentration of trace elements has been widely investigated[1]. The large choice of chelating agents together with the high possible selectivity, which can be achieved by careful control of the experimental conditions, has allowed several preconcentration processes to be carried out even in the presence of large amounts of other metal ions[1].

Obviously, it is an advantage if the chelating agent can be rendered immobile by retention on or in a solid support, because the liquid–liquid extraction process can then be replaced by a batch solid–liquid extraction or by a column retention process. A column technique which is originally based on a series of successive equilibrations between the metal ion in the aqueous solution (mobile phase) and the chelating agent on or in the solid support (stationary phase) is generally considered to have considerable advantage over a liquid–liquid extraction process, especially when elements of small distribution ratios are collected.

The first attempt to retain a chelating agent on a solid support was described in 1952[2]. It was claimed that cross-linked polystyrene beads can be swelled in a carbon tetrachloride solution of a water-insoluble complexing agent, *e.g.* dithizone, and the swollen resin may be used for the collection and separation of metals from aqueous acid solutions. In 1953, Carritt[3] suggested the application of cellulose acetate loaded with dithizone for the preconcentration of heavy metal ions from sea water. Since the appearance of these works, several investigations have been published describing the application of dithizone loaded on different supports for the separation and preconcentration of trace elements, as summarized in Table I. Although the results obtained in batch and column experiments were very promising, the slowness of operation and the low capacity of these supports are considered serious disadvantages.

In previous publications from this laboratory, the application of open-cell type polyurethane foams in reversed-phase[19–22] and in ion-exchange[23, 24] chromatography has been reported. It was proved that partition, exchange and adsorption processes on the relatively large available surface of the foam material are fast and the hydrodynamic properties of foam-filled columns extremely favourable.

TABLE I

CHRONOLOGICAL SURVEY OF THE ANALYTICAL APPLICATIONS OF SUPPORTED AND SELF-SUPPORTED DITHIZONE

Support (collector)	*Geometrical form*	*Observations*	*Reference*
Styrene–divinylbenzene	beads	collection of traces of metals	2
Styrene–divinylbenzene base anion exchangers	beads	collection of traces of metals	3
Cellulose acetate	granules	sepn. of traces	4
Silica gel	granules	retention of Hg	5, 7
Active carbon	powder	sepn. and enrichment of Pb	6
Cellulose acetate	granules	sepn. of trace In	8
Precipitated dithizone	amorphous precipitate	collection of some metal traces	9
Precipitated dithizone	amorphous precipitate	collection of traces of Ag	10
Cellulose[a]	granules	collection of traces of metals	11
Active carbon	powder	sepn. and enrichment of Ge	12
Active carbon	powder	collection of Cu and Pb traces	13
Kieselguhr	granules	sepn. of some metal traces	14
Dithizone powder	powder	collection of traces of Ag with ultrasonics	15
Styrene–divinylbenzene	beads	macroporous resin 10%. DVB, sepn. of Fe and Co	16
Styrene–divinylbenzene	beads	2% DVB, collection of Hg	17
Trifluoromonochloropolyethylene (Voltalef 300 PL)	granules	sepn. of traces of Hg	18

[a] Dithizone coupled to cellulose was used.

Also, the break-through and the overall capacities of foam columns are quite high in comparison with columns packed with granular supports.

As no work seems to have been done on the application of foam material as a matrix for chelating agents, it was decided to study the possibility of loading open-cell type polyurethane foam with various chelating agents. The present work was also based on the idea of using plasticizers as solvents for the chelating agents. It was expected that loading the foam material with these plasticizers would produce chelating polyurethane foams with higher capacity and better permeability. It was hoped that this new general approach to the preparation of plasticized chelating foams would allow a more rapid separation and preconcentration of a large variety of trace elements.

Plasticizers are defined as essentially non-volatile liquids used to modify synthetic resins[25]. They do not chemically react with the resins, but modify them through the reduction of Van der Waals forces[25]. The plasticized resin is a dynamic system in which the individual plasticizer molecules have varying degrees of mobility within the resin matrix. In recent years, considerable attention has been directed towards the application of plasticizers in the so-called "solvent membranes"[26–28]

As a model for the present study, a solution of dithizone dissolved in various plastizicers and loaded on polyurethane foam was tested for the collection of traces of silver. The effect of different plasticizers, the concentration of dithizone and the pH of the aqueous solution on the collection of silver on the proposed dithizone foam was investigated.

EXPERIMENTAL

Reagents and materials

Unless otherwise stated, all chemicals used were of analytical-reagent grade. Tri-n-butyl phosphate (TBP) was purified as described by Hamlin *et al.*[29]. α-Dinonylphthalate and di-n-octylphthalate (pure grade) were used without further purifications. Polyurethane foam, a polyether of open-cell type was supplied by the North Hungarian Chemical Works, Sajóbábony, Hungary.

Zinc dithizonate stock solutions were prepared by shaking 0.2 g of dithizone in 20-ml solutions of plasticizer or chloroform with 20 ml of 0.2 *M* aqueous solution of zinc sulfate for about 1 h. After the separation of the two phases, the organic layer was washed twice with distilled water. Dilute solutions of zinc dithizonate were prepared daily from the stock solution. Silver nitrate solutions were spiked with carrier-free ^{111}Ag (Institute of Isotopes, Budapest, Hungary). Water purified by distillation and ion exchange was used throughout the work.

Instrumentation

For activity measurement a NaI(Tl) detector and an energy-selective counting device (type NK-107/B, Gamma, Budapest ,Hungary) were employed.

Dithizone foam preparation

The polyurethane foam (cubes of about 5-mm edge) was washed with 1 *M* hydrochloric acid solution followed by distilled water until the washings were free from chloride ion. Then the foam material was washed with acetone and dried at 80°. The dried foam cubes were equilibrated with the required concentration of zinc dithizonate solution (6 ml g^{-1} dry foam) with efficient stirring and then allowed to remain in the zinc dithizonate solution for about 2 h to ensure complete saturation. The loaded foam material was dried between two sheets of filter paper to remove the excess of the zinc dithizonate solution. The red dithizonate foam material was not affected by light or air.

Column preparation

Glass columns of 25-mm diameter and 12-cm length were used; 5 g of the dried loaded foam was packed in the column, by the procedure previously described[20], to produce a 5-cm bed height.

Separation of silver from copper or lead in batch experiments

Aliquots (10 ml) of aqueous solutions, containing different relative concentrations of silver and lead or copper were shaken with 0.1 g of the loaded foam material for 30 min in a mechanical shaker. The uncollected silver was determined by measuring the radioactivity of 2 ml of the aqueous solution, and the extracted silver was calculated by difference. In the case of the separation of silver from copper, an EDTA solution was used as a masking agent for copper. In some experiments the amount of silver retained on the foam was determined directly by shaking the foam material with 10 ml of 0.5 *M* sodium thiosulfate solution for about 30 min and measuring the activity of the leached silver in the aqueous solution.

Preconcentration of silver on foam columns

The spiked silver nitrate solution (1 ml of 1 μg Ag^+ ml^{-1}) was diluted to 1 l with distilled water. This solution was allowed to pass through the foam column at a flow-rate of 50–60 ml min^{-1}. The collected silver was eluted from the foam material by shaking it with 100 ml of 0.5 *M* sodium thiosulfate solution for about 30 min.

RESULTS AND DISCUSSION

Although many methods have been published for the collection of trace elements with dithizone loaded on different solid supports (see Table 1), the general use of these methods is limited by the following factors: the instability of the dithizone columns, the relatively low capacity possible, and the slowness of the rate processes. A reasonable solution to the first problem, involving the use of the dithizone in a chelated form (*e.g.* as zinc dithizonate which is stable for several months) has recently been given[16, 17]. For the other two problems, the application of plasticized polyurethane foam as a matrix for dithizone seemed to offer an acceptable solution.

As expected, polyurethane foam retains considerable amounts of the different plasticizers tested. It was found that the dry loaded foam materials contain 65–70% (w/w) plasticizer solution. Worth mentioning is that the plasticizer solutions containing dithizone are firmly retained in the foam material and they are not leached from columns packed with these loaded foams even when flow-rates as high as 50–60 ml min^{-1} are applied. In order to examine these plasticized dithizone foams for the rapid collection of silver traces from aqueous solution, a detailed study of various factors was carried out.

Effect of the shaking time on collection of silver on polyurethane foam loaded with zinc dithizonate dissolved in various plasticizers and chloroform

The dependence of the percentage collection of silver with zinc dithizonate-loaded polyurethane foam on shaking time was investigated for three solutions of 1% zinc dithizonate in different plasticizers and chloroform. The curves of Fig. 1 represent the results obtained for the extraction of 1 μg of silver in 10 ml of aqueous solution (pH *ca.* 6.5) with 0.1 g of loaded foam. The curves show that the collection rate of silver with plasticized foam materials is much faster than with the unplasticized one (chloroform). Also the effect of the plasticizer decreases in the order, α-dinonylphthalate > tri-n-butylphosphate > di-n-octylphthalate.

These results may be explained on the basis of the following general considerations. In liquid–liquid extraction, the rate of extraction of a metal chelate depends on the rate of the chemical reactions occurring in the system and the rate of mass transfer between the two phases[30]. Furthermore, it has been assumed[30] that the rate-controlling step is the rate of formation of the chelate complex. However, in the case of dithizone foam an additional factor must be considered.

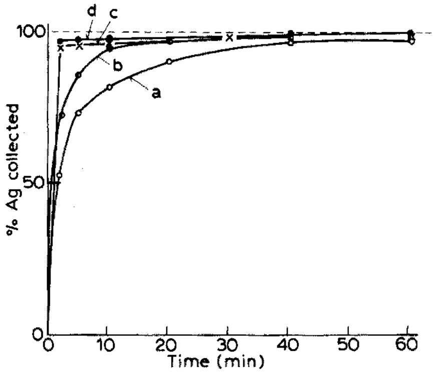


Fig. 1. Effect of shaking time on collection of silver by polyurethane foam loaded with zinc dithizonate in various plasticizers and chloroform. The loaded foam contained in each case 1% zinc dithizonate, in (a) $CHCl_3$, (b) di-n-octylphthalate, (c) TBP and (d) α-di-n-nonylphthalate.

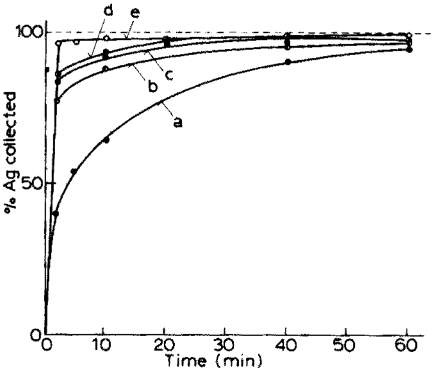
This is the diffusion of the metal ions in the gel phase, which in turn depends on the degree of permeability of the plastic foam material. The larger the permeability of the foam material, the higher the possibility of metal ion–complexing agent interaction and consequently the higher the rate of extraction.

Kumins *et al.*[31] studied the diffusion of water vapour through unplasticized and plasticized vinyl chloride–vinyl acetate copolymer. They found that water moves faster through the plasticized film than through the unplasticized one. A similar effect is considered to be present in the case of plasticized dithizone foam, and consequently rapid equilibrium between the metal ion in the aqueous solution and the chelating agent in the organic one is attained.

As is evident from Fig. 1, silver recoveries with plasticized foam reach about 98% after shaking times of *ca.* 10 min. These results are even better than those obtained by Fukuda and Mizuike[15] who proposed the collection of silver traces on powdered dithizone in an ultrasonic field; the application of ultrasonics accelerated the rate of retention of silver on dithizone powder. One of the most serious disadvantages of this method is the increase in the solubility of dithizone powder with increase in ultrasonic irradiation time, which causes a sharp decrease in silver recoveries. This together with the unavailability of ultrasonic generators in normal analytical laboratories limits the application of this method, especially for routine purposes. No such disadvantages were encountered in the case of the proposed dithizone foam. It is worth mentioning that the collection of silver from neutral, aqueous and nitric acid solutions on plasticized polyurethane foam, in the absence of dithizone, was found to be negligible under the experimental conditions used.

Effect of pH on the collection rate of silver by zinc dithizonate foam plasticized with α-dinonylphthalate and TBP

The results obtained for the collection of 1 μg of silver by 0.1 g of foam plasticized with α-dinonylphthalate or TBP (zinc form) at various pH values are given in Figs. 2 and 3, respectively. As is obvious from the curves, the collection rate of silver by the plasticized foam was generally decreased by lowering



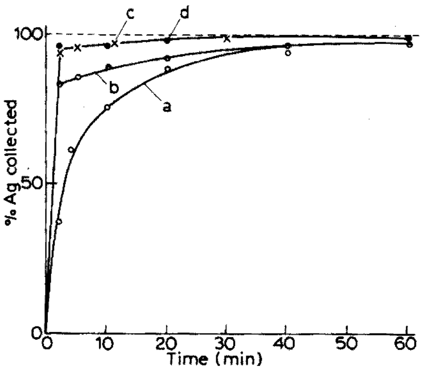
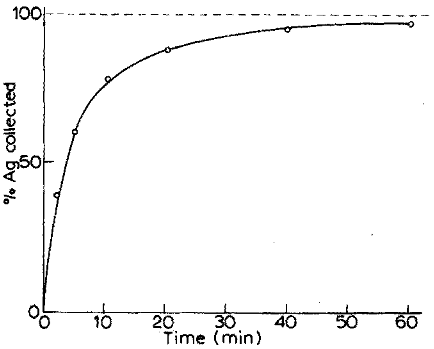


Fig. 2. Effect of pH on the collection rate of silver by α-di-n-nonylphthalate-plasticized zinc dithizonate foam. pH: (a) 0.16, (b) 1.82, (c) 3.72, (d) 5.32 and (e) 6.59.

Fig. 3. Effect of pH on the collection rate of silver by TBP-plasticized zinc dithizonate foam. pH: (a) 0.16, (b) 2.84, (c) 5.47 and (d) 6.59.

the pH of the aqueous solution. At pH values between 4.5 and 6.5, the best extraction rate was obtained. At lower pH values (<4.5), the collection rate was significantly affected. Also, zinc was leached from the foam material, as indicated by a change in the colour of the foam from red to green caused by the instability of zinc dithizonate at low pH values.

The extraction of silver with TBP-plasticized dithizone foams was studied under the same experimental conditions as those applied above. The curves of the collection rate of silver were found to be more or less the same at all the pH values tested. For example, Fig. 4. shows the results obtained at pH 6.6. It is clear that the rate curve is identical to that obtained with the plasticized zinc dithizonate foam at pH 0.16 (*cf*. Fig. 3). These results suggest that zinc leached from the foam materials at lower pH values has no effect on the collection of silver.



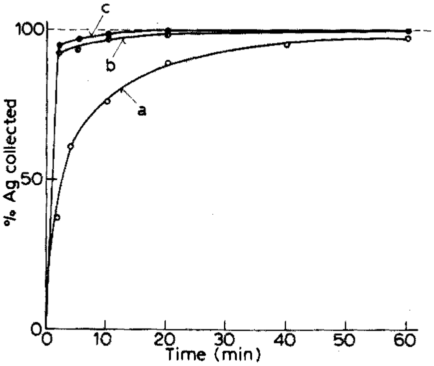


Fig. 4. Collection rate of silver by TBP-plasticized dithizone foam (in the free form) from neutral aqueous solution, pH 6.6.

Fig. 5. Effect of zinc dithizonate concentration in TBP-plasticized foam on the collection rate of silver. (a) $3.9 \cdot 10^{-2}$, (b) $3.9 \cdot 10^{-3}$ and (c) $3.9 \cdot 10^{-4}$ *M*.

The lower retention rate of free dithizone foams may be attributed to the probable formation of an intermolecular hydrogen-bonding between the free dithizone molecule and the electron-donating atoms of the plasticizers. Evidently, this hydrogen-bonding, which is not present for zinc dithizonate foam, delays the formation of the silver dithizonate complex and consequently affects the collection rate.

It is of interest to note that the collection of silver with chloroform-swollen dithizone foam in the free or zinc form was found to be the same. This is probably related to the fact that the possibility of formation of hydrogen-bonding between dithizone and chloroform is relatively low.

Effect of dithizone concentration in the plasticized foam matrix on the collection rate of silver

Owing to the extremely high collection rate of silver from neutral solutions on the plasticized zinc dithizonate foam, the collection experiments were made from 0.1 *M* nitric acid solutions in attempts to establish the effect of the concentration of zinc dithizonate on the foam. The results (Fig. 5) indicate that the collection rate for 0.01 μg of silver, in 10 ml of 0.1 *M* nitric acid solution with 0.1 g of TBP-plasticized foam containing various concentrations of zinc dithizonate, is increased by a decrease in the chelating agent concentration. This may be due to the concomitant increase in the plasticizer/dithizone ratio. As can be seen from curve c, the retention of silver on polyurethane foam loaded with $3.9 \cdot 10^{-4}$ *M* zinc dithizonate in TBP solution reaches *ca.* 99% after 10-min shaking. In fact, this result extends the useful application of the plasticized zinc dithizonate foam to the collection of silver from dilute acid solutions, which is considered of special practical importance.

Effect of dithizone concentration on the rate of recovery of silver from TBP-plasticized dithizone foam

In preliminary experiments, attempts were made to recover silver from the plasticized-foam material with a wide range of concentrations of mineral acids, ammonia solution and complexing agents, *e.g.* potassium cyanide, potassium thiocyanate and sodium thiosulfate. It was found that 0.5 *M* sodium thiosulfate solution gave the best recovery of silver in the shortest time of shaking. Consequently, the dependence of the rate of recovery of silver on the concentration of dithizone in the plasticized foam was investigated for this reagent. In separate experiments, 0.1 g of loaded foam containing 0.5 μg of silver was mixed with 10 ml of 0.5 *M* sodium thiosulfate solution in 25-ml stoppered flasks. The flasks were then shaken for different times (1–30 min) and silver was determined in the aqueous phase by measuring its radioactivity. The amount of silver retained on the foam was calculated by difference. As is evident from Fig. 6., the rate of recovery of silver from the foam material which contained the lowest concentration of dithizone was the highest.

Break-through capacity

The break-through capacity of a column (5-cm length and 1-cm internal diameter) packed with 1 g of TBP-plasticized zinc dithizonate foam (loaded with 1%

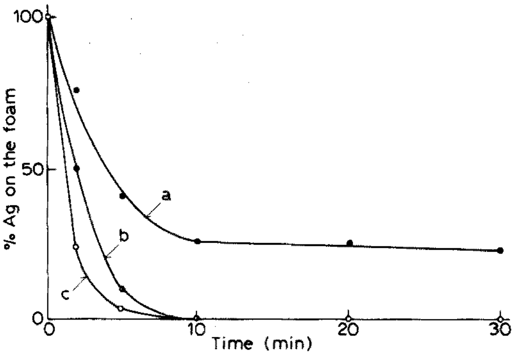


Fig. 6. Effect of zinc dithizonate concentration on the rate of recovery of silver from TBP-plasticized foam with 0.5 M $Na_2S_2O_3$ solution. (a) $3.9\cdot10^{-2}$, (b) $3.9\cdot10^{-3}$ and (c) $3.9\cdot10^{-4}$ M.

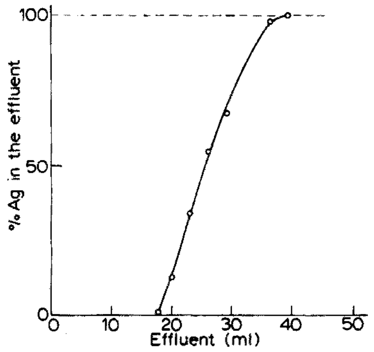


Fig. 7. Break-through capacity curve.

zinc dithizonate solution) was determined by allowing a solution of silver containing 100 μg ml^{-1} to pass through it at a flow-rate of 2 ml min^{-1}. The results are represented in Fig. 7. The break-through and the overall capacities as calculated from this curve are 1.25 and 2 mg of silver ion, respectively. These capacities are quite reasonable in comparison with those reported previously with different dithizone-loaded supports[2–17].

Separation of traces of silver from lead and copper

Separation of silver from a wide range of relative concentrations of lead and copper was carried out in batch experiments. Complete collection of silver was obtained in the presence of as high as six orders of magnitude of lead (*cf.* Table II) but in the case of copper, low recoveries were observed. However, addition of a masking agent, *e.g.* EDTA, yielded a quantitative separation of silver from copper even in the presence of a high excess of the latter (Table II).

As is clear from the results reported in Table II, the collection of carrier-free silver in the presence of 1 mg of lead or copper by the TBP-plasticized zinc dithizonate foam is also possible.

Collection of silver traces by columns packed with TBP-plasticized zinc dithizonate foam

Obviously, the practical usefulness of the TBP-plasticized dithizone foam will be extended by applying it in column operation. The collection of various concentrations of silver on short foam columns was successfully carried out. The rapid attainment of equilibrium between silver and the plasticized foam material allowed the application of relatively high flow-rates (50–60 ml min^{-1}) without affecting the quantitativeness of the operation (Table III).

Finally, preconcentration of small amounts of silver from dilute solutions was examined with 5 g of TBP-plasticized foam (loaded with $3.9\cdot10^{-4}$ M zinc dithizonate solution) in a short column. More than 95% of the 1 μg of silver in 1 l aqueous solution was rapidly collected by passing it through the foam columns at a flow-rate of 50–60 ml min^{-1}.

TABLE II

COLLECTION OF TRACES OF SILVER IN THE PRESENCE OF VARIOUS CONCENTRATIONS OF LEAD AND COPPER

Matrix	*Amount*		*Ag in matrix (p.p.m.)*	*Average Ag^+ in aq. soln.*[a] *(%)*	*Average Ag^+ on foam (%)*	*Variance*	s^b	s_r^c	*r.s.e.*[d] *t: 0.05*	*Bias*
	Ag^+ (µg)	*matrix (mg)*								
H_2O	100	–	10	0.7	99.3	0.05	0.224	0.226	0.36	−0.7±0.06
H_2O	1	–	0.1	1.4	98.6	0.43	0.656	0.665	1.06	−1.4±1.06
H_2O	0.01	–	0.001	1.2	98.8	0.44	0.663	0.671	1.08	−1.2±1.08
H_2O	carrier-free	–	–	1.9	98.1	0.64	0.800	0.812	1.29	−1.9±1.29
Cu^{2+}	0.01	0.1	100	1.6	98.4	0.36	0.600	0.609	0.97	−1.6±0.97
Cu^{2+}	0.01	1.0	10	2.2	97.8	0.26	0.509	0.520	0.83	−2.2±0.83
Cu^{2+}	0.01	10.0	1	2.5	97.5	2.45	1.565	1.616	2.57	−2.5±2.57
Cu^{2+}	carrier-free	1.0	–	2.0	98.0	0.58	0.761	0.777	1.24	−2.0±1.24
Pb^{2+}	0.01	0.1	100	2.3	97.7	0.34	0.583	0.597	0.95	−2.3±0.95
Pb^{2+}	0.01	1.0	10	1.8	98.2	1.29	1.179	1.201	1.91	−1.8±1.91
Pb^{2+}	0.01	10.0	1	2.9	97.1	3.56	1.887	1.943	3.09	−2.9±3.09
Pb^{2+}	carrier-free	1.0	–	0.8	99.2	0.07	0.265	0.261	0.42	−0.8±0.42

[a] Average of 4 determinations.
[b] s: standard deviation.
[c] s_r: standard error of the average$=(s/\bar{x})\times 100$.
[d] *r.s.e.*: relative standard error of the average$=s_r\times t/\sqrt{N}$.

TABLE III

COLLECTION OF VARIOUS CONCENTRATIONS OF SILVER ON COLUMNS PACKED WITH TBP-PLASTICIZED ZINC DITHIZONATE FOAM

(The foam contained a 1% solution. The flow rate was 50–60 ml min^{-1}).

Amount of Ag taken (μg)	*No. of determinations*	*Average Ag^+ in effluent (%)*	*Average Ag^+ retained on foam*	*Variance*	*s*	s_r	*r.s.e. t: 0.05*	*Bias*
100.0	5	0.5	99.5	0.023	0.15	0.15	0.19	0.5 ± 0.19
1.0	5	2.4	97.6	0.488	0.70	0.72	0.89	2.4 ± 0.89
0.01	4	2.4	97.6	0.217	0.47	0.49	0.76	2.4 ± 0.76

SUMMARY

The preparation of plasticized foam containing dithizone or zinc dithizonate is described. The foam was used successfully for the rapid and quantitative collection of trace amounts of silver in batch and column experiments. The preconcentration of small amounts of silver from dilute aqueous solutions was found to be possible. The effects of different plasticizers, pH of the aqueous phase, and dithizone concentration in the foam, on the collection rate of silver were critically investigated.

RÉSUMÉ

Une méthode est décrite pour la préparation d'une mousse plastifiée contenant dithizone ou dithizonate de zinc. La mousse est utilisée avec succès pour retenir quantitativement et rapidement des traces d'argent. Une préconcentration est possible. On examine différents facteurs pouvant influence la vitesse d'absorption de l'argent: nature des plastifiants, pH de la phase aqueuse, concentration de dithizone dans la mousse.

ZUSAMMENFASSUNG

Die Herstellung eines plastifizierten, Dithizon oder Zinkdithizonat enthaltenden Schaums wird beschrieben. Der Schaum wurde mit Erfolg für die schnelle und quantitative Sammlung von Silberspuren nach dem satzweisen und nach dem Säulenverfahren verwendet. Die Anreicherung kleiner Mengen Silber aus verdünnten wässrigen Lösungen ist möglich. Der Einfluss verschiedener Weichmacher, des pH-Wertes der wässrigen Phase und der Dithizonkonzentration im Schaum auf die Geschwindigkeit der Silberansammlung wurde kritisch untersucht.

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ANALYSIS FOR CHROMIUM TRACES IN NATURAL WATERS

PART I. PRECONCENTRATION OF CHROMATE FROM P.P.B. LEVELS IN AQUEOUS SOLUTIONS BY ION EXCHANGE*

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Chromium(VI) is recognized as a serious health hazard for workers in chromium-plating plants[1], but has so far received little attention as an environmental pollutant in natural waters. Virtually nothing is known with regard to possible long-range toxic effects which low concentrations of chromates may exert upon aquatic life and, through the food chain, upon humans. However, chromium(VI) inhibits the growth of algae over a concentration range of 0.03–6.4 p.p.m., while it may stimulate growth when present at lower concentrations[2,3]. The mechanism of the toxicity of sub-p.p.m. concentrations of chromate is not yet understood, but may have to do with chromate masquerading as sulfate and thus interfering with the enzymatic sulfur-uptake mechanism of the cell.

Sources of chromium in the environment are waste chromates from electroplating, corrosion inhibitors from water-cooled heat-exchange systems, waste solutions from oxidative dyeing, and leachings from sanitary land fills. Although the actual concentration of chromate in cooling liquids ranges only from 250 to 5.000 p.p.m., a single cooling tower of moderate size (recirculation rate of 10.000 gallons $\min^{-1}$ and blow-down rate of 100 gallons $\min^{-1}$) will discharge more than 7 tons of chromate annually[4] which would have to be dissolved in more than 100 billion liters of water to meet U.S. Public Health Service standards for potable water[5]. Fortunately, not all of the chromium released by industrial plants is chromium(VI). In many cases, waste solutions are subjected to reduction so that the less toxic chromium(III) enters the environment. Chromium(III) probably exists in natural waters in the form of many different species, hydrolyzed, complexed, and some even adsorbed on colloidal matter. Such species decompose on the addition of acid, so that in the analysis of waters, one will have a mixture of anionic chromate and cationic chromium(III) species, of which the highly toxic chromates are of primary concern.

An analytical method, in order to be suitable for use in a systematic study of the fate of, and the effects arising from, chromium traces in natural water

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systems, must be extremely sensitive. The (total) chromium concentration in "unpolluted" rivers is in the range 0–50 p.p.b.[6], while sea water contains around 0.04 p.p.b.[7]. The only method suitable for the direct detection of such low concentration levels would be chemiluminescence[8] which, unfortunately, suffers from serious interferences. Conventional methods[9–11] for sub-p.p.b. levels of chromium and even neutron activation analysis[12] must employ a pre-concentration step. A convenient, simple, and very efficient procedure for the concentration of chromate from aqueous solution by ion exchange is described in this paper.

EXPERIMENTAL

Equipment

Chromium determinations were done on a Perkin-Elmer 303 Atomic Absorption Spectrophotometer, with a Honeywell Electronix 194 recorder. A "Fractomat" automatic fraction collector (Buchler Instruments, Fort Lee, New Jersey) with siphon delivery tubes was used in most of the experiments. The exact volumes of the fractions were determined by weighing (Oertling balance, Model R-20). A Beckman Expandomatic pH meter was used for all pH measurements.

Materials

Potassium dichromate and all other chemicals used were reagent grade. The macroporous anion-exchange resins XN 1002 and IRA 900 (Rohm and Haas, Inc.; experimental samples) and the regular ion-exchange resin AG1-X4 (strongly basic anion exchanger, analytical grade; Bio-Rad Laboratories) were used in the chloride form. Deionized water was distilled from an all-Pyrex still and then used for dilutions and for rinsing the nitric acid-washed glassware.

Procedures

General. Ion-exchange resins were allowed to swell for at least 48 h in deionized distilled water before use. Except for cases where the effects of pH were of interest, all solutions containing chromate were adjusted to pH 5.0 with 0.1 *M* nitric acid and were made 20 p.p.m. in sulfate. Thus when the concentration of the chromate ion was 1 p.p.m. (as chromium), the sulfate ion was in *ca.* 10-fold (molar) excess. When the chromate concentration was 0.050 p.p.m. (50 p.p.b.), sulfate was present in *ca.* 200-fold excess. The sulfate was included in order to simulate one of the characteristics of natural waters which would have to be taken into account when concentrating the chromate on an anion-exchange resin.

Atomic-absorption spectrometry. A reducing flame (yellow) was used to maximize the chromium signal[13] at the 357.7-nm line. Eluent blanks were used for zeroing, and separate calibration curves were constructed for different runs. The linear portion of the calibration curves extended to about 15 p.p.m.

Column experiments. In preliminary runs, 1-l samples containing 1 p.p.m. or 50 p.p.b. chromate were adjusted to pH values over the range 3.0–7.0 and passed down 4 × 0.5-cm columns packed with AG1-X4 resin. The resin beds were then pushed out of the columns and divided into several segments of equal thickness, each of which was eluted separately with 50 ml of 1 *M* sodium chloride solution;

15-ml fractions were collected and analyzed by atomic absorption. The chromate was found to be adsorbed in a reasonably narrow band on top of the resin beds. Some oxidation of the resin was observed at pH 3.0 and 4.0. Therefore, two different macroporous resins which, on the basis of informal information, exhibit good stability against oxidation, were also tried. However, the results were not too encouraging (see Results and Discussion). At pH 5.0, the 100–200-mesh regular AG1-X4 resin gave good adsorption characteristics and was used in most subsequent experiments. The major remaining drawback was the long time required for the elution of chromate from the columns, a problem common to most ion-exchange procedures. This was, however, alleviated by using a modified column and an inverted flow technique: as illustrated schematically in Fig. 1, the chromate solution was passed through the resin bed in ascending flow so that sorption occurred in a narrow band at the bottom. After a few ml of acidified water had been passed, the siphon tube was removed from the upper reservoir, and the reservoir was emptied and filled with eluent (1 *M* sodium chloride). The 2-way stopcock was turned and elution was carried out in the descending mode. A further significant improvement in elution time and concentration factor for chromate was achieved by means of *in situ* reduction of chromate on the column during elution. This new

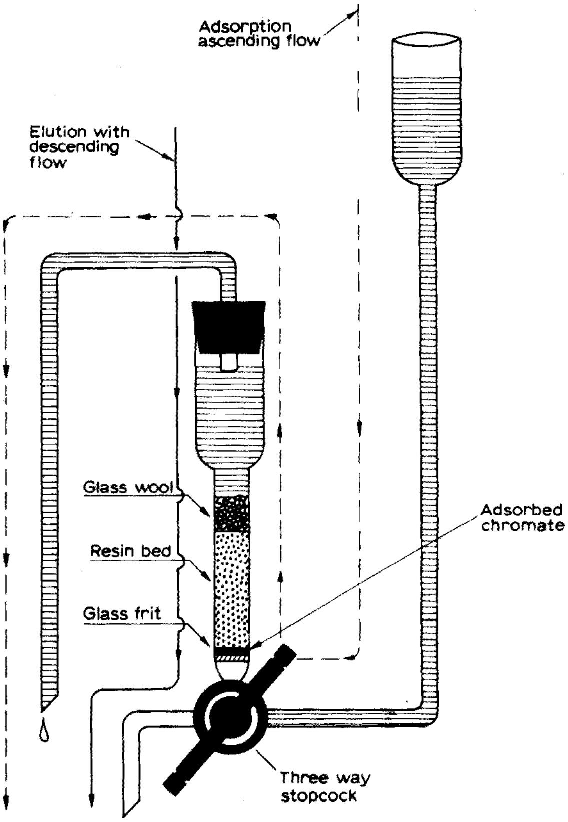

Fig. 1. Adsorption and elution in opposite flow directions; this minimizes elution volume, contact time (oxidation of resin), and band spreading.

approach will henceforth be termed reactive ion exchange elution. Various reducing eluents (iron(II), tin(II), formaldehyde, etc.) were successful, and the choice will depend to some extent on the method to be used for the final determination of chromium. The results shown below were obtained when the reducing eluent was a 0.5 *M* iron(II) ammonium sulfate solution in 1.0 *M* hydrochloric acid.

Suggested preconcentration procedure. Adjust the water sample to pH 5.0 with nitric acid*, and, using the modified column (Fig. 1), adsorb chromate in the ascending mode at a flow rate of 4 ml min^{-1} (*e.g.* overnight). Wash with 10 ml of deionized distilled water acidified* to pH 5.0, empty the upper part of the column, and refill with eluent. Elute in descending flow at a rate of about 0.5 ml min^{-1}, starting to collect immediately. About 30 ml of 1.0 *M* sodium chloride (conventional eluent) or 10 ml of 0.5 *M* iron(II) ammonium sulfate in 1.0 *M* hydrochloric acid suffice to elute up to 1000 μg of chromium adsorbed as chromate.

Repeat runs showed this procedure to have a reproducibility of better than ±5% relative and an average yield of 90% or better of the chromate present at the p.p.b. level. [The substitution of 2.0 *M* formaldehyde solution for iron(II) eliminated signal depression if atomic absorption was used for analysis. However, in the absence of iron, the hydrochloric acid should be replaced by 1.0 *M* sulfuric acid in order to avoid formation of chlorocomplexes of chromium(III) on the column.]

RESULTS AND DISCUSSION

Figures 2–4 illustrate typical elution behavior under the different conditions studied. Table I contains representative data from the experiments discussed here as well as some data from additional experiments not specifically mentioned.

TABLE I

REPRESENTATIVE RESULTS

(A: adsorption of chromate in descending flow; elution down with 1 *M* NaCl. B: adsorption of chromate in ascending flow; elution with 1 *M* NaCl. C: adsorption of chromate in ascending flow with reactive ion-exchange elution with iron(II) solution.)

	Resin	*Mesh*	*Cr (p.p.b.)*	*Concentration factor*	*Yield (%)*
A	XN 1002	20–40	50	20	43
	XN 1002	20–40	50	15	61
	IRA 900	20–40	50	22	71
	AG1-X4	100–200	50	Resin shrinking caused extensive spreading, very poor	
	AG1-X4	100–200	50		
B	XN 1002	20–40	50	30	89
	IRA 900	20–40	50	13	60
	XN 1002	30–40	50	30	91
	AG1-X4	100–200	50	60	88
C	AG1-X4	100–200	50	100	90
	AG1-X4	100–200	1000	100	90

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* No acid was added to natural river waters after it had been established that in the time from sampling through the entire procedure until final determination no losses occurred.

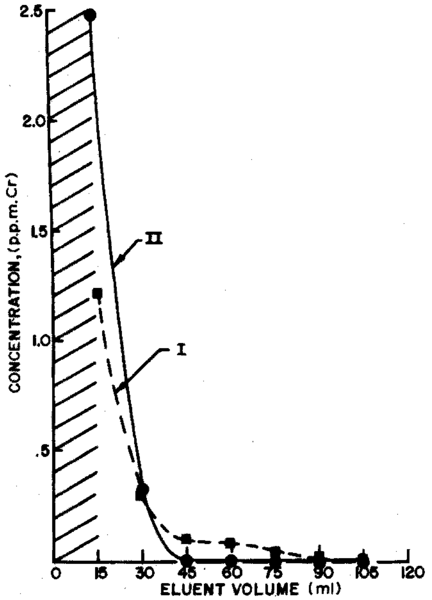

Fig. 2. Elution from macroporous resin XN 1002 (20–40-mesh) after adsorption of chromate during descending flow (I) or ascending flow (II). [Cr], 50 p.p.b.; pH 5.0; eluent, 1 *M* NaCl. The yield was 61.4% for I, and 89.4% for II.

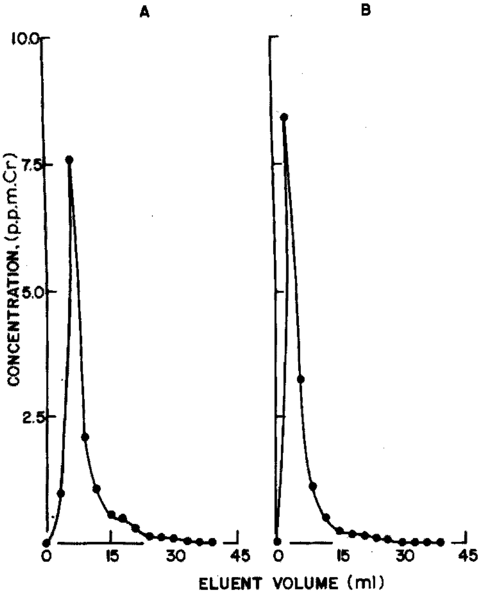

Fig. 3. Performance of regular 100–200-mesh, AG1-X4 resin (B) and of 40-mesh macroporous resin XN 1002 (A) with ascending flow during adsorption in both cases. [Cr], 50 p.p.b.; pH 5.0; eluent, 1 *M* NaCl. The yield was 88.2% for B and 90.5% for A.

Adsorption of chromate during ascending flow always gave better results than adsorption in descending flow. In both modes, the chromate peaks were quite sharp on macroreticular resin beds at pH values above 5.0, while some peak broadening occurred at pH 4 and 3. This is readily understood since at lower pH the equilibrium $CrO_4^{2-} + H^+ \rightleftharpoons HCrO_4^-$ will be shifted more to the right, resulting in a reduced preference at the anion-exchange resin for chromium(VI) compared to chloride and sulfate. $HCrO_4^-$ spends a much greater time in the mobile phase than CrO_4^{2-}, and peak spreading results. Because of the broadening and because of some oxidation of resin by chromate at the lower pH values (as evidenced by discoloration of the resin), a pH of 5.0 was selected*. Another reason for this choice was that the procedure for chromium(VI) preconcentration should lend itself to incorporation into future Cr(III)/Cr(VI) differential analytical methods (now being developed). In such a case the danger of formation of extensively hydrolyzed aquochromium(III) species would arise at pH values much greater than 5. Neutral species might be formed which could sorb onto the anion-exchange resin (neutral adsorption) instead of passing through. High chromate [and low chromium(III)] values would be the result.

As noted before, a number of macroporous resins were first tried in yield studies, since it had been suggested that they are of superior resistance to attack

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* However, see footnote on page 100.

by oxidants. As seen from Fig. 2 with XN-1002 resin, the ascending adsorption procedure (curve II) was superior to the descending adsorption technique (curve I), although 45 ml of eluent were still required. A significant improvement was expectedly achieved for the XN-1002 resin when, instead of all bead sizes (20–40 mesh), only a narrow fraction ($\geqslant$40 mesh) of particle sizes was used (Fig. 3A). However, as shown by Fig. 3B, a regular strongly basic anion-exchange resin gave similar results in the ascending mode. In all these experiments, artificial water samples of 1-l volume with a concentration of 50 p.p.b. chromium (present as chromate) and 20 p.p.m. sulfate were used. The recovery of chromium was usually in the low 90% range, the *ca.* 10% loss apparently being due to inevitable reduction of a constant fraction of chromium(VI) during the adsorption process (any chromium(III) formed presumably passes through the column and is lost).

The descending adsorption procedure suffered from a particular shortcoming, owing to the tremendous change in ionic strength from virtually zero (water sample) to 1 *M* sodium chloride solution. At the moment of introduction of the eluent solution, sudden dehydration of the AG1-X4 resin occurred and the beads shrank about 10–15% in volume. At the same time, the yellow chromate band visibly broadened to a considerable degree. It is presumed that this band-spreading was caused by formation of channels in the resin bed while each individual bead was shrinking. When the resin beads had reached swelling equilibrium and the beads had settled, the elution proceeded with 2 distinct yellow chromate bands (may be due to different species) moving down the column. All effects of resin shrinkage are negligible in the ascending procedure, since the chromate is then adsorbed at the very exit of the column during the elution step, and channelling cannot produce band separation or any significant broadening. This effect was not observed with the macroporous exchange resins, presumably because these resins possess a rigid matrix which is not subject to the normal swelling and shrinking phenomena.

The adverse effects in the descending adsorption mode were more evident at the higher concentration levels of chromate used (*ca.* 1000 p.p.b.). In these experiments, more than 100 ml of 1 *M* sodium chloride had to be passed through the resin bed to remove the chromate. Thus, only the use of ascending adsorption seemed encouraging for practical applications. However, a very important improvement was made by the introduction of the reactive ion-exchange concept.

The reduction of adsorbed chromate *in situ* on the column made elution significantly easier, because it converted the counter-ion (chromate) to a co-ion (chromium(III)) which has no tendency at all to adsorb on the anion exchanger. With iron(II), the reduction of chromate requires a large quantity of acid. It is interesting to watch the elution when this reactive ion-exchange procedure is used. The yellow chromate band turns green and begins to elute quickly; it then turns light brown and then dark brown. Some chlorocomplexes of iron(III) may be formed during the elution. The use of this reactive ion exchange is not limited to chromate, anion exchangers, and iron(II). It has recently been shown that this principle can be applied with various species in many different situations[14]. In the present case, the use of formaldehyde instead of iron(II) has some merit, because signal depression is caused in atomic absorption by the presence of iron in the flame. The use of reactive ion exchange increases the overall efficiency of the

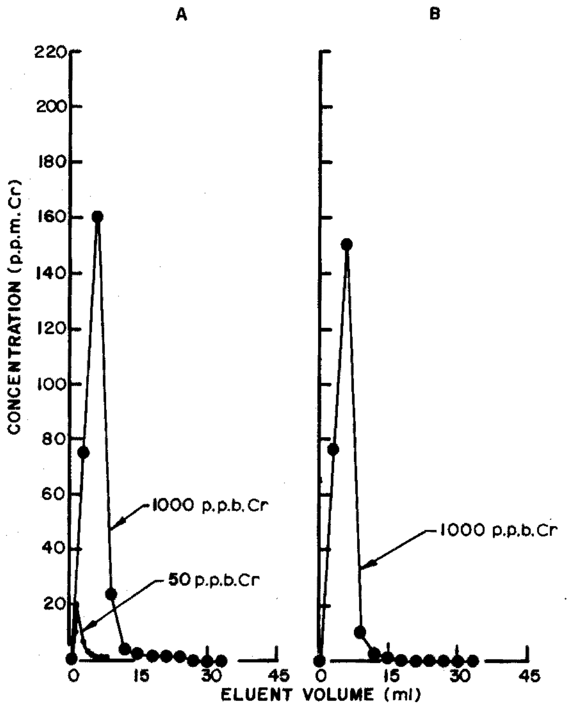

Fig. 4. Elution peaks obtained with the reactive ion-exchange procedure by ascending adsorption (A) or descending adsorption (B). Resin, AG1-X4, 100–200 mesh; pH 5.0; eluent, 0.5 *M* iron(II) in 1 *M* HCl. The yield was 77.5% for descending adsorption but 89.5–89.6% for ascending adsorption.

preconcentration procedure and cuts down elution time significantly. Actually, Fig. 4 illustrates the fact that when reactive ion exchange is used, the descending mode (Fig. 4B) is as rapid as the descending mode (Fig. 4A), although the yield of the descending mode was rather low at the concentration of 1000 p.p.b. The loss, exceeding 10%, may be due to re-adsorption on the anion-exchange column of chromium(III) in the form of anionic chloro-complexes during the elution through the entire column (after descending adsorption), as was suggested by the results of prolonged elutions.

In conclusion, it can be said that the use of the proposed procedure in conjunction with a Perkin-Elmer 303 atomic absorption spectrophotometer has made possible the determination of 0.1 p.p.b. chromate in 1-l samples of water with a precision of ±20% or better. The use of larger water samples should permit the determination of even lower chromate concentrations.

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SUMMARY

A new procedure for the preconcentration of p.p.b. concentrations of chromate from aqueous solutions has been developed. Water samples containing chromate are acidified to pH 5 and passed through an anion-exchange resin bed

(AG1-X4, 100–200 mesh, Cl^- form) in ascending flow, so that the chromate is adsorbed in a narrow zone at the lower end of the resin bed. The chromate is eluted rapidly with small volumes of an acidic reductant solution which reacts with chromate on the column to form chromium(III) during the actual elution step, thus producing very high concentration factors.

RÉSUMÉ

On propose un nouveau procédé de préconcentration de teneurs en chromate dans des solutions aqueuses, de l'ordre du p.p.b. Les échantillons d'eau à analyser sont acidifiés à un pH de 5 et traités sur résine échangeuse d'anions (AG1-X4, 100–200 mesh, forme Cl^-) en courant ascendant; le chromate est ainsi adsorbé dans une zone étroite à la partie inférieure de la colonne de résine. Le chromate est alors elué rapidement avec de petits volumes d'une solution réductrice acide qui réagit sur la colonne pour donner le chrome(III) au cours de l'élution, produisant ainsi des facteurs de concentration très élevés.

ZUSAMMENFASSUNG

Es wurde ein neues Verfahren für die Anreicherung von Chromat aus wässrigen Lösungen von p.p.b.-Konzentrationen entwickelt. Die chromathaltigen Wasserproben werden auf pH 5 angesäuert und durch ein Anionenaustauscherharz-Bett (AG1-X4, 100–200 mesh, Cl^--Form) in aufsteigender Fliessrichtung gegeben, so dass das Chromat in einer schmalen Zone am unteren Ende des Harzbettes adsorbiert wird. Das Chromat wird schnell mit kleinen Volumina einer sauren Reduktionsmittellösung eluiert, die mit dem Chromat in den Säulen während der wirklichen Elutionsstufe unter Bildung von Chrom(III) reagiert, so dass sich sehr hohe Anreicherungsfaktoren ergeben.

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SEPARATION OF COPPER(II) FROM URANIUM(VI) AND MANY OTHER ELEMENTS BY CATION-EXCHANGE CHROMATOGRAPHY IN ACETONE–HYDROBROMIC ACID MEDIA

IMPROVED SELECTIVE SEPARATION OF COPPER

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It has been shown recently[1] that Zn, Cd, In, Ga, Tl(III), Fe(III), Sn(IV), Se(IV), Te(IV), As(III), Sb(III), Bi(III), Au(III), Pt(IV), Hg(II), Rh(III) and some other elements which form relatively stable chloride complexes or oxy-anions, can be eluted with 0.20 *M* hydrochloric acid in 85% acetone from a column of AG 50W-X8 resin, while copper(II) is retained together with many other elements which have less strong tendencies to chloride complex formation. Copper(II) can then be eluted selectively with 0.50 *M* hydrochloric acid in 85% acetone, while Co(II), Ni(II), Mn(II), the alkali metals, the alkaline earths, the rare earths and many other elements are still retained by the column quantitatively. The only element found to accompany copper(II) was uranium(VI)[1].

Recent systematic studies of cation-exchange distribution coefficients in hydrobromic acid–acetone mixtures[2,3] indicate that at high acetone concentrations uranium(VI) is much more strongly adsorbed from hydrobromic acid–acetone than from hydrochloric acid–acetone mixtures, while copper(II) is even slightly less strongly adsorbed. As a result, the separation of copper(II) from uranium(VI) becomes very easy in hydrobromic acid–acetone media.

Since the merits of hydrobromic acid–acetone mixtures for the selective separation of copper(II) have apparently not received much attention so far, the quantitative aspects of the separation of copper(II) from U(VI), Co(II), Li and the other elements of the group more strongly retained than copper(II) in 0.20 *M* hydrochloric acid in 85% acetone, were investigated in detail.

EXPERIMENTAL

Reagents, solutions and apparatus

Analytical reagent-grade chemicals were used throughout. Standard solutions of the elements were prepared by dissolving the chlorides or other suitable compounds of the elements in 0.2 *M* hydrochloric acid. The solutions contained 1 mmole of the element per 10 ml. Solutions containing smaller concentrations of the elements were prepared by dilution when required.

The resin used was the AG 50W-X8 cation exchanger (Bio-Rad Laboratories, Richmond, Calif.) of 200–400 mesh particle size. Borosilicate glass tubes about 20 mm diameter, fitted with a No. 2 porosity glass sinter and a burette tap at the bottom and a B19 joint at the top, were used as columns.

Distribution coefficients

After elution of the elements forming relatively stable chloride complexes with 0.20 *M* hydrochloric acid in 85% acetone, the following elements will remain adsorbed on the column: Cu(II), U(VI), Co(II), Mn(II), Ni(IV), Zr, Hf, Th, Ti(IV), Al, Sc, Y, La and the rare earths, the alkali and the alkaline earth elements[1]. Vanadium(IV) will also be retained when hydrogen peroxide has not been used in the first elution step. In 0.50 *M* hydrochloric acid containing 85% acetone, copper(II) has a distribution coefficient of about 3.5 while those for uranium(VI) and cobalt(II)—the lowest of the other elements—are 5.8 and about 50, respectively. This promises a good separation of copper(II) from cobalt(II) but not from uranium(VI). The situation changes completely when 0.50 *M* hydrobromic acid in 85% acetone is used. The distribution coefficient for copper(II) drops to about 2.3, while those for uranium(VI) and cobalt(II) rise to about 500 and 450, respectively. The element with the lowest distribution coefficient of those to be separated now is lithium with a coefficient of about 50. Because hydrobromic acid–organic solvent mixtures sometimes tend to have more unfavourable ion-exchange kinetics than the corresponding hydrochloric acid–organic solvent mixtures, some of the relevant elution curves were investigated, and some analyses of synthetic mixtures were carried out.

Elution curves

An experimental elution curve was prepared for the pair copper(II)–lithium because lithium has the lowest separation factor from copper(II) of all the elements more strongly retained than copper(II). A column containing 60 ml (20 g) of AG 50W-X8 resin of 200–400 mesh particle size was prepared and equilibrated with 0.1 *M* hydrochloric acid in 50% acetone. The resin column was about 19 cm in length and had a diameter of 2.0 cm. Adsorption was carried out from about 40 ml of solution containing about 1 mmole of each copper(II) and lithium in 0.1 *M* hydrochloric acid in 50% acetone. The cations were washed onto the resin with 0.1 *M* hydrochloric acid in 50% acetone, and copper(II) was then eluted with 0.50 *M* hydrobromic acid in 85% acetone. Fractions of 25 ml volume were taken with an automatic fractionator, and the elution was continued until the lithium peak had appeared in order to evaluate the amount of free volume between the peaks. A flow-rate of 3.5 ± 0.5 ml min^{-1} was maintained for elution. The fractions were evaporated to dryness and the elements in the fractions were determined by suitable procedures. The experimental elution curve is shown in Fig. 1. No uranium(VI) or cobalt(II) could be detected in the first 1,000 ml of eluate when similar experiments were carried out with the Cu(II)–U(VI) and Cu(II)–Co(II) pairs.

Figure 2 shows an experimental elution curve for the copper(II)–uranium(VI) pair. In this case the elution of copper(II) was stopped after 400 ml of the eluting agent had passed through, and the uranium(VI) was then eluted with 3.0 *M* hydrochloric acid.

An elution curve for a mixture containing 1 mmole each of gallium, which is the most strongly adsorbed element of the group eluted with 0.2 *M* hydrochloric acid in 85% acetone, copper(II) and cobalt(II) is shown in Fig. 3; 400 ml of the respective eluting agents were used for gallium and for copper(II),

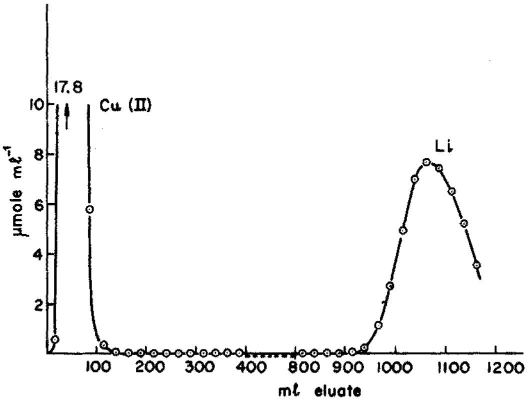

Fig. 1. Elution curve for Cu(II)–Li with 0.50 *M* HBr in 85% acetone. Column of 60 ml (19 × 2.0 cm) AG 50W-X8, resin 200–400 mesh. Flow-rate 3.5 ± 0.5 ml min^{-1}

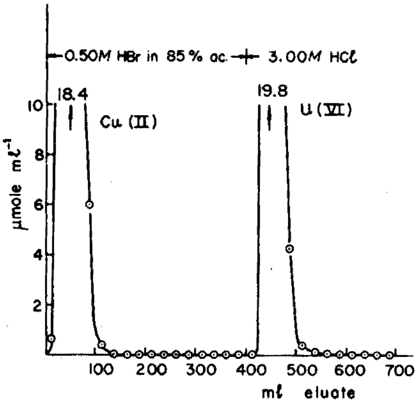

Fig. 2. Elution curve for Cu(II)–U(VI). Column and flow-rate as for Fig. 1.

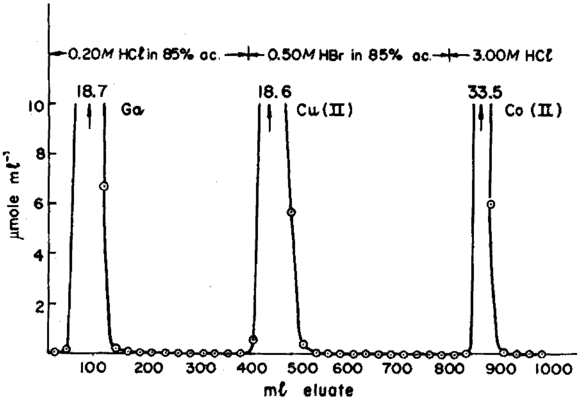

Fig. 3. Elution curve for Ga–Cu(II)–Co(II). Column and flow-rate as for Fig. 1.

but the elution of gallium is quantitative (> 99.9%) with 350 ml and that of copper(II) with less than 250 ml.

Quantitative separations of synthetic mixtures

Amounts of standard solutions of copper(II) and one other element in 0.2 *M* hydrochloric acid were measured out and mixed. An equal volume of acetone was added, and the cations were adsorbed on a column of 60 ml (20 g) of AG 50W-X8 resin of 200–400 mesh particle size, previously equilibrated with 0.1 *M* hydrochloric acid in 50% acetone. Beakers were rinsed three times with the same reagent and the cations were washed on to the columns and completely adsorbed on the resin from this medium before elution was started. Copper(II) was eluted with 250 ml of 0.50 *M* hydrobromic acid in 85% acetone. The eluates were taken from the beginning of the elution step. The acetone was then washed from the column by passing through about 50 ml of 0.1 *M* hydrochloric acid.

Finally lithium and sodium were eluted with 250 ml of 1.00 *M* hydrochloric acid, while U(VI), Co(II), Ni(II), Mn(II), Be, Mg, Ca, Ti(IV) and Al(III) were eluted with 250 ml of 3.00 *M* hydrochloric acid. Flow-rates were kept at 3.5±0.5 ml min^{-1}.

Excess of acetone and acid in the eluates was evaporated on the steam-bath after about 150 ml of water had been added to minimize formation of

TABLE I

ANALYTICAL PROCEDURES

Element	*Method*
Cu(II)	Titration with EDTA, methylthymol blue (MTB) or xylenol orange (XO) indicator in the presence of 1,10-phenanthroline. Small amounts by atomic-absorption spectrometry.
Co(II)	Titration with EDTA, naphthylazoxine S indicator. Small amounts by atomic-absorption spectrometry.
U(VI)	Gravimetrically as U_3O_8 after precipitation with CO_2-free ammonia solution. Small amounts spectrophotometrically with PAR as reagent.
Li, Na	Gravimetrically as Li_2SO_4 and Na_2SO_4. Small amounts by atomic-absorption spectrometry.
Ni(II)	Titration with EDTA, murexide indicator.
Ca, Mn(II)	Titration with EDTA, MTB indicator.
Mg	Titration with EDTA, eriochrome blueblack B indicator.
Al	Excess of EDTA, back-titration with zinc sulphate, xylenol orange indicator.
Ti(IV)	Spectrophotometrically as H_2O_2 complex at high optical density.
Be	Gravimetrically as benzoylacetonate.

TABLE II

RESULTS OF QUANTITATIVE SEPARATIONS

Taken (mg)			*Found*[a] *(mg)*	
Cu	*Other element*		*Cu*	*Other element*
128.0	Co(II)	0.123	128.0 ±0.02	0.123±0.0002
63.99	Co(II)	59.52	63.99 ±0.02	59.52 ±0.02
0.120	Co(II)	119.0	0.120±0.0003	119.0 ±0.02
128.0	U(VI)	0.215	128.0 ±0.02	0.215±0.0006
63.99	U(VI)	239.8	63.99 ±0.02	239.9 ±0.1
0.120	U(VI)	480.9	0.120±0.0003	480.9 ±0.2
63.75	Mg	25.26	63.73 ±0.05	25.23 ±0.05
63.75	Mn(II)	55.04	63.73 ±0.05	55.05 ±0.04
63.75	Ni(II)	58.75	63.76 ±0.03	58.73 ±0.03
63.75	Ca	40.31	63.75 ±0.04	40.31 ±0.02
63.75	Li	34.52	63.76 ±0.05	34.54 ±0.05
63.75	Na	67.21	63.74 ±0.03	67.25 ±0.08
63.75	Ti(IV)	48.14	63.75 ±0.05	48.15 ±0.08
63.75	Be	18.22	63.73 ±0.05	18.21 ±0.02
63.75	Al	54.28	63.74 ±0.03	54.29 ±0.03

[a] Means of triplicate determinations.

condensation products of acetone. Residual organic matter was destroyed when necessary. The amounts of the elements were then determined by suitable analytical procedures. The procedures are listed in Table I and the results are presented in Table II.

DISCUSSION

The method described demonstrates how additional selectivity in a separation by ion-exchange chromatography can be obtained by changing from one eluting system giving a certain selectivity sequence to another system giving a different sequence. When one uses the acetone concentration at which formation of anionic halide complexes becomes prominent and distribution coefficients as a result drop below a value of 10, as an indication of selectivity, the following selectivity sequences with increasing acetone concentrations are obtained.

0.5 M HCl:

$$\mathrm{Tl(III)} \approx \mathrm{Au(III)} \approx \mathrm{Bi(III)} < \mathrm{Cd} \approx \mathrm{In} \approx \mathrm{Sn(IV)} < \mathrm{Zn} < \mathrm{Pb(II)}$$
$$< \mathrm{Fe(III)} < \mathrm{Ga} < \boxed{\mathrm{Cu(II)}} \approx \boxed{\mathrm{U(VI)}} < \mathrm{Co(II)} < \mathrm{Mn(II)} < \mathrm{Ni(II)} \approx \mathrm{Mg}$$

0.5 M HBr:

$$\mathrm{Tl(III)} \approx \mathrm{Au(III)} \approx \mathrm{Bi(III)} < \mathrm{Cd} < \mathrm{Pb(II)} < \mathrm{In} < \mathrm{Sn(IV)} < \mathrm{Zn}$$
$$< \boxed{\mathrm{(Cu(II)}} \approx \mathrm{Fe(III)} < \mathrm{Ga} < \mathrm{Co(II)} < \mathrm{Mn(II)} < \boxed{\mathrm{U(VI)}} \approx \mathrm{Ni(II)} \approx \mathrm{Mg}$$

In hydrochloric acid–acetone mixtures, both 0.5 *M* and also 0.2 *M* in hydrochloric acid, copper (II) is placed between gallium and cobalt(II), and the separation factors from both elements are large enough for satisfactory separations from both by selecting the appropriate acetone concentration[1]. But uranium(VI) shows approximately the same behaviour as copper(II) and quantitative separation of these two is not possible[1]. In the hydrobromic acid–acetone system, copper(II) is adsorbed about as strongly as iron(III) while uranium(VI) shows about no tendency to bromide complex formation. It is therefore possible to elute all elements up to and including gallium, in the hydrochloric acid system, leaving copper(II) and the elements above it in the sequence on the column. After this, a selective elution of copper(II) with hydrobromic acid–acetone can take place with large separation factors. It should be noted that by using the hydrobromic acid–acetone system alone, separation of copper(II) from iron(III) would present difficulties. One advantage of the described approach is that the major part of the resin remains in the hydrogen form in both systems and that the hydrogen ion concentration in the outside solution remains fairly low. The conditions in the resin bed therefore do not change very much and a smooth continuation of the elution process is guaranteed.

The procedure described provides an excellent means for the selective separation of copper from all the elements retained together with it, after the group forming more stable chloride complexes has been eluted with 0.2 *M* hydrochloric acid in 85% acetone[1]. Elution of copper(II) is sharp and quantitative while U(VI), Co(II), Li, Na, Mg(II), Ni(II), Mn(II), Ti(IV), Be and Al are quantitatively retained by the column used (Table II). The separation factors are quite large and considerably smaller columns can be used when only about 1 mequiv. or less of lithium, the element with the smallest separation factor is present. Up to 10 mequiv. of lithium or even larger amounts of the other elements can be accommodated on a 60-ml column.

K, Rb, Cs, Ca, Sr, Ba, V(IV), Zr, Hf, Th, Sc, Y, La and the lanthanides have not been investigated quantitatively, but should accompany uranium(VI), cobalt(II), etc., according to their distribution coefficients which are much higher than those of lithium. By adsorbing from 0.50 *M* hydrobromic acid it should be possible to separate microgram amounts of U(VI), Co(II), Mn(II), Ni(II), Mg, Ca, Al, Ti(IV) and many other elements from several grams of copper(II), which passes through the column.

SUMMARY

Co(II), Ni(II), Mn(II), Li, Na, K, Rb, Cs, Be, Mg, Ca, Sr, Ba, Ti(IV), V(IV), Zr, Hf, Th, Al, Sc, Y, La, the lanthanides and also U(VI), which accompany copper(II) in hydrochloric acid–acetone mixtures, can be separated from copper by eluting copper(II) with 0.50 *M* hydrobromic acid in 85% acetone from a column of AG 50W-X8 resin, 200–400 mesh, while all these elements are retained by the column quantitatively. Separations are sharp and quantitative, as is demonstrated by results for some synthetic mixtures. Some relevant elution curves are presented.

RÉSUMÉ

Le cuivre(II), en milieu acide chlorhydrique–acétone, peut être séparé d'avec l'uranium(VI) et de nombreux autres éléments par chromatographie sur colonne de résine AG 50W-X8, 200–400 mesh, et élution au moyen d'acide bromhydrique 0.5 *M* dans l'acétone à 85%; tous les autres éléments sont retenus quantitativement sur la colonne. Les séparations sont nettes et quantitatives comme le prouvent les résultats obtenues pour un certain nombre de mélanges synthétiques. Quelques courbes d'élution sont présentées.

ZUSAMMENFASSUNG

Co(II), Ni(II), Mn(II), Li, Na, K, Rb, Cs, Be, Mg, Ca, Sr, Ba, Ti(IV), V(IV), Zr, Hf, Th, Al, Sc, Y, La, die Lanthaniden und auch U(VI), das Kupfer(II) in Salzsäure–Aceton-Gemischen begleitet, können von Kupfer abgetrennt werden, indem dieses mit 0.50 *M* Bromwasserstoffsäure in 85% Aceton aus einer mit AG 50W-X8-Harz, 200–400 mesh, gefüllten Säule eluiert wird, während die anderen Elemente von der Säule quantitativ zurückgehalten werden. Wie die Ergebnisse bei einigen synthetischen Gemischen zeigen, sind die Trennungen scharf und quantitativ. Einige wesentliche Elutionskurven werden vorgelegt.

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THE DETERMINATION OF SULFURIC ACID AEROSOLS

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A reliable, sensitive method for the determination of sulfuric acid aerosol has been developed based on samples collected on filter tapes and processed on a ring oven. The stoichiometric liberation of bromine is achieved by the aerosol protons with subsequent bromination of fluorescein to produce distinctive eosin.

The significance of sulfuric acid aerosol and other acidic species in the atmosphere relates to their effect on the ecologic system. Acidic atmospheres can cause corrosion of metals and construction materials such as limestone and marble. In some acidic atmospheres, vegetation may be damaged and animals killed[1,2]. However, the most important effect of sulfuric acid and other acidic species is almost undoubtedly their health effects on humans. Although definitive data are lacking, animal studies indicate that sulfuric acid increases resistance to pulmonary flow and suppresses responses of the central nervous system[2].

The presence of sulfuric acid in air is mainly due to the burning of fuels containing sulfur. Although the primary combustion product is sulfur dioxide, from 1 to 10% of the sulfur is emitted as the trioxide, depending on the combustion conditions[3]. The sulfur trioxide reacts rapidly with atmospheric moisture producing sulfuric acid aerosol. Sulfuric acid aerosols are also produced from sulfur dioxide in the environment by photochemical and catalytic reactions[4]. Thus, the conversion to sulfuric acid is the ultimate fate for most atmospheric sulfur dioxide.

In the major air pollution episodes of the past, sulfur dioxide has always been present in relatively high concentrations. It is now believed that aerosols of sulfuric acid and other acidic species must also have been present and must have been at least partially responsible for fogs and reduced visibility. It is also believed that the presence of sulfuric acid in the air contributed to the high death rates[5] during these episodes, although no accurate data are available to establish the actual concentrations that may have been involved.

To date, the most commonly used method of determining sulfuric acid aerosol has consisted of determining total sulfate by means of a barium sulfate turbimetric measurement on an aqueous extract of the sample[6]. Thus, all water-soluble sulfates such as calcium, zinc and ammonium sulfates are included together with sulfuric acid, and therefore, the actual concentration of sulfuric acid may be far less than the data indicates. Other available methods are based on some form of acid–base titration[7,8]. Recently, Scaringelli and Rehme[9] have suggested decom-

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position of sulfuric acid at controlled temperatures in a stream of nitrogen to separate the acid from metal sulfates. The liberated sulfur trioxide is reduced to sulfur dioxide by passing it over heated copper in a stream of nitrogen and finally measured spectrophotometrically, coulometrically, or flame-photometrically. This approach requires a careful technique and employs relatively complicated and expensive equipment. A specific method has been reported by Dubois *et al.*[10], based on the selective separation of sulfuric acid from the air sample by means of a microdiffusion technique. Final determination of the separated fraction is made by barium chloroanilate titration. This method is obviously selective but requires considerable time for acid separation and involves several critical analytical steps.

The method presented here for the determination of sulfuric acid is based on the stoichiometric reactions of its dissociated protons. Use is made of the inexpensive ring oven for separating and concentrating the samples and reaction products. The technique, because it is simple, sensitive and rapid, offers special advantages in air pollution studies. Relative errors are in the range 10–15%, which at microgram or nanogram levels compares favorably with sophisticated and expensive methods such as emission and X-ray spectrometry. An obvious advantage of the ring oven technique is that direct analysis is made on the sample spot without special manipulation or preparation.

EXPERIMENTAL

Materials

The following equipment was required: sequential tape sampler (e.g. Bendix-Unico, Paper tape sampler; Research Appliance Co., AISI Automatic tape sampler); ring oven with accessories (Arthur H. Thomas Co., Trace Oven); surface thermometer (Pacific Transducer Corp., Model 311F); micropipets, assorted; capillary pipets, uncalibrated. The filter paper for tape samplers was Whatman 41 or S and S 595; these grades are recommended because of their desirable diffusion characteristics, wet strength and superior properties as the reaction matrix for test procedures.

Standard stock sulfuric acid, 0.100 N. A solution containing 4.9 μg of sulfuric acid per μl was prepared. This was diluted as desired to prepare standard working solutions.

Sample. Collect 0.1–2 m^3 of sample (depending on anticipated acid levels) using a tape sampler and tapes as described above. An areal portion stamped with a cork borer from a sample collected with a high-volume sampler can also be used, provided that the aliquot is representative. In such a procedure, the sample disc is centered dust side down on a piece of Whatman 41 or S and S 595 filter paper, properly positioned on a ring oven.

Procedure A. Determination of sulfuric acid (net non-volatile acid)

Place the filter tape on the ring oven with the sample spot centered exactly over the annular space of the heated (90°) surface. Add to the center 30 μl of 4.0% (w/v) potassium bromide solution and wash to the ring zone with distilled water. Allow the ring to dry completely and then add 6 μl of aqueous

0.05% (w/v) sodium fluorescein solution (this is stable for 10 days). Wash the dye to the ring zone area with methanol. Remove the tape from the ring oven and add 4.0% (w/v) potassium bromate solution around the ring zone (from the outside) using a capillary tube. Return the tape to the hot ring oven and wait 3 min for full color development. Compare the color intensity of the ring with standard rings.

By following the above procedure, net non-volatile protons, which are most likely contributed by the sulfuric acid in the sample, can be determined. If volatile acids such as nitric or hydrochloric acid are suspected to be present, heat the sample spot for 4 h at 100° to remove the volatile acids completely (see below) and proceed as outlined above. The intensity of the ring is a direct measure of the residual protons contributed by sulfuric acid as well as any protons derived from hydrolyzed metal salts. Any phosphoric acid, being non-volatile, would respond in the same way as sulfuric acid.

Procedure B. Determination of total protons

Center the dust spot from the tape sampler on the hot (90°) ring oven. Add 30 μl of the 4.0% potassium bromide solution and enough distilled water to wet the entire dust spot. Add 6 μl of the fluorescein solution and 30 μl of the potassium bromate solution, and allow the paper to dry completely. Wash the reaction product (eosin) to the ring zone with methanol. Compare the intensity with standard rings.

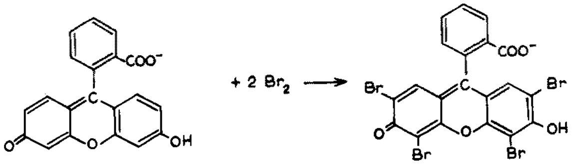
This method determines total protons, contributed by volatile and non-volatile acids as well as protons derived from the hydrolysis of metal salts.

Standard acid rings are conveniently prepared by using 0, 2, 4, 6, 8 and 10 μl of standard sulfuric acid solution. Rings can be made by following procedure A or B, provided that the same sequence of addition of reagent solutions is maintained. Standard rings are stable and no visible decrease in the intensity of color was observed even after a one-month period. The limit of detection by the recommended procedures (A or B) is 0.5 μg of sulfuric acid, while the range for optimal accuracy is 1.0–10 μg. These values are based on the final 33-mm ring.

RESULTS AND DISCUSSION

The determination of sulfuric acid aerosol (Procedure A) and total protons (Procedure B) involves the same basic reactions which may be represented as follows:

$$5\,Br^- + BrO_3^- + 6\,H^+ \rightarrow 3\,Br_2 + 3\,H_2O \qquad (1)$$



Fluorescein Eosin

(2)

Equivalent amounts of bromine are liberated by protons from a bromide–bromate mixture as shown by eqn. (1). The liberated bromine reacts with fluorescein to produce the distinctive reaction product eosin. These are classic reactions of established reliability.

Although the reactions for both procedures are the same, there exists a definite distinction between the two: Procedure A describing the sulfuric acid determination does not include the contribution of protons from volatile acids. Therefore, the intensity of the reaction product is a direct measure of the net non-volatile proton concentration. In Procedure B, the reaction is once again controlled by the protons, but in this case, the protons react in place to produce the final product, eosin, before heat can drive off volatile acids such as nitric acid and hydrochloric acid. The second procedure also includes the contribution of protons resulting from the hydrolysis of metal salts such as ammonium chloride, ammonium sulfate, zinc sulfate, etc.

In order to optimize the color development of the eosin, ring-oven temperatures were varied from 60° to 130°. The lowest temperature produced diffuse rings, because of the slow evaporation of solvents, while higher temperatures (110° and 130°) resulted in an erratic color development. The temperatures between 90° and 95° gave sharp and homogeneous rings. Various filter papers were tried, but Whatman 41 and S and S 595 were found to be most satisfactory. Whatman's dust tapes were also found to be equally good.

At first, it was considered that volatile acids, such as hydrochloric and nitric acids, would not interfere in the sulfuric acid determination, since heat from the ring oven would drive them off. But when experiments were actually carried out, both these acids were found to give positive interferences. Surprisingly enough, the color intensity of the ring produced from 5 μg of hydrochloric or nitric acid was the same as when 100 μg of these acids were spotted and processed on the filter paper. Separate experiments showed that these two acids vaporize rather quickly from the filter paper because of the heat from the ring oven but a small portion of them was found to remain even when heated at 90° for 2 h or more on the hot ring oven. Various attempts to induce vaporization of these acids failed. However, when air samples containing volatile acids were heated in an oven for 4 h at 100°, no interference was observed. Another series of experiments with radioactive sulfuric acid (^{35}S-labeled H_2SO_4) proved that no sulfuric acid was lost during the 4-h heating time. Therefore, it is recommended that if the presence of hydrochloric or nitric acid is suspected, the air sample should be heated at $100 \pm 5°$ for at least 4 h, before its sulfuric acid content is determined. The presence of nitric acid can be detected by its oxidizing capacity which results in the formation of a yellowish-green ring when eluted to the ring zone with potassium bromide solution and water.

The precision of the methods developed for determining sulfuric acid aerosol and total protons was ascertained by following the procedure described by Weisz[11]. The results obtained showed the same degree of accuracy (*i.e.*, about 10–15% relative error) as other ring-oven methods developed in this laboratory[12,13]

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SUMMARY

A reliable method has been developed for the chemical determination of sulfuric acid aerosol by simple technique and inexpensive equipment. A similar method is also presented for determining total acidity, including sulfuric acid and volatile acids together with salts that hydrolyze to give protons. The determinations can be performed directly on air samples collected with a sequential tape sampler. The basic chemical reactions involved are specifically controlled by the protons: stoichiometric amounts of bromine are released from a mixture of bromate-bromide by the protons, and the liberated bromine reacts with fluorescein to produce eosin, the color intensity of which is a direct measure of proton concentration. The detection limit for both the methods is 0.5 μg in terms of sulfuric acid and the range for quantitative measurement is 1–10 μg. The new methods are of special significance because of previous lack of reliable methods for determining acidic species in a polluted atmosphere.

RÉSUMÉ

Une technique simple et peu coûteuse est développée pour le dosage chimique des aérosols sulfuriques. Une méthode similaire est également proposée pour déterminer l'acidité totale, comprenant acide sulfurique, acides volatils, ainsi que sels libérant des protons par hydrolyse. Le brome, formé par réaction des protons sur un mélange bromate–bromure, réagit avec la fluorescéine pour donner de l'éosine; l'intensité de sa coloration permet de mesurer directement la concentration en protons. La limite de détection, exprimée en acide sulfurique est de 0.5 μg. Pour des mesures quantitatives, la tenure doit être comprise entre 1 et 10 μg. Cette méthode peut s'appliquer au dosage de particules acides dans une atmosphère polluée.

ZUSAMMENFASSUNG

Es wurde eine einfache, wenig aufwendige, zuverlässige Methode für die chemische Bestimmung von Schwefelsäure-Aerosol entwickelt. Eine ähnliche Methode wird beschrieben für die Bestimmung der Gesamt-Acidität einschliesslich Schwefelsäure und flüchtigen Säuren zusammen mit Salzen, die unter Bildung von Protonen hydrolysieren. Die Bestimmungen können unmittelbar an Luftproben ausgeführt werden, die von einem Probensammler mit fortlaufendem Band gesammelt werden. Die angewendeten chemischen Reaktionen werden spezifisch durch die Protonen kontrolliert: Stöchiometrische Mengen von Brom werden durch die Protonen aus einem Gemisch von Bromat-Bromid freigesetzt, und das entstandene Brom reagiert mit Fluoreszein unter Bildung von Eosin, dessen Farbintensität ein direktes Mass für die Protonenkonzentration ist. Die Nachweisgrenze ist bei beiden Methoden 0.5 μg (bezogen auf Schwefelsäure), und der Bereich für quantitative Messungen ist 1–10 μg. Die neuen Methoden sind für die Bestimmung saurer Spezies in einer verunreinigten Atmosphäre wertvoll.

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THE COLLECTION AND DETERMINATION OF ATMOSPHERIC GASEOUS BROMINE AND IODINE

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In recent years increasing interest and attention has been paid to measuring, defining and understanding the trace chemical composition of the atmosphere. As a result of this interest a number of procedures and techniques for collecting and analyzing atmospheric constituents have been described. For the most part, filtration and impaction techniques are used to collect particulate samples, and gas washing and adsorption for gas sampling. From these collection techniques and suitable analytical procedures, a wealth of information has been obtained about atmospheric trace metals, organic compounds, NO_x, SO_2 and CO in various polluted and clean air sheds. Species which have received relatively little attention are the halogens and halogenated compounds. The absence of data for atmospheric gaseous halogens is especially noticeable. This may be attributed largely to the absence of collection and analytical techniques sensitive and selective enough to measure these elements in the atmosphere.

The chemical cycle of atmospheric halogens in both clean and polluted air sheds is extremely important. The chemical cycle of marine air halogens has been studied for several years by a number of investigators, yet certain basic questions must be answered before an understanding of these elements in the marine atmosphere is forthcoming[1–5]. The significance of the cycle of chlorine and bromine in air sheds polluted by automobile exhaust is due to the addition of ethyl bromide and ethyl chloride to leaded gasolines. These produce volatile lead compounds on combustion[6–9]. Several workers have discussed the possibility of atmospheric iodine reacting with air pollution particles (especially lead compounds) to form artificial ice nuclei which may alter the weather structure of the atmosphere[10–12]. In each of the above-mentioned areas, suggestions have been made that various gaseous halogen compounds may play an important role in the atmospheric cycle and chemistry of these elements. This paper describes a procedure for the collection and analysis of atmospheric gaseous bromine and iodine.

The proposed method involves the adsorption of gaseous iodine and/or gaseous bromine on pre-cleaned activated charcoal. Particulate matter is removed from the air stream to be sampled by electrostatic precipitation. In air where the concentration of gaseous halogen species is large compared to that of the particulate halogen, as for example in a clean marine atmosphere[3,4], it has been found that

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electrostatic precipitation provides a convenient and efficient mechanism for the removal of particulate halogen before the air is passed over the activated charcoal. On the other hand, if the particulate halide concentrations are high relative to the gaseous halogen (*e.g.* in a polluted urban atmosphere), the oxidants produced by the electrostatic precipitation can oxidize the halides and generate gaseous halogens. Under such conditions, it is necessary to account for any alterations of the true gaseous halogen concentrations.

EXPERIMENTAL

Collection of samples

Samples are collected by pulling an airstream through the electrostatic precipitator and across a 1.5-g bed of pre-cleaned activated charcoal (Type AC, 8–12 mesh, Barnebey-Cheney, Columbus, Ohio) *ca.* 5 cm in depth. A small vacuum pump is used to maintain an air sampling rate of 1–1.5 m^3 h^{-1}. Sampling time is determined by the ambient concentration of the atmospheric halogens, but must be sufficient to exceed the charcoal halogen blanks (see below). Normally, sampling times of 4–6 h and 2–3 h are required for non-urban marine air and urban polluted air, respectively.

Analytical procedure

After completion of sample collection, transfer the charcoal to a 2-dram polyethylene vial, which has been previously cleaned in nitric acid and distilled demineralized water, and heat-seal it. Place this vial, along with two chloride flux monitors (see Standards, below), in a clean 4-dram polyethylene vial; heat-seal this vial and store it in a freezer. Prepare charcoal blanks periodically for each batch of cleaned charcoal.

Irradiate the samples for 20 min with thermal neutrons at a flux of *ca.* $5 \cdot 10^{12}$ n cm^{-2} s^{-1}. Immediately after irradiation, cut open the vial containing the charcoal and quantitatively transfer the charcoal to a hot solution of 5 ml of 1 *M* sodium hydroxide containing iodate and bromate carriers (AgI and AgBr equivalents of 50.00 mg each) and one or two drops of 5% sodium hypochlorite solution. Digest the solution for 1–2 min on a hot plate adding distilled water periodically in *ca.* 0.5-ml increments to prevent the solution from boiling too vigorously. Then remove the flask from the hot plate and allow to cool for 1–2 min. Acidify the solution to a phenolphthalein end-point with 16 *M* nitric acid, and while still in the presence of the charcoal, reduce the iodate and bromate to iodide and bromide by dropwise addition of 0.05 *M* sodium metabisulfite. Then filter the solution through a 24-mm Millipore Filter (Type HA) and make basic to phenolphthalein with 12 *M* sodium hydroxide. It is important to filter the solution while acidic; if the solution is basic, the Millipore filter is saponified and, under these conditions, the solution is filtered with extreme difficulty. After filtration, adjust the pH of the solution to *ca.* 4 by addition of *ca.* 2 ml of acetic acid, and transfer the solution to a separatory funnel containing 20 ml of carbon tetrachloride.

Separate and purify the bromide and iodide by using selective oxidizing and reducing agents, solvent extraction and precipitation as described by Duce and Winchester[13]. Count the purified silver halides along with a chloride standard (or flux monitor) for gross β-activity on a low background, anti-coincidence, gas-flow proportional counter in conjunction with an automatic sample changer and timer.

The sensitivity of this method is dependent upon the thermal neutron flux used to irradiate the samples. In the work described in this paper, the reactor at the Rhode Island Nuclear Science Center ($\phi_{th} \approx 5 \times 10^{12}$ n cm^{-2} s^{-1}) was used. At this neutron flux, the sensitivities for the bromine and iodine are *ca.* 0.12 ng and 0.15 ng, respectively. Sensitivity as used here is that amount of bromide and iodide required to produce ^{80}Br and ^{128}I activities of 10 disintegration s^{-1} after 20 min of thermal neutron irradiation.

Standards. Prepare chloride flux monitors and iodide and bromide standards by dissolving the appropriate weight of dried potassium halide salt in distilled demineralized water to give solutions of appropriate chloride, bromide and iodide concentrations (*e.g.* 1000 p.p.m., 200 p.p.m. and 200 p.p.m., respectively). Make small polyethylene vials (*ca.* 2–3 cm in length) from 0.2-cm diameter polyethylene tubing by heat-sealing the ends. Clean the standard vials with nitric acid and distilled demineralized water, and rinse with the standard solution. Heat-seal *ca.* 200 μl of standard in the vial. Analyse the chloride, bromide and iodide standards periodically. Duce and Winchester[13] have described the significance of using chloride flux monitors and the data treatment required to relate flux monitor information to the standard iodine and bromine solutions.

Electrostatic precipitation. An electrostatic precipitator was constructed so that a charcoal trap could be mounted at the air exit. Figure 1 shows a schematic diagram of such an instrument. The collector electrode is made from 1.35-in. i.d. type 304 stainless steel pipe 7-in. in length, which is threaded at each end. Teflon collars are constructed to mount on either end of the collection tube. The center charging wire is 0.01-in. diameter tungsten wire which is mounted on brass rods through the Teflon collars. The brass rods also serve as electrical connections to the high-voltage power supply. The Teflon collar at the exit end of the precipitator is machined so that a pyrex tube which holds the activated charcoal can be attached by means of an O-ring seal and clamp.

The precipitator is operated with a suitable high-voltage power supply at a positive voltage of 11,000 V and at a current of 0.3 mA.

Activated charcoal. Activated charcoal is well known as an excellent quantitative adsorbing medium for small quantities of gaseous iodine and bromine compounds. Since carbon does not form radioisotopes to any extent when irradiated with thermal neutrons, activated charcoal offers the advantage of direct neutron irradiation of iodine and bromine while they are still adsorbed to the charcoal. Thus, gaseous halogen samples collected on activated charcoal require only a minimum of handling and the chances for sample contamination are small.

The largest problem encountered in attempting to use activated charcoal for this work lay in obtaining activated charcoal with low enough iodine and bromine blanks to be usable. Various samples of activated charcoal from several sources were obtained and the iodine and bromine content of these samples were determined by neutron activation analysis. Most samples contained parts per million amounts (by weight) of iodine and bromine. One type of charcoal (coconut shell, Type AC, 8–12 mesh, Barnebey-Cheney Activated Charcoal Company, Columbus,

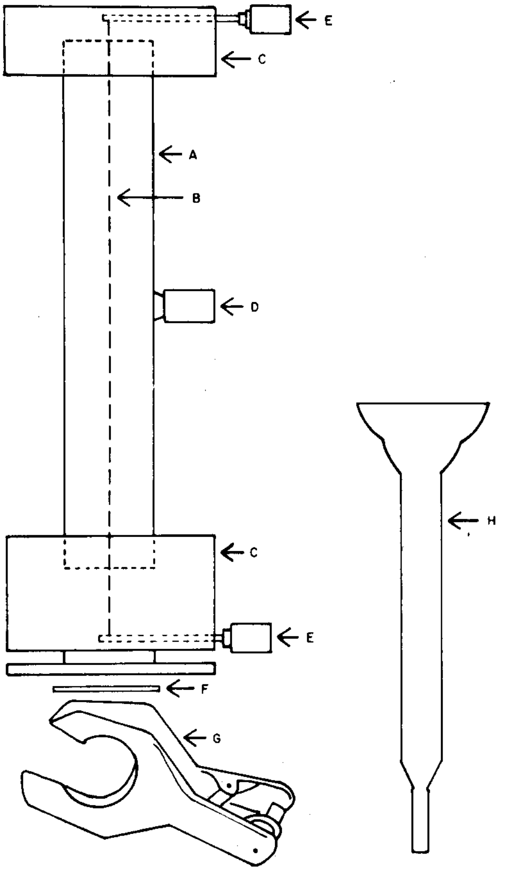

Fig. 1. Schematic view of electrostatic precipitator and charcoal sample holder. (A) Collector electrode (1.35 in. i.d. stainless steel), (B) discharge electrode wire (0.01 in. tungsten), (C) teflon insulators, (D) grounding plug, (E) high voltage plug, (F) rubber O-ring, (G) clamp, (H) pyrex charcoal sample holder.

Ohio) had iodine concentrations between 50 and 300 p.p.b. and bromine concentrations between 500 and 1,000 p.p.b.

Heating the charcoal to *ca.* 750° under a high vacuum (*ca.* 10^{-5} cm Hg) proved to be an extremely effective method of reducing the iodine and bromine blanks. The charcoal blanks for iodine and bromine were lowered by one to two orders of magnitude as a result of the vacuum heat treatment. Table I shows typical iodine and bromine concentrations (in units of 10^{-9} g per g of carbon) for Barnebey-Cheney Type AC 8–12 mesh coconut shell activated charcoal before and after the vacuum heat treatment.

In order to obtain and maintain low chloride and bromide blanks the charcoal was cleaned and handled in the following manner. About 100 g of the activated charcoal were placed in a quartz tube and attached to a high-vacuum system. The pressure of the system was lowered to *ca.* 10^{-5} cm Hg. The temperature of the charcoal was then raised slowly (over a period of 2 days) to 750°. This temperature was held for one week, and was then lowered slowly (25°

TABLE I

IODINE AND BROMINE CONCENTRATIONS OF ACTIVATED CHARCOAL BEFORE AND AFTER VACUUM TREATMENT

Sample[a]	*Iodine* (10^{-9} *g/g C*)	*Bromine* (10^{-9} *g/g C*)
1. Untreated	256	702
Treated	3.5	4.7
Treated (duplicate)	5.8	7.2
2. Untreated	61.2	800
Treated	6.5	11.7
3. Untreated	104	946
Treated	2.5	16.7

[a] Samples listed are from different 1-lb bags of charcoal obtained from Barnebey-Cheney Activated Charcoal Company, Columbus, Ohio. Different numbered samples were treated at different times.

h^{-1}) to ambient laboratory temperature. An activated charcoal trap was placed on the vacuum system before it was opened to the atmosphere; this was done to adsorb any gaseous iodine and bromine in the laboratory air before the air reached the cleaned charcoal. After opening the vacuum system to the atmosphere, the charcoal was immediately transferred to a clean vacuum desiccator (no desiccant). A vacuum was drawn on the desiccator and the charcoal was stored in this manner until it was used for sample collection. When the desiccator was opened to the atmosphere to remove the charcoal, an activated charcoal trap was placed on the air inlet to clean the air before it entered the desiccator. After removing enough charcoal for a sample, a vacuum was again drawn on the desiccator.

TABLE II

IODINE AND BROMINE CONCENTRATIONS OF CHARCOAL BLANKS

Batch No.	*Blank No.*	*Iodine* (10^{-9} *g/g C*)	*Bromine* (10^{-9} *g/g C*)
A	1	4.1	15.9
	2	4.0	18.1
	3	3.7	19.6
	4	5.9	13.3
	5	3.2	7.5
Average and s.d.		4.2 ± 1.1	14.9 ± 4.8
B	1	12.4	19.1
	2	16.2	22.7
	3	15.7	18.8
	4	9.9	8.4
	5	8.0	17.1
Average and s.d.		12.5 ± 3.4	17.2 ± 5.3
C	1	5.6	4.4
	2	4.2	6.9
	3	5.5	6.7
	4	5.5	6.0
Average and s.d.		5.2 ± 0.7	6.2 ± 1.1

A series of blank concentrations of iodine and bromine for three different batches of charcoal is given in Table II. The blank concentrations from batch to batch varied by more than a factor of three for both iodine and bromine. However, the blank concentrations of samples within an individual batch were generally in good agreement.

RESULTS AND DISCUSSION

It is known that the particle collection efficiency of an electrostatic precipitator is a function of particle size and flow rate of the airstream through the precipitator[14]. By using the electrostatic precipitator followed by a cascade impactor (Sciotech CI-6, Columbus, Ohio), it was possible to determine the effect of both particle size and flow rate on the collection efficiency of the precipitator used for this work.

In addition to the six collection stages of the cascade impactor, it was possible to place a filter immediately behind the sixth stage to collect particles smaller than those collected on the sixth stage. In this manner, it was possible to examine collection efficiencies of 7 ranges of particle size. Since the cascade impactor has an orifice after the sixth stage which limits the flow rate through the impactor to 0.75 $m^3\ h^{-1}$, the changes in flow rate were obtained by the following method. Efficiencies for the various size particles were first obtained by using the cascade impactor at a flow rate of 0.75 $m^3\ h^{-1}$. The limiting orifice was then removed and the tests were repeated with the jet for the sixth stage then being the limiting orifice. Under these conditions the flowrate was about 1.1 $m^3\ h^{-1}$. Removal of the sixth stage allowed efficiency tests at a flow rate of about 2 $m^3\ h^{-1}$. Collection efficiencies of each impactor stage at the higher flow rate were calculated as described by Ranz and Wong[15].

Initially, this procedure was designed to measure gaseous iodine and bromine in the marine atmosphere and in order to duplicate as nearly as possible the spectrum of particles produced over the open ocean, the particles produced for the study of precipitator efficiency were made in the following manner. A solution of sea water spiked with radioactive ^{22}Na was placed in a fine sintered-glass filter tower. Air was then forced through the frit producing bubbles in the solution which rose to the surface and broke, thus ejecting saline particles into the air, similar to wave and bubble action in the sea. These particles were then drawn through the precipitator and into the cascade impactor by means of a vacuum pump. The ^{22}Na activities for each stage and the filter were then γ-counted with a sodium iodide well detector and single-channel analyzer (Versa/Matic II Spectrometer SC-81, Tracerlab, Inc.). Thus, by comparing the ^{22}Na activity on the different stages of the impactor with and without the precipitator running, the precipitator efficiency for each stage (or particle size) could be calculated. This was done several times under various conditions *e.g.*, varying flow rate, varying collection periods, and varying ^{22}Na concentration. Table III gives an example of the results obtained. In all cases, the efficiency was high enough that no activity could be detected on the impactor samples collected with the precipitator running. The efficiency, therefore, could statistically be observed. Because the maximal amount of ^{22}Na which could be calculated only as greater than the minimal activity which

E III

TROSTATIC PRECIPITATOR EFFICIENCIES

? r	*Stage number*	*Size of particle collected at 100% efficiency (μm)*	*Flowrate (m^3 h^{-1})*	*Na concentration (cpm)*		*Efficiency (%)*
				Precipitator off	*Precipitator on*	
	A	10.0	0.75	600	0	>99.3
	B	5.0	0.75	2031	0	>98.8
	C	2.5	0.75	6820	0	>99.9
	D	1.2	0.75	6004	0	>99.9
	E	0.6	0.75	1481	0	>99.5
	F	0.3	0.75	212	0	>96
	Filter[a]	0.05	0.75	209	0	>90
	A	8.4	1.1	750	0	>99.4
	B	4.2	1.1	3335	0	>99.8
	C	2.1	1.1	14699	0	>99.9
	D	1.05	1.1	8001	0	>99.9
	E	0.5	1.1	1006	0	>99.5
	F	0.25	1.1	104	0	>95
	Filter[a]	0.05	1.1	50	0	>90
	A	6.4	1.9	38905	0	>99.9
	B	3.2	1.9	39137	0	>99.9
	C	1.6	1.9	27762	0	>99.9
	D	0.8	1.9	5987	0	>99.7
	E	0.4	1.9	730	0	>98
	Filter[a]	0.05	1.9	529	0	>97
	Filter[a] 0.45 μm	0.05	2.1	730000	6±3	>99.9

collects all particles greater than 0.05 μm with an efficiency equal to or greater than 90%.

could be used economically and safely gave no activity on the impactor stages with the precipitator on, a bulk sample was collected on a filter. To do this a 0.45-μm HA Millipore Filter was used in place of the impactor. This filter will collect all particles of radius 0.45 μm or greater, and about 90% of the particles down to a radius of 0.05 μm. It can be seen in Table III that by using the filter, all particulate matter was again removed from the airstream. These results indicated that essentially all particulate matter in the size range of interest was removed by the precipitator. Since the spectrum of particle size produced by this method was in close agreement with that found in the marine atmosphere, it was decided that this was an acceptable way of removing particulate matter from the air to be sampled.

It was also necessary to study the effect of the electrostatic precipitator on both particulate and gaseous halogens in an airstream. For gaseous iodine, carrier-free ^{131}I was generated as $I_2(g)$. Air was drawn over 0.5 ml of a 0.1 *M* nitric acid solution containing various concentrations of iodide-131. This air was passed through the electrostatic precipitator and then over a bed of activated charcoal. It was found that generation of gaseous iodine under these conditions was slow and quite constant. At the end of the sampling period the iodine activity remaining in the flask and the iodine activity on the activated charcoal were γ-counted with the sodium iodide well detector and the single-channel analyzer. About 5% of the gaseous iodine in the airstream was lost (presumably because of ionization of iodine with subsequent precipitation onto the walls of the precipitator). Similar tests with gaseous bromine produced essentially the same results *i.e.*, *ca* 5% gaseous bromine loss.

Tests were also performed to determine if either gaseous iodine or bromine would be released from particulate matter as a result of the gaseous oxidants produced by the discharging wire (ozone is produced in relatively large quantities by a corona discharge). A Teflon collar was constructed for the precipitator which allowed a 0.47-mm stainless-steel Millipore filter holder to be attached to the exit end of the precipitator. With this equipment it was possible to test the effect of the precipitator on particulate iodine in the following manner: particles were generated in the same manner as was described for the precipitator efficiency tests from a solution of sea water containing ^{131}I; the generated particles were pulled through the precipitator by means of a vacuum pump. A 47-mm HA Millipore filter (0.45 μm) was placed at the exit end of the electrostatic precipitator, and the filter was followed by an activated charcoal trap. Samples were collected on both the filter and the charcoal. Collecting samples without the precipitator operating allowed an estimate of particulate iodine production rate (by measuring activity collected by the filter) and a "natural" production rate of gaseous iodine (activity on charcoal). Collecting samples with the precipitator operating allowed an estimate of the degree to which gaseous iodine production rate was affected by the corona discharge. Tests were performed to check the retentivity of the filter for iodine vapor. It was found that the specified filter would retain *ca.* 25% of the gaseous iodine passed through it, and this number was reasonably reproducible if the flow rates and relative humidity were not changed and if the filters all came from the same box. By collecting samples with and without the precipitator operating, it was determined that statistically, no difference in the production rate of gaseous iodine could be detected between these two groups of samples.

Tests for the release of gaseous bromine from particles were conducted in exactly the same manner as for gaseous iodine by using 36-h ^{82}Br as bromide in sea water. For the bromine tests, it was found that there is a substantial release of gaseous bromine when the precipitator is operating. Table IV summarizes the results. With the precipitator not operating, these results would indicate that 2–3% of the particulate bromine was lost to the gaseous phase. With the precipitator operating, *ca.* 20–25% of the particulate bromine was released as gaseous bromine. If it is possible to extrapolate these results to the ambient marine atmosphere, it would then be expected that sampling of air containing 10 ng m^{-3} of particulate bromine would result in a sample showing a gaseous bromine concentration of 2 ng m^{-3} greater than the true gaseous bromine concentration, if it is assumed that the bromine in the particles is present as bromide. In the case of air containing particulate bromine and gaseous bromine in the relative proportions found for clean

TABLE IV

THE EFFECT OF THE ELECTROSTATIC PRECIPITATOR ON THE RATE OF RELEASE OF GASEOUS BROMINE FROM SEA SALT PARTICLES

(All activities were standardized with 0.1 ml of parent solution and counted with each sample to correct for decay of ^{80}Br. Collection time was 1 h except where stated).

Sample	*Precipitator status*	*Particulate Br activity (c.p.m.)*[a]	*Total gaseous activity (c.p.m.)*[b]	*Rate of gaseous Br release (c.p.m. h^{-1})*	*%$Br_2(g)$ released from particles ($Br_2(g)$/total Br) × 100*
1	off	7800	220	220	2.7
2	on	Average of 1 and 3	2300	2300	23.5
3	off	6950	170	170	2.4
4	on	Average of 3 and 5	1900	1900	21.2
5	off	7150	250	250	3.5
6	on	Average of 5 and 8	2370	2370	23.7
7	on[c]	Average of 5 and 8	4180	2090	21.6
8	off	8100	1120	1120[d]	12.2[d]
9	on	Average of 8 and 10	2280	2280	22.4
10	off	7680	280	280	3.4

[a] Corrected for amount of gaseous bromine adsorbed to filter.

[b] Including amount adsorbed to filter.

[c] Collection time, 2 h.

[d] High gaseous bromine activity for sample 8 is due to release of nitric acid fumes in the laboratory.

marine air, the effect of the precipitator on the gaseous bromine concentration of the air being sampled is small, since the gaseous bromine concentration is about 6–10 times higher than the particulate bromine concentration[5]. This means that if 20% of the particulate bromine is released to the gaseous phase, the measured concentration of gaseous bromine would be only 2–3% higher than the true gaseous bromine concentration.

It is assumed that a difference in oxidation states for particulate iodine and bromine accounts for the fact that bromine is released to the gaseous phase from particles while iodine is not. The thermodynamically stable forms for atmospheric particulate Br and I are bromide and iodate.

The charcoal was tested for adsorption efficiency for gaseous iodine and gaseous bromide by placing two charcoal traps in tandem. Iodine gas was generated from a 0.01 *M* solution of nitric acid containing iodide-131 as described previously. Bromine gas was generated from a solution of *M* nitric acid *ca.* 0.01 *M* potassium permanganate containing bromide-82. The activity on the two charcoal traps was measured by γ-counting, and for both iodine and bromine, 100% of the activity was found to be retained on the first trap.

These results indicate that particulate removal by electrostatic precipitation followed by adsorption on to clean activated charcoal provides a convenient and reliable method for measuring and studying gaseous halogens in clean atmospheres. This procedure can also be used for gaseous bromine and iodine in polluted urban atmospheres; however, alterations in the true gaseous halogen concentrations can occur and must be accounted for in such situations[8].

Since the electrostatic precipitator can seriously alter the concentration of gaseous halogens in airsheds containing large concentrations of particulate halide, we are presently investigating alternative methods of particulate removal for such situations. Studies are being conducted with a variety of filter materials and several gaseous halogen species to estimate the degree of interaction of the filters with the gaseous halogen compounds under different collection conditions *e.g.* flow rates, relative humidity and particulate loading.

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SUMMARY

A procedure has been developed which allows for the isolation, collection and analysis of atmospheric gaseous iodine and bromine. Particulate matter is removed from the airstream to be sampled by electrostatic precipitation. The gaseous halogen species are adsorbed onto specially cleaned activated charcoal. After irradiation of the charcoal sample with thermal neutrons, the iodine and bromine are removed from the charcoal, isolated, and purified as the AgX precipitate. The amounts of gaseous iodine and bromine are then determined by β-counting. The system works well for air sheds with low particulate iodine and bromine concentrations relative to that in the gaseous phase. In air sheds where the particulate halogen predominates, the gas phase measurements are more uncertain.

RÉSUMÉ

Une méthode est mise au point pour la séparation, la concentration et le dosage de l'iode et du brome dans l'atmosphère. On procède a une séparation des particules solides de l'échantillon d'air prélevé, par précipitation électrostatique, puis à une absorption sur un charbon actif spécialement purifié. Après irradiation de l'échantillon de charbon par neutrons thermiques, l'iode et le brome sont isolés et purifiés par précipitation sous forme de AgX. Les quantités de brome et d'iode sont finalement déterminées par comptage β.

ZUSAMMENFASSUNG

Es wurde ein Verfahren entwickelt, nach dem atmosphärisches gasförmiges Jod und Brom isoliert, gesammelt und analysiert werden können. Das aus Teilchen bestehende Material wird aus dem zu analysierenden Luftstrom mittels elektrostatischer Abscheidung entfernt. Die gasförmigen Halogenspezies werden an besonders gereinigter Aktivkohle sorbiert. Nach Bestrahlung der Aktivkohle-Probe mit thermischen Neutronen werden das Jod und das Brom von der Aktivkohle

entfernt, abgetrennt und gereinigt durch eine AgX-Fällung. Die Mengen des gasförmigen Jods und Broms werden dann durch β-Zählung bestimmt. Das System eignet sich gut für Abluft-Proben, deren Konzentrationen an Jod- und Bromteilchen niedrig sind im Vergleich zu denen in der Gasphase. Bei Luft, in der das in Teilchen vorliegende Halogen vorherrscht, sind die Gasphasen-Messungen weniger sicher.

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A NEW POTENTIOMETRIC GAS SENSOR—THE AIR-GAP ELECTRODE

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The recent development of potentiometric gas sensors is a logical continuation of the successful research in the field of ion-selective electrodes. The commercial availability of these sensors, promoted by Orion[1,2] and EIL[3,4], reflects a growing demand for simple, rapid and selective measuring tools in pollution, industrial and clinical analysis. A broad research programme aimed at the development of new sensors, *e.g.* for H_2S, HCN and NO_2[5], as well as for further applications of existing ones (ammonia electrode for Kjeldahl nitrogen analysis, and nitrate after reduction)[2], is carried out at Orion Research Inc.[6]. This trend is indeed justified not only because of the actual needs in practical analytical work, but also because the gas sensors are likely to be selective, owing to the selectivity of the volatilization by means of which the gas to be measured is separated from the aqueous sample.

All existing gas electrodes are based on the same principle as the well known carbon dioxide electrode[7–9], utilizing a glass electrode and a reference electrode joined by an electrolyte containing hydrogen carbonate ions[10]. The two electrodes and the electrolyte are separated from the sample medium by means of a hydrophobic membrane which is permeable to the gas (carbon dioxide), but impermeable to the aqueous solution. The glass electrode in conjunction with the reference electrode measures the pH of the inner electrolyte solution in the usual manner. As this pH_e is proportional to the logarithm of the partial pressure of carbon dioxide (p_{CO_2}) within the gas-permeable membrane, it can be further related to the concentration of carbon dioxide or carbonate dissolved in the sample solution, into which the sensor is immersed during the measurement. Typical use of the carbon dioxide sensor is in determination of the acid–base status of blood, which involves measurement of either plasma total CO_2, plasma hydrogen carbonate or p_{CO_2}[11]. Recently available sulphur dioxide and ammonia electrodes utilize the same principle, except that sodium hydrogensulphite and ammonium chloride solutions serve as the inner electrolytes, respectively.

The main efforts in this field have been focused on the development of suitable gas-permeable membranes, separating the inner electrolyte from the sample solution. Various membrane materials have been suggested, such as thin rubber film[7], polypropylene, Teflon[9], fluorinated ethylenepropylene, and, most recently, polyvinylidene fluoride microporous filter material[12] (which has been used for both the EIL and the Orion ammonia electrodes). The requirements for membrane properties are not only demanding but also incompatible. To secure reliable mounting and durability a mechanical strength is necessary, yet to allow a fast passage of gas the membrane must be thin, and up to 60% of its area should consist of straight open pores. The gas should penetrate these pores freely, though neither the inner electrolyte nor the sample solution must enter the membrane and meet, thus causing the sensor to cease to function. Therefore these pores have to be very narrow (diameter less than 1.5 μm is recommended[12]), the membrane material must be hydrophobic (*e.g.*, Teflon), and the sample solution must not contain any wetting agent[5]. For the same reasons, measurements in blood, serum, milk, beer, or effluent waters have to be carefully considered, while samples containing organic solvents have to be avoided[1]. Another problem in constructing a gas sensor is related to the thickness of the electrolyte layer covering the glass electrode. Ideally, this layer should be extremely thin in order to yield a fast electrode response, and recovery. This is, however, difficult to achieve owing to surface irregularities of "flat" glass electrodes at their edges, and also because the electrolyte usually has to be imbibed in a relatively thick cellophane membrane, which is sandwiched between the glass electrode and the gas membrane in order to give a reliable coverage of the pH-sensitive surface. Therefore, although considerably improved, the performance of the present gas sensors is still impaired by the shortcomings of available membrane materials.

There is, however, a different way of constructing a gas sensor, namely by replacing the gas-permeable membrane by an air gap which then separates the electrolyte layer from the sample solution[13]. Such an air-gap electrode will have the following advantages.

1. Since the diffusion of gases in air is much more rapid than in solid, aqueous, or even porous media, the air-gap electrode will exhibit a faster response than any porous membrane electrode. Even if the air gap is as wide as 3 cm, equilibrium within the gas phase, in measurement of, *e.g.*, ammonia (gaseous diffusion coefficient 16.9 at 20°) will be reached within 0.34 min[19]

2. The air-gap electrode utilizes only a very thin layer of electrolyte adsorbed at the surface of the indicator electrode. Therefore the speed of response is higher than that of electrodes employing cellophane layers, which are thicker and furthermore allow the formation of dead spaces at the edges of the electrodes.

3. The life time of the sensor and the reliability of the measurement are substantially increased, as the electrode does not come into direct contact with the sample solution; thus there will be no interference from surfactants, particulate matter or organic solvents.

4. The electrolyte layer can be easily renewed or even changed according to the requirements of a particular analysis; the same electrode can be used for measurements of, *e.g.*, ammonia, carbon dioxide or sulphur dioxide, as the peculiarities of the membrane material do not need to be considered.

5. The construction of the electrode is very simple.

The purpose of the present publication is then to review the basic theory of gas–solution equilibria in relation to the air-gap electrode, and to describe the construction of and experience with this newly developed gas sensor, subjected to measurements of carbon dioxide and ammonia.

THEORY

A weak acid H_2A which can exist in chemical equilibrium with a gas, g, is considered (*e.g.* H_2CO_3/CO_2), the partial pressure of the gas being p_g. According to the Henderson–Hasselbach equation, the pH of the solution is given by:

$$\mathrm{pH_s} = \mathrm{p}K_1 + \log([\mathrm{HA}^-]_s/[\mathrm{H_2A}]_s) \tag{1}$$

where the symbols have their usual meanings and index s denotes the sample solution. If the gas is in equilibrium with the component H_2A through a hydration reaction:

$$\mathrm{H_2A} \rightleftarrows \mathrm{g_d} + \mathrm{H_2O} \tag{2}$$

and the dissolved gas, g_d, in turn is in equilibrium with the partial pressure p_g of the same gas above the sample solution, Henry's law yields:

$$p_g = x_{g_d} \cdot S = c_{g_d} \cdot S_1 \tag{3}$$

where S_1 is the solubility constant. Thus, by combining eqns. (1)–(3):

$$\mathrm{pH_s} = \mathrm{p}K_1 + \log[\mathrm{HA}^-]_s - \log(p_g/k_1 S_1) \tag{4}$$

where k_1 is the equilibrium constant of eqn. (2). If the gas sensor system—equipped with a glass electrode, the surface of which is covered with an electrolyte solution containing the anion of the same weak acid, HA^-, (Fig. 1)—is placed in the gas medium above the sample solution, the pH measured by this electrode, $\mathrm{pH_e}$, will be governed by a similar equation:

$$\mathrm{pH_e} = \mathrm{p}K_1 + \log[\mathrm{HA}^-]_e - \log(p_g/k_2 S_2) \tag{5}$$

where the last term of eqn. (5) will be a linear function of the corresponding term of eqn. (4).

Thus, on the conditions that: (a) the electrolyte solution contains the common ion HA^- and has a constant ionic strength; (b) the volume of the sample is much larger than that of the solution constituting the electrolyte layer ($V_s \gg V_e$); and (c) the ionic strength of the samples is kept constant, eqns. (4) and (5) may be combined to yield the following expression:

$$\mathrm{pH_e} = -\log[\mathrm{HA}^-]_s + \log[\mathrm{HA}^-]_e + \mathrm{pH_s} + C \tag{6}$$

where C is a constant term accounting primarily for the ratio of sample volume to air gap volume, but also for the difference in ionic strength of electrolyte and sample solution.

If the terms $\log[\mathrm{HA}^-]_e$ and $\mathrm{pH_s}$ are kept constant, *i.e.*, the composition of the electrolyte solution is maintained unchanged and the pH of the sample solutions is fixed at an appropriate value, eqn. (6) may be written:

$$\mathrm{pH_e} = -\log[\mathrm{HA}^-]_s + \text{constant} \tag{7}$$

Accordingly, provided that: (a) the total pressure P and the partial pressure of water vapour p_{H_2O} above the sample solutions can be kept constant; and (b) the ratio of the volume of the sample solutions (V_s) and the volume of the gas above it (V_g) is fixed, *i.e.*, the geometry during a series of measurements remains constant, eqn. (7) predicts that $\mathrm{pH_e}$ as measured by the gas sensor will be a linear function of $\log[\mathrm{HA}^-]_s$. In order to obtain optimal measuring conditions, the choice of values for $\log[\mathrm{HA}^-]_e$ and $\mathrm{pH_s}$ may be evaluated on the basis of eqns. (1) and

(5). As to pH_s, there are two possibilities: HA^- is converted into the gaseous state either quantitatively or partially. For quantitative conversion, *i.e.* a 99% or better liberation of the total latent gas content, a value of $pH_s \lesssim pK_1 - 2$ is required, and as practically no additional gas is generated by further acidification of the sample solutions, changes in pH_s in this region will not influence pH_e. An advantage of this approach is the higher sensitivity of measurement which is obviously beneficial at low HA^- contents. The second approach, the partial conversion of HA^- into gas, occurring at $pH \geqslant pK_1$, requires a very good buffering of the sample solutions as changes of pH_s are directly reflected in pH_e. However, smaller electrode responses will be observed, depending of course on the proportion of gas liberated.

Another factor influencing the sensitivity of measurement is the composition of the electrolyte solution, that is, its H_2A content, originating from HA^- by hydrolysis. Electrolyte solutions containing high concentrations of HA^- will thus be less suited for measuring dilute samples. It is therefore an obvious advantage of the present system that the composition of the electrolyte solution can be readily adjusted according to the expected range of measurement.

To summarize, all the above-mentioned advantages of the air-gap electrode can be utilized if it is possible to keep the value of $\log[HA^-]_e$ constant, while the layer of electrolyte is kept extremely thin but still continuously covering the surface of the glass electrode.

In order to present the capacity and versatility of the air-gap electrode, the electrode has been tested in measurements of carbon dioxide and ammonia. Two methods, a surface renewal technique and a surface equilibration technique, were adopted for testing the electrode during these experiments.

EXPERIMENTAL

Apparatus

The air-gap sensor was made as indicated in Fig. 1 which is a partial longitudinal sectional view of the cylindrical Teflon body (diam. 50 mm; *h* 80 mm) which accommodates the glass and reference electrodes. For this purpose, two holes were drilled, one smooth, coaxial (diam. 10 mm), furnished with an O-ring (8), and the other (diam. 10 mm) with a threaded wall. A reference electrode (6) was mounted in the body by means of a Teflon tube of suitable diameter, having an outer thread matching that in the Teflon body. The cavity (5) was filled with solid, humidified potassium chloride, a saturated solution of which was also applied into the ceramic porous pin (4). The object of this arrangement was to avoid any leakage of potassium chloride solution from the reference solution as this would influence the composition of the electrolyte layer (1). The glass electrode (7) was mounted in a Perspex tube (outer diam. 10 mm; length *ca.* 45 mm; material thickness, 1 mm) by means of Parafilm foil.

The glass electrode used (Radiometer E 5036/0) with a "flat" surface (sensitive area *ca.* 13 mm^2) was adapted for the present purpose by removing the outer Ag/AgCl reference strip and insulating the reference connection with Araldite. The electrode (7) was then mounted in the Teflon body so that *ca.* 2 mm of it protruded from the flat surface (2). As reference, a Radiometer K 401 calomel electrode was used (6).

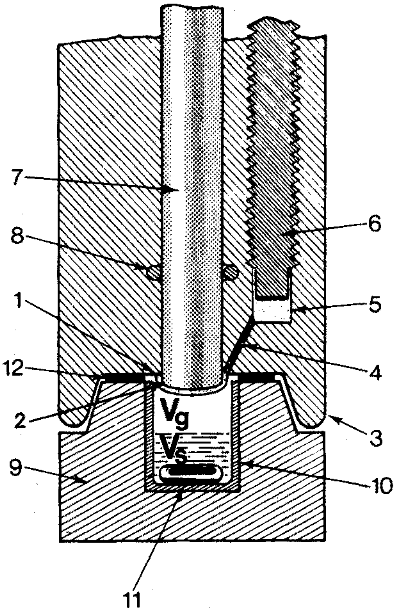

Fig. 1. The construction and the mode of operation of the air-gap electrode (for details see text).

The electrolyte layer was applied on the electrode by means of a Perspex electrode holder which had a cavity containing a polyurethane sponge accommodated in such a way that the glass electrode just touched its surface. The sponge was soaked with the electrolyte solution which contained a wetting agent. When operated in the surface-renewal mode, the gas electrode was simply rested in the electrode holder in between each measurement.

A Radiometer Servograph 310 and a Radiometer pH meter PHM 26 were used for measuring and recording the electrode response.

Measuring technique for carbon dioxide

An aliquot (50 μl) was pipetted into a small polyethylene cup (Fig. 1, 10; volume *ca.* 1 ml) which was placed in the sample holder (9) provided with a washer (12). The electrolyte solution used in the polyurethane sponge was $2 \cdot 10^{-2}$ *M* sodium hydrogencarbonate, saturated with a nonionic wetting agent (Victaret 12, Stauffer Co., New York). To the sample, 100 μl of 0.1 *M* lactic acid solution were added and the chamber of the air-gap electrode was closed. The sample was agitated by means of a magnetic stirrer (11) during the measurement. Readings were taken when equilibrium was reached after 3–4 min.

RESULTS AND DISCUSSION

Measurement of carbon dioxide

The measurement of carbon dioxide was carried out by the surface renewal technique and tested in determining sodium hydrogencarbonate by means of the total conversion method. Thus H_2CO_3 can be substituted for H_2A and HCO_3^- for

HA^- in eqn. (6), *i.e.*, $pK_1 = 6.1$, $S = 0.03$ mol l^{-1}, and $k = 800$. Therefore at $pH \leqslant 4.1$ nearly all hydrogen carbonate is converted into CO_2, which in turn determines the pH_e value recorded. The calibration curve which has a slope of -1.0 pH unit/decade obtained with hydrogen carbonate concentrations covering the clinically important range of concentrations is shown in Fig. 2. The electrolyte solution employed consisted of $2 \cdot 10^{-2}$ *M* $NaHCO_3$ to which wetting agent was added. When the air-gap electrode was used as described under Experimental, the equilibrium pH_e value was reached within 3–4 min. It was found necessary to agitate the sample during the measurement, otherwise poor reproducibility was obtained owing to a tendency of the sample to retain tiny bubbles of CO_2. Comparative experiments with premixed solutions suggested that the main factor affecting the speed of reaching the final steady pH_e value was not the slowness of the electrode response, but the time necessary for mixing the sample with the acid and expelling the carbon dioxide from the mixture. The use of lactic acid was chosen in order to make the experimental conditions as similar as possible to the Van Slyke method[11], originally employed for the determination of the acid–base status of blood. The fair reproducibility of these initial experiments (± 0.01 pH_e) might be sufficient for clinical purposes; experiments on determination of the total CO_2 content in plasma and whole blood are presently in progress*. The renewal of the electrolyte layer by the surface renewal technique was easy and reproducible. Over a month of operation, variations of ± 0.05 pH_e in the position of the calibration curve were experienced, probably because of changes in the ambient temperature ($\pm 1.0°$).

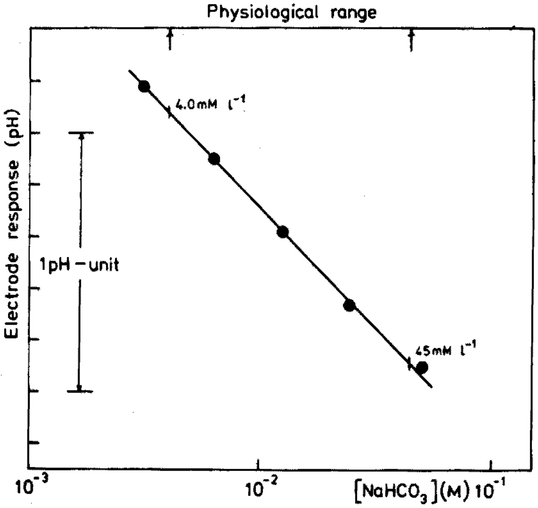

Fig. 2. Calibration curve for the air-gap electrode used as a carbon dioxide-sensitive electrode, calibrated with hydrogen carbonate solutions in the clinically important range of concentrations. The electrode was operated by the surface renewal technique, with the total conversion method.

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* Since the submission of this paper the response time (95%) has been decreased to 30 s, with a standard deviation of ± 0.006 pH_e.

Measurement of ammonia

The measurements of ammonia, performed by the surface renewal technique, were tested in determinations of ammonia in ammonium chloride standards, by both the partial and the total conversion method, and in determinations of ammonium ion in human serum and blood. For the present purpose, eqns. (6) and (7) were transformed into the following expressions, respectively:

$$pH_e = \log[NH_4^+]_s - \log[NH_4^+]_e + pH_s + C \tag{8}$$

and

$$pH_e = \log[NH_4^+]_s + \text{constant} \tag{9}$$

(Although the S value for ammonia can vary from 15 mol l^{-1} in concentrated solutions to 27 mol l^{-1} in 0.1 M solutions, the S value can be considered constant in dilute solutions and thus eqns. (8) and (9) will hold true.) In cooperation with

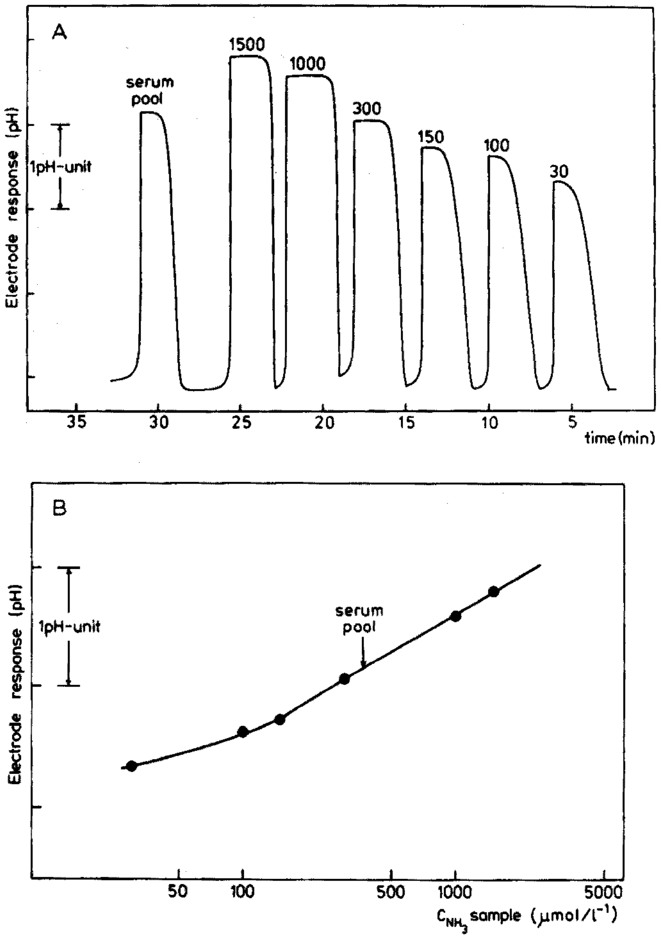

Fig. 3. The air-gap electrode used as an ammonia-sensitive electrode, calibrated with ammonium chloride solutions in the concentration range 30–1500 μmol l^{-1}. The electrode was operated by the surface renewal technique, with the total conversion method. A, electrode responses, including that of human serum from a serum pool; B, calibration curve.

the University Hospital of Copenhagen, the total conversion method was first tested in the range encompassing that of human serum (serum pool), *i.e.*, the electrode was calibrated in the range 30–1500 μmol NH_4^+ l^{-1}. The same electrode, microchamber and measurement technique as used for the carbon dioxide determinations were employed, except that an electrolyte solution containing 0.01 *M* ammonium chloride, 0.1 *M* potassium nitrate and wetting agent was used. The standard samples of ammonium chloride (volume 100 μl) were mixed with an equal volume of 0.1 *M* sodium hydroxide, and the signal of the ammonia sensitive air-gap electrode was recorded (Fig. 3A); the 100-μl samples of human serum pool were measured in an identical manner. The calibration curve (Fig. 3B) was linear with a slope of + 1.0 pH unit per tenfold increase of ammonium chloride concentration (above *ca.* 100 μmol l^{-1}), and the amount of ammonium in the serum pool was found to be 360 μmol l^{-1} compared with a value of 355 μmol l^{-1} as found by the conventional method of ion exchange and colorimetry[15].

Secondly, the air-gap sensor was used in determinations of the ammonium level in human blood plasma. As the ammonia level of freshly sampled blood from a normal human subject is below 100 μmol l^{-1}, and since the gas sensor applied with the electrolyte solution in the previous series of experiments indicated a limit of sensitivity of *ca.* 100 μmol l^{-1} (Fig. 3B), a new electrolyte solution was used, containing 0.001 *M* ammonium chloride, 0.01 *M* potassium nitrate and wetting agent. A series of standards in the range 30–600 μmol NH_4^+ l^{-1} were prepared, all standards being adjusted to an ionic strength of 0.14 with sodium chloride. The sample volume was 150 μl to which 25 μl of 1.0 *M* sodium hydroxide was added; otherwise the measuring conditions were similar to those described previously. The blood sample was measured within 30 min of sampling, after centrifugation. The actual air-gap electrode signals are reproduced in Fig. 4A, and the calibration curve is drawn in Fig. 4B. The ammonium content of the plasma was found to

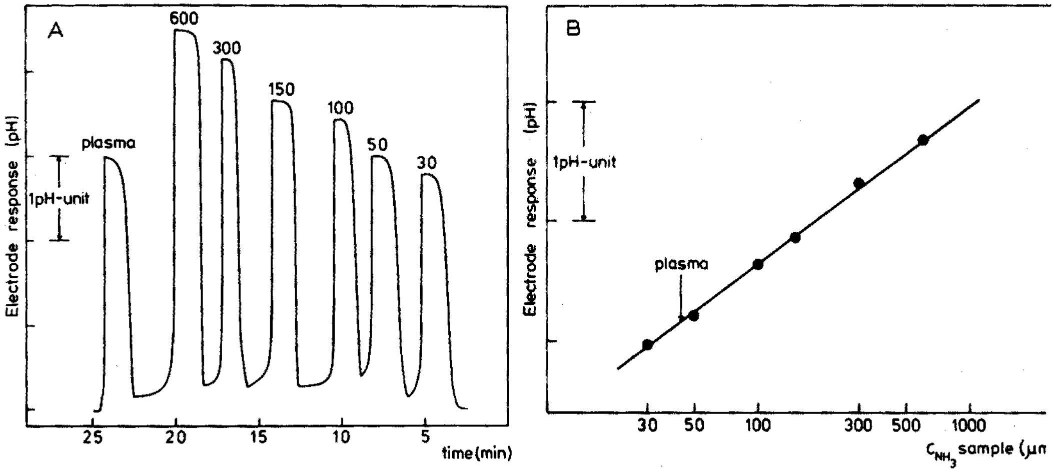

Fig. 4. The air-gap electrode used as an ammonia-sensitive electrode, calibrated with ammonium chloride solutions in the concentration range 30–600 μmol l^{-1}. The electrode was operated by the surface renewal technique, with the total conversion method. A, electrode responses, including that of plasma freshly sampled from a normal human subject. B, calibration curve.

be 43 μmol l^{-1}, which is in accordance with values generally recorded for normal human subjects (20–45 μmol l^{-1})[15].

In both series, the speed of electrode response allowed a steady state to be reached within 1–2 min, thus fulfilling even the most demanding practical requirements, and confirming the inherent capabilities of the air-gap sensor. Moreover, the surface renewal technique allowed an extremely fast return of the signal to a steady, well defined baseline, because this level is not reached via rediffusion of the gas from the electrolyte as in the conventional gas sensors, but by renewing the electrolyte layer entirely between each measurement, when the electrode is in the resting position.

In order to test the capability of the air-gap electrode further and under less favourable conditions, a series of standard solutions encompassing a range from $5 \cdot 10^{-2}$ *M* to $5 \cdot 10^{-5}$ *M* ammonium chloride, buffered by means of borax at a concentration of 0.05 *M* in the samples, was measured. The solutions were premixed in closed polyethylene vials, and after 15 min the lid was replaced by the air-gap electrode. This time the sensor was operated in the surface equilibration mode, *i.e.*, the electrolyte solution (0.2 *M* ammonium chloride, 0.2 *M* potassium nitrate and wetting agent) was applied in the usual manner, but the sensor was stored for at least 1 h above a solution of 0.1 *M* borax before the first measurement was made. During the recording of the calibration curve (Fig. 5A), the electrode was transferred as quickly as possible by the shortest route from one sample to the next, and after the most concentrated sample back above the surface of the borax solution. A linear calibration curve (Fig. 5B) with a slope of close to 1.0 pH unit per tenfold change of ammonium ion was obtained. Over a period of one week, the slope and sensitivity remained unchanged, without renewing the electrolyte layer; in addition, the speed of response was consistently very fast. However, a drift of the reference potential was observed and identified with a change in log $[NH_4^+]_e$ (eqn. 8), which might be altered from the initial concentration until it equilibrates with the relative humidity in the air-gap chamber. Subsequent drift can probably be ascribed to slow leakage of potassium chloride from the reference electrode into the electrolyte layer. This drift amounted to 0.05 pH units within the first 30 min after applying the electrolyte layer, then further increased 0.05 pH units after another hour, but thereafter remained practically unchanged for 3 days, whereupon it slowly decreased approximately 0.2 pH units over a period of one week (all values measured above a sample containing $5 \cdot 10^{-2}$ *M* ammonium chloride and 0.05 *M* borax). Thus, if the air-gap electrode were to be used for continuous monitoring in the surface equilibration mode, fairly frequent recalibrations would be necessary in order to recheck the position of the standard curve. Also the speed of recovery from high to low samples is not entirely favourable if fast changes of a measured species within a wide concentration range are to be measured. Fortunately, when a fast response is required as in AutoAnalyzer systems operating at a sample rate of 60 samples h^{-1} or more, a narrow concentration range of about one decade is likely to be encountered. Furthermore, in automated systems, a response only about 90% is often sufficient, as mixing and transport of all samples occur at exactly the same rate. In any case, although the recovery of the air-gap electrode operated by the surface equilibration technique is slower than in the surface renewal mode, it is still the fastest obtainable with a gas sensor, simply because

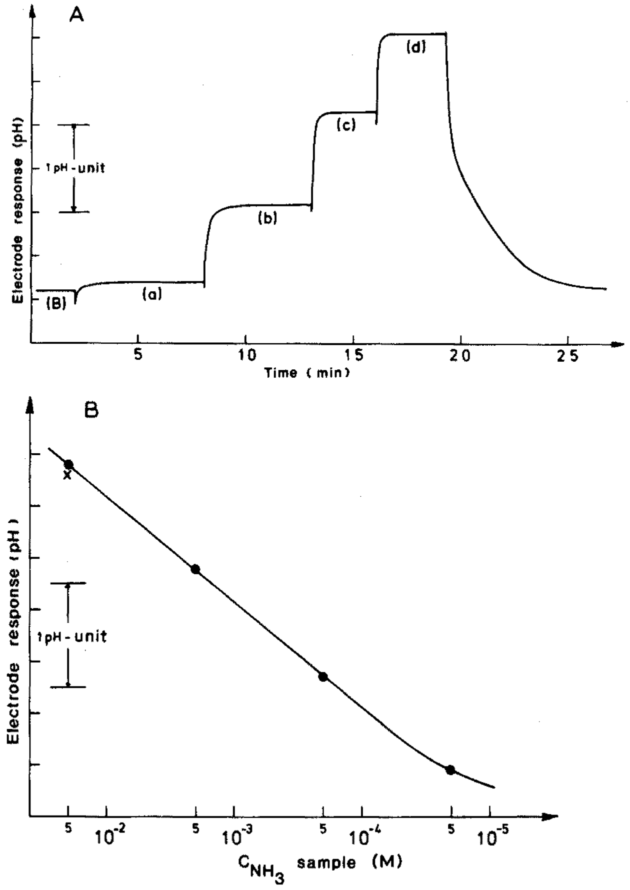

Fig. 5. The air-gap electrode used as an ammonia-sensitive electrode, calibrated with ammonium chloride solutions in the concentration range $5 \cdot 10^{-5}$–$5 \cdot 10^{-2}$ *M*. The electrode was operated by the surface equilibration technique, with the partial conversion method, *i.e.* buffered with a borax solution of pH 9.20. A, electrode responses; B, calibration curve (the pH of all sample solutions, except (d), was 9.20; the response of sample (d) is thus converted to this value).

the electrolyte layer is the thinnest formable and the rediffusion of the gas is not hindered by a porous membrane.

Conclusion

The first experiences with the air-gap electrode showed that this new measuring device can indeed be made to function satisfactorily and with sufficient precision, high speed of response and surprisingly long lifetime, even in the more demanding surface equilibration operational mode. There is no doubt that the advantages of this electrode will lead to many future applications. As to an imperfection of the system, attention should be drawn to the possibility of contaminating the electrode surface with the sample solution. If this happens, not only is the shape of the response curve distorted, but also erroneous readings are positioned conspicuously outside the range of the calibration curve. Thus faulty readings may immediately be identified and, when the surface renewal technique is used, the electrode will, of course, be restored automatically.

Intrinsically, the application of the air-gap electrode is not limited to measurements of ammonia and carbon dioxide and their inorganic salts in aqueous solutions. It is well known that especially ammonia is a product of numerous enzymatic reactions, through which either the substrate (urea, glutamine, asparagine, amino acids, amines or glutamic acid)[16] or the enzyme (by reaction-rate measurements) can be determined. Several urea electrodes have been suggested, yet all of them apparently suffer from sensitivity towards alkali metal cations[17], which is a serious drawback when measuring in biological fluids. As ammonia is the only monovalent cation which can be volatilized and thus sensed by a gas electrode, the sensitivity of the air-gap electrode and its speed of response may be suitable for such measurements.

Besides measurements of gases, which cause a change of pH_e, also other gases may potentially be sensed via redox changes or pM or pA changes in the electrolyte layer, as recently reviewed by Ross and Riseman[5]. For this purpose, the flat glass electrode must be replaced by a flat redox pM or pA sensitive electrode and covered by a layer of a suitable electrolyte. The solid-state Selectrode[18] activated by metallic platinum for redox measurements, may very well prove appropriate for this purpose. Preliminary experiments on determinations of small amounts of sulphides, via hydrogen sulphide measurements by means of a Selectrode activated with metallic silver, are presently in progress.

The authors wish to express their appreciation to Radiometer A/S, Copenhagen, for the gift of many glass electrodes; and to T. Frederiksen and G. Møller of the workshop of this Department for technical assistance. Thanks are also due to J. Melchior-Rasmussen, B. Clausen and K. Rasmussen of the University Hospital of Copenhagen for cooperation in the measurements of the serum and plasma samples.

SUMMARY

The concept and construction of a newly developed gas sensor, the air-gap electrode, is described. The hydrophobic gas-permeable membrane generally employed in gas electrodes is replaced by an air gap which separates the electrolyte layer from the sample solution. By avoiding the membrane construction and utilizing a very thin layer of electrolyte at the surface of the indicator electrode, a very high speed of response is obtained. Furthermore, the life time of the sensor is substantially increased, as the electrode does not come into direct physical contact with the sample solutions; thus there is no interference from surfactants, particulate matter or organic solvents. The electrolyte layer can be easily renewed or even changed according to the requirements of a particular analysis, so that the same electrode can be used for measurements of a variety of gases. Two different techniques of applying the air-gap electrode are described. The air-gap electrode was used to determine the carbon dioxide and ammonia contents of a series of samples, using sample volumes from 50–150 μl to several ml. The electrode was also used to determine the ammonium ion content in serum and plasma samples, yielding highly satisfactory results. The speed of response was of the order of 1–3 min. Some potential applications of the sensor are mentioned.

RÉSUMÉ

On décrit un nouveau senseur à gaz, l'électrode à intervalle d'air. La membrane hydrophobe perméable aux gaz généralement employée dans les électrodes à gaz est remplacée par un intervalle d'air séparant la couche d'électrolyte d'avec la solution de l'échantillon. La couche de l'électrolyte peut être facilement renouvelée ou même changée suivant l'analyse à effectuer; ce qui permet d'utiliser la même électrode pour les mesures de divers gaz. Deux techniques différentes d'application de l'électrode à intervalle d'air sont décrites. Cette électrode a été utilisée pour le dosage du dioxyde de carbone et de l'ammonia dans une série d'échantillons, de volumes allant de 50–150 μl à plusieurs ml. Elle a permis également de doser l'ion ammonium dans le sérum et le plasma, avec des résultats tout à fait satisfaisants. La vitesse du réponse est de l'ordre de 1–3 min. Quelques applications potentielles sont mentionnées.

ZUSAMMENFASSUNG

Die Grundlage und der Aufbau eines neu entwickelten Gas-Sensors, der Luftspalt-Elektrode, werden beschrieben. Die im allgemeinen bei Gas-Elektroden verwendete hydrophobe gasdurchlässige Membran ist durch einen Luftspalt ersetzt, der die Elektrolytschicht von der Probelösung trennt. Durch Vermeidung der Membran-Konstruktion und Verwendung einer sehr dünnen hydrophilen Elektrolytschicht an der Oberfläche der Indikatorelektrode wird eine sehr hohe Ansprechgeschwindigkeit erreicht. Darüber hinaus wird die Lebensdauer des Sensors wesentlich verbessert, da die Elektrode keinen unmittelbaren physikalischen Kontakt mit den Probelösungen hat; deshalb gibt es keine Störung durch grenzflächenaktive Stoffe, einzelne Teilchen enthaltende Proben oder organische Lösungsmittel. Die Elektrolytschicht kann leicht erneuert oder sogar entsprechend den Erfordernissen einer besonderen Analyse gewechselt werden, so dass dieselbe Elektrode für die Bestimmung verschiedener Gase verwendet werden kann. Zwei verschiedene Verfahren der Anwendung der Luftspalt-Elektrode werden beschrieben. Sie wurde für die Bestimmung des Kohlendioxid- und Ammoniakgehalts einer Reihe von Proben benutzt, wobei Probenvolumina von 50–150 μl bis zu einigen ml verwendet wurden. Die Elektrode wurde ebenfalls auf die Bestimmung des Ammoniumionen-Gehalts in Serum- und Plasmaproben mit sehr zufriedenstellenden Ergebnissen angewendet. Die Ansprechgeschwindigkeit war in der Grössenordnung 1–3 min. Einige mögliche Anwendungen des Sensors werden erwähnt.

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TITRIMETRIC APPLICATIONS OF MULTIPARAMETRIC CURVE-FITTING

PART II. POTENTIOMETRIC TITRATION WITH AN UNSTANDARDIZED REAGENT

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The first paper of this series[1] showed that multiparametric curve-fitting can be used to locate the equivalence point of a potentiometric titration of a weak base with a previously standardized solution of a strong acid, regardless of whether there is a point of maximum slope on the titration curve or not. The present paper shows that this technique can also be used to obtain both the amount of the base titrated and the concentration of the acid used as reagent from data obtained in a single titration with acid which has not been previously standardized.

The notion that the concentration of one of the two solutions involved in a titrimetric analysis must be determined in a separate titration, lies very close to the heart of analytical titrimetry. The usual situation is that in which the unknown solution is titrated with a previously standardized reagent, but "reverse" or "inverse" titrations, in which a known volume of previously standardized reagent is titrated with the unknown solution, are well known though much less common. There are circumstances in which the notion gives rise to much inconvenience. One arises with reagents of low stability, whose solutions are standardized repeatedly during a series of titrations, because their concentrations cannot be relied on to remain even approximately constant over a limited period of time. Another arises with reagents that are fairly stable but that are very rarely used; in some laboratories solutions of these are freshly prepared whenever they are needed, in order to save the space that they would consume during the long periods when they are not needed, while in others their solutions are stored but are restandardized whenever they are used because their concentrations cannot be trusted to remain constant for many weeks, months, or years. Repeated restandardization has given rise to much literature on the storage and stability of each new reagent. The work described here was undertaken in the hope of mitigating the time and effort expended in standardizations.

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* This paper is based on a report submitted by Dana M. Barry to the Faculty of Clarkson College of Technology in partial fulfillment of the requirements for the degree of M.S. in Chemistry, October, 1973.

EXPERIMENTAL

Barry and Meites[1] performed potentiometric titrations of a number of cF solutions of potassium acetate ($6.5 \cdot 10^{-5} \leqslant c \leqslant 0.1$) with 10.7 cF hydrochloric acid, and the values of the parameters K_a and y_{H^+} used here were taken from the results of their computations. Their stock solutions were used to prepare new solutions of acetate and acid: that of acetate contained $9.923 \cdot 10^{-3}$ F potassium acetate and 2.99 F potassium chloride, while that of acid contained 0.1691_4 F hydrochloric acid and 2.83 F potassium chloride. The potassium chloride serves to keep the liquid-junction potential, the ionic activity coefficients, and the conditional dissociation constant K_a of acetic acid constant throughout the titration, and its concentrations in these two solutions were so chosen that the ionic strength would always be exactly 3 M. The original solution of hydrochloric acid had been standardized against tris(hydroxymethyl)aminomethane; that of potassium acetate had been standardized against the hydrochloric acid in two different ways.

A volume of 79.98 cm^3 of the acetate solution was taken for each titration, and the conditions described by Barry and Meites[1] were exactly reproduced, except that the temperature was 25.2±0.3°.

Calculations

The coordinates of the data points (volume of acid and measured pH) were transferred to punched cards or paper tape and combined with the multiparametric curve-fitting program[2,*] now in general use in this laboratory and with an overlay that solved eqns. (1) and (2) below to yield, for each experimental point, the value of the pH that corresponded to the values of the parameters furnished by the main body of the program. Some of the computations were performed on a PDP8/I computer (Digital Equipment Corporation, Maynard, Mass.) operated in an early version of EduSystem 25 BASIC and in a multiuser configuration that made 4096 words of core available for this work; others were performed on an IBM 360/44 computer.

THEORY

The titration curve for the potentiometric titration of a weak monoacidic base with a strong monobasic acid is described by the equations:

$$[\mathrm{H}^+]^2 + \left(\frac{V_b^0 c_b^0 - V_a c_a}{V_b^0 + V_a} + K_a\right)[\mathrm{H}^+] - \frac{V_a c_a K_a}{V_b^0 + V_a} = 0 \tag{1}$$

$$\mathrm{pH} = -\log_{10}(y_{\mathrm{H}^+}[\mathrm{H}^+]) \tag{2}$$

where $[\mathrm{H}^+]$ is the concentration of hydrogen ion at the point where V_a cm^3 of

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[*] Copies of this program, CFT3, in both BASIC and FORTRAN-IV, with detailed documentation and instructions for use, may be obtained by remitting $4.00, to cover the cost of duplication and postage, to the Computing Laboratory of the Department of Chemistry, Clarkson College of Technology, Potsdam, New York 13676, U.S.A.

$c_a F$ acid has been added to V_b^0 cm^3 of $c_b^0 F$ base; K_a is the conditional dissociation constant of the conjugate acid HX of the base X^- being titrated and is defined by

$$K_a = [H^+][X^-]/[HX] \tag{3}$$

and y_{H^+} is an apparent single-ion molarity activity coefficient of hydrogen ion. It is assumed that V_b^0 is known and that V_a and the pH have been measured at each experimental point.

Equations (1) and (2) contain four parameters: c_b^0, c_a, K_a and y_{H^+}. It is assumed that both c_b^0 and c_a are unknown; K_a and y_{H^+} may or may not be known. The value of y_{H^+} is a property of the medium in which the titration is carried out, and depends on the temperature but not on the nature or identity of the base being titrated. It may therefore be deduced, in the fashion described by Barry and Meites, from data obtained in prior titrations of the same or another base in the same medium. The value of K_a is a property of the base titrated as well as of the titration medium, and also depends on temperature. It may be deduced from data obtained in prior titrations of the same base in the same medium. Values of y_{H^+} and K_a for acetic acid in potassium chloride solutions of various different concentrations at 23.5±0.5° are given in Table 1: those for 3 *F* potassium chloride are taken from ref. 1 but the others are new. Both vary in the expected fashion. Moreover, it is of some interest to note that combining the Harned-rule coefficient given by Hawkins[3] for hydrochloric acid in mixtures with potassium chloride to obtain the trace mean ionic molality activity coefficient of hydrochloric acid in 4 *F* potassium chloride, dividing the square of the result by the single-ion molality activity coefficient of chloride ion in 4 *m* potassium chloride, according to Robinson and Bates[4], and converting this value to the corresponding molarity activity coefficient, yields $y_{H^+} = 2.23$ in 4 *F* potassium chloride. This procedure ignores the small difference of temperature and the difference between the activity coefficients of chloride ion in 4 *m* and in 4 *F* potassium chloride, although it may be inferred from the behavior of the mean ionic activity coefficient of potassium chloride in this range of concentrations that the difference is probably small; it also neglects the assumptions regarding the liquid-junction potential which are embodied in y_{H^+} as we have defined it; and Robinson and Bates felt that their method was somewhat strained in applying it to the highly hydrated hydrogen ion even in 3 *m* solutions. In view of these reservations, the agreement between this value and the one given in Table I is little short of astonishing.

In any event, there are three situations that may be contemplated if both c_b^0 and c_a are unknown. One is that in which there is no prior or published information regarding the value of y_{H^+} in the titration medium employed, and in which the appropriate value of K_a will also be unknown regardless of whether the identity of the base being titrated is known or not. A second is that in which a value of y_{H^+} is available from the results of other titrations in the same medium, but in which a value of K_a is not, either because the base has not previously been titrated in that medium or because the identity of the base is unknown. A third is that in which values of both y_{H^+} and K_a are available, which requires that the identity of the base be known and also that the data obtained in prior titrations of the base in the same medium have been

TABLE I

VALUES OF y_{H^+} AND K_a FOR ACETIC ACID IN VARIOUS SOLUTIONS OF POTASSIUM CHLORIDE AT 23.5°

Concentration of KCl (F)	y_{H^+}	$10^5\ K_a$ *(HOAc)*
0.300	0.8352	3.093
0.500	0.8190	3.115
1.00	0.9366	2.817
2.00	1.2400	2.038
3.00	1.7460	1.313
4.00	2.30	0.8136

used to evaluate both of these parameters. Each of these three cases has practical analytical significance, and the following section will indicate what can be achieved in each.

RESULTS AND DISCUSSION

To place the results of the approach here proposed in their proper perspective, it is useful to consider the accuracy and precision that could be secured if the same titrations had been performed in the ordinary way, locating the point of maximum slope on the titration curve and calculating the amount of acetate titrated from the known concentration of the hydrochloric acid and the volume required to reach the inflection point. Acetate ion is so weak a base in aqueous solutions, and the concentrations of acetate and acid were so low in these titrations, that the point of maximum slope is neither very close to the equivalence point nor very well defined. It is easily shown[5] that it occurs 0.40% before the equivalence point under the conditions of these titrations. The precision of inflection-point location was estimated as follows. From twelve values obtained in titrations of 0.0993 *F* acetate with 1.057 *F* hydrochloric acid the standard error of location of a single point of maximum slope was found to be ±0.30%. The ratio of the computed slope at the inflection point in the titration of 0.00992 *F* acetate with 0.169 *F* hydrochloric acid to that in the titration of 0.0993 *F* acetate with 1.057 *F* acid is 0.329. If it is assumed that the precision is inversely proportional to the slope at the inflection point, the standard error of location of a single point of maximum slope may be estimated to be ±0.91% for the titrations of 0.00992 *F* acetate with 0.169 *F* acid.

The first four lines of Table II show the mean values, together with their standard deviations and deviations from the expected values, obtained from the data of 11 titrations on assuming both y_{H^+} and K_a to be unknown. The standard deviation of a single acetate concentration from the mean is *ca.* ±0.2% and the mean of these eleven values deviates less than 0.2% from the expected value. Each of these figures is about one-fifth of the corresponding one for titration to the point of maximum slope. For the concentration of acid, the precision and accuracy are roughly the same as the best that can be secured in volume (as opposed to weight) titrations against a standard for which the

TABLE II

RESULTS OBTAINED

(Each line summarizes the results of the computations for 11 titrations of 0.009923_3 *F* acetate with 0.16914 *F* hydrochloric acid under the conditions described in the text and with the prior information given in the first column. The precision measure given in parentheses in the third column is the absolute or relative standard error of a single result; the error given in column 4 is the absolute or relative deviation of the mean from the expected value.)

Parameters taken as known	*Parameters*	*Values (and standard deviations obtained)*	*Error*
none	c_b^0	0.0099072 (±0.19%)[a]	−0.16%
	c_a	0.16918 (±0.06%)[a]	+0.023%
	K_a	$1.3102\ (\pm 0.0132) \times 10^{-5}$	
	y_{H^+}	1.688 (±0.049)	
$y_{H^+} = 1.688$	c_b^0	0.0099277 (±1.55%)[b]	−0.044%
	c_a	0.16949 (±0.21%)[b]	+0.21%
	K_a	$1.3114\ (\pm 0.0289) \times 10^{-5}$	
$y_{H^+} = 1.688$, $K_a = 1.310 \cdot 10^{-5}$	c_b^0	0.0099102 (±1.08%)[c]	−0.13%
	c_a	0.16923 (±0.23%)[c]	+0.052%

[a] $c_a/c_b^0 = 17.076_5$. [b] $c_a/c_b^0 = 17.072_4$. [c] $c_a/c_b^0 = 17.076_4$.

titration curve is far more favorable than this gently sloping one. It may be remarked, in passing, that each of these 4-parameter fits consumed *ca.* 40 s on an IBM 360/44 computer operated in FORTRAN-IV.

Despite the success of a generally similar procedure[1] in which the concentration of acid was taken as known in the computations, the quality of these results was unexpected. The most scrupulous care was taken in calibrating and cleaning the glassware, in the two-point standardization of the pH-meter before each titration, in expelling carbon dioxide from all the solutions and from the titration vessel, and in making sure that a true equilibrium value of the pH was obtained at every point, but it remains hardly credible that results of such extraordinary precision can be obtained from titrations of such dilute solutions of such a weak base. The results suggest that a host of titrations, which have heretofore scarcely, if at all, been given serious consideration because the slopes of the titration curves at their equivalence points are too small to render reasonable precision and accuracy attainable by conventional techniques of end-point location, deserve reconsideration in the light of this new procedure.

The mean value of K_a differs by only 0.25% from that reported previously[1], but that of y_{H^+} is appreciably smaller. The obvious interpretation of these differences is that they reflect the difference between the temperatures at which these and the prior experiments were performed, 25.2±0.3° and 23.5±0.5°, respectively. This appears to be supported by the facts that the thermodynamic dissociation constant of acetic acid is known to be nearly, though not quite perfectly, independent of temperature[6], while the mean ionic activity coefficient of hydrochloric acid is known to decrease slightly as the temperature increases[7], over this range. Against it is the fact that the coefficient of correlation between y_{H^+} and the temperature is only 0.3 for the values obtained in these experiments, and only −0.2 for those previously reported. Though the variation of temperature was not large in either set of experiments, the range of values of y_{H^+} obtained here (1.611–1.762) corresponds to a shift of the whole titration curve by 0.04 pH-unit in a direction parallel to the pH-axis. This is approximately 20 times the standard deviation from regression of a single pH measurement, a ratio that certainly ought to permit detecting a correlation if one did exist. We conclude that the variations of y_{H^+} from one experiment to another are attributable to real variations of some unknown experimental variable, most likely the liquid-junction potential, among different titrations, and that the difference between the mean value given in Table II and that reported previously is due to a change in the concentration profile through the liquid junction in the reference electrode employed.

In titrations of extremely dilute acetate solutions[1], it is advantageous to constrain the fit by assigning fixed values to y_{H^+} and K_a even though small variations of them may be real; if this is not done, they may take on values impossibly far from the truth and far outside their normal ranges of variation. In the somewhat less unfavorable case discussed here, wide variations of these values do not occur, and preventing the smaller variations that do have some physical significance tends to degrade the fit. This may be seen from the second and third parts of Table II. The second part gives the results obtained on inserting the mean value of y_{H^+} into the subroutine that solved eqns. (1) for values of V_a, c_a, c_b, and K_a provided by the main body of the program; the third part gives those obtained on employing the mean value of K_a as well. In these two parts of the Table the precision of the acetate concentration is about as bad as if the titrations had been made in the ordinary way, although the accuracy is much better (because the inflection point is not involved) and the values of c_a are hard to fault.

From the fact that the 3-parameter fits yield values of the concentrations that are inferior to those obtained from the 2-parameter fits, it may be concluded that the variations of y_{H^+} and K_a are interrelated, so that fixing either while permitting variation of the other violates the physical reality of the situation. Since K_a is defined in such a way that it depends on the activity coefficients of acetate ion and acetic acid as well as on the thermodynamic dissociation constant of the acid, it is by no means unexpected that variations of K_a should accompany variations of y_{H^+}

There is only one advantage to constraining the fit by inserting previously established values of parameters that, like y_{H^+} and K_a, are both susceptible to prior evaluation and of little or no interest in any one practical titration. This is that the original 4-parameter fit can be simplified to one involving three or even only two parameters, with a consequent saving in computer time. Since the 4-parameter fit can be made in 40 s on a large computer, so that even with computer time at $200 per hour, it would cost less than the actual performance of the titration, a persuasive argument for shortening it further at the cost of degrading the results would be difficult to find. The same thing would be true if the computation were made on a dedicated minicomputer operating in a reasonably efficient interpretive language, for then it would be possible to complete even the 4-parameter fit long before the subsequent titration was complete.

Consequently, even if prior information about both y_{H^+} and K_a is available for a particular titration, we recommend that the data be subjected to a 4-parameter fit in which both are evaluated along with the two unknown concentrations. A substantial deviation of the value of either y_{H^+} or K_a from the one previously obtained would serve as a valuable indication that the experimental conditions have not been controlled as carefully as is desirable. It appears from the first part of Table II that this procedure is capable of yielding titrimetric results very much superior to any heretofore attainable. It appears further that there is no advantage to be gained from prior standardization of the reagent as long as the stoichiometry of the titration is known to be exact.

It may be advantageous to indicate how the results depend on the way in which the experimental points are distributed along the titration curve. This can be deduced by considering how changes in the values of the individual parameters would affect the curve. Altering the value of y_{H^+} would alter each measured pH value by the same amount. Altering the value of K_a would alter the pH values at points preceding the equivalence point, but would have much less effect on those at points where excess of acid was present. Altering the value of c_a would have little effect on the first few pH values, but thereafter the effect would increase to a maximum in the vicinity of the equivalence point and then decrease again, but to a level well above the initial one, as excess of acid is added. Altering the value of c_b^0 would have a substantial effect on the first few pH values, a much larger one on those in the general vicinity of the equivalence point, and only a small one on those well beyond the equivalence point. All these assertions may be confirmed by sketching the titration curve, first with any desired set of values of the four parameters, and then with a different value of any one but identical values of the other three.

It follows from these considerations that the distribution of the experimental points has no effect on the precision with which y_{H^+} can be evaluated. Greater precision in the evaluation of K_a might be secured by taking many more points before the equivalence point than after it, while the precisions of both c_b^0 and c_a may be maximized by taking more points near the equivalence point than in the regions of the curve remote from it. However, it is not clear that there is any practical use for results more precise than those obtained here in an unfavorable case and shown in Table II, and if the concentrations of both base and acid are unknown it is not so simple to take data most densely in the neighborhood of an equivalence point whose location is unknown.

In this work the first measurement of pH was made after 0.5 cm^3 of acid had been added, and then after the addition of each successive 0.5-cm^3 portion of acid until a total of 10 cm^3 had been added. This gave 20 points evenly spaced along the volume axis. From the values in Table II it may be calculated that 4.73 cm^3 of acid was required to reach the equivalence point in each titration of 80 cm^3 of acetate. There were thus nearly equal numbers of points before and after the equivalence point, and it may be noted that the two points nearest to the equivalence point were each about 5% away from it.

Although we strove by expedients like this to avoid the possibility of overestimating the accuracy and precision attainable, and although the titration curve on which the results are based was so nearly devoid of an inflection point that an attempt to obtain even reasonably precise results from it in any other way could arise only from ignorance, recklessness, or desperation, it must be said that the conditions employed here were favorable in one respect: matters were arranged so that the concentration of potassium chloride not only remained exactly constant throughout each titration, but was at least two orders of magnitude larger than the concentration of acetate ion, hydrogen ion, or acetic acid at any point. An exactly equivalent arrangement could be made in determining, say, a small concentration of sodium acetate in a solution known to contain about 0.1 *F* sodium chloride. A known volume of the unknown solution could be transferred to a volumetric flask, and diluted to the mark with water after adding enough solid potassium chloride to make the final solution 2.9 *F* in this salt; then a measured volume of it could be titrated with a reagent containing hydrochloric acid, 0.1 *F* sodium chloride, and 2.9 *F* potassium chloride. If the concentration of sodium chloride were unknown, as it often would be in practical analysis, or if it varied appreciably between successive samples, a much more attractive expedient would probably be to dilute the sample so much while making it 3 *F* in potassium chloride that the concentration of sodium chloride was sure to be negligible by comparison with that of potassium chloride, and then to titrate with acid containing enough potassium chloride to make its ionic strength equal to 3 *M*. This would render the sodium chloride innocuous and knowledge of its concentration unnecessary, but if the sample originally contained only 0.001 *F* sodium acetate and perhaps as much as 5 *F* sodium chloride, dilution sufficient to render the sodium-ion concentration negligible would decrease the acetate-ion concentration below the level at which good results could be secured. To balance this argument it may be noted that the titration of 0.001 *F* acetate with acid in aqueous solutions is also unlikely to be notably successful by any other technique.

It is therefore too early to attempt an assessment of the utility and scope of this technique in practical analysis. Nevertheless, we believe it may fairly be claimed that its achievements to date entitle it to consideration as a real and important advance in the art of analytical titrimetry.

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SUMMARY

The execution of a titration rarely performed, or of one with a reagent of limited stability, may be facilitated by applying multiparametric curve-fitting to titration-curve data in such a way that the amount of the substance titrated and the concentration of the reagent are evaluated simultaneously; the necessity for prior standardization of the reagent is thereby eliminated. In the potentiometric titration of acetate ion, a very weak base, with unstandardized hydro-

chloric acid, the accuracy and precision of the concentration of acetate thus obtained are approximately five times better than those which can be secured by titration with standardized acid and location of the point of maximum slope. The accuracy and precision of the concentration of acid are comparable to those that can be secured in very careful standardizations against a primary standard that gives a titration curve of far more favorable shape.

RÉSUMÉ

Un titrage rarement exécuté, ou utilisant un réactif de stabilité limitée, peut être facilité en appliquant l'ajustement d'une courbe multiparamétrique aux valeurs de la courbe de titrage, de façon que la quantité de substance titrée et la concentration du réactif soient évaluées simultanément. La nécessité d'un étalonnage pre-alable est ainsi supprimée. La précision obtenue lors d'un titrage potentiométrique de l'ion acétate, base très faible, au moyen d'acide chlorhydrique non étalonné, est excellente. L'exactitude et la précision de la concentration d'un acide sont comparables à celles obtenues par titrage très soigné au moyen d'un étalon primaire donnant une courbe de titrage de tracé beaucoup plus favorable.

ZUSAMMENFASSUNG

Die Ausführung einer Titration, die nur selten angewendet wird, oder einer solchen mit einem Reagenz begrenzter Haltbarkeit kann erleichtert werden, wenn eine Multiparameter-Kurvenanpassung auf die Titrationskurvenwerte in der Weise angewendet wird, dass die Menge der titrierten Substanz und die Reagenzkonzentration simultan ausgewertet werden. Hierdurch entfällt die Notwendigkeit einer vorausgehenden Einstellung der Masslösung. Bei der potentiometrischen Titration von Acetation, einer sehr schwachen Base, mit nicht eingestellter Salzsäure sind die Genauigkeit und Reproduzierbarkeit der so erhaltenen Acetatkonzentration nahe zu fünfmal besser als die, die durch Titration mit eingestellter Säure und Ermittlung des Punktes maximaler Steigung erzielt werden können. Die Genauigkeit und die Reproduzierbarkeit der Säurekonzentration sind vergleichbar mit jenen, die bei sehr sorgfältigen Einstellungen gegen einen Urtiter erreicht werden, der eine sehr viel günstiger verlaufende Titrationskurve ergibt.

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KINETIC PARAMETERS FOR THE ANODIC OXIDATION OF THIOCYANATE AT THE GLASSY CARBON ELECTRODE

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The electrochemical oxidation of the thiocyanate ion has been studied for many years in both non-aqueous and aqueous media. The reaction product from the oxidation in a non-aqueous solution has been identified as thiocyanogen[1] with the structure NCS–SCN[2]. The thiocyanogen is stable in anhydrous solvents such as methanol or acetic acid, but in the free state it rapidly and irreversibly polymerizes to parathiocyanogen[3]. The oxidation of the thiocyanate ion in aqueous solution produces cyanide and sulfate ions, and small amounts of a polymeric substance, pseudothiocyanogen[4].

Gauguin[5] has shown that the anodic process fits the totally irreversible situation. From potentiometric data obtained at the rotating platinum electrode, he concluded that two electrons were simultaneously removed from two thiocyanate ions, and that the slow step in the reaction mechanism was the dimerization of the two thiocyanate radicals, or the dismutation process: $2\,NCS^{\bullet}+H_2O \rightarrow SCNOH+SCN^-+H^+$. The existence of this latter process, however, has been refuted by Seel and Müller[6]. The effects of supporting electrolytes of cyanide, bromide, and iodide ions on thiocyanate oxidation at the stationary platinum electrode have been investigated by Nicholson[7], who found the current sufficiently reproducible for analytical purposes. However, progressive contamination of the electrode during the electrolysis process prevented the determination of αn_a, the product of the transfer coefficient and the number of electrons involved in the rate-controlling step.

In the present study the kinetic parameters for the anodic oxidation of thiocyanate ions at a stationary glassy carbon electrode in aqueous solution were determined by means of linear-sweep voltammetry.

EXPERIMENTAL

A Beckman Electroscan 30 was used in the mode prescribed for chronoamperometry with potential sweep. Measurements were taken from strip-chart recordings of the polarograms.

The platinum electrode consisted of a platinum wire sealed into a soft glass tube, the end of which was polished with a #400 emery cloth. Before each scan the electrode was pretreated with concentrated nitric acid for 15 min.

The Chemtrix glassy carbon electrode was pretreated by setting the potential about 0.5 V negative of the oxidation wave, and an occasional (weekly) pretreatment with nitric acid proved necessary.

The surface areas of the electrodes were determined from peak polarograms for the reduction of potassium hexacyanoferrate(III) ($D = 1.83 \cdot 10^{-5}$ cm^2), and found to be 0.0365 cm^2 for the platinum electrode and 0.0961 cm^2 for the glassy carbon electrode.

Reagent-grade potassium thiocyanate was recrystallized once, and all other reagent-grade solids were used directly.

RESULTS AND DISCUSSION

A typical peak voltammogram is shown in Fig. 1. The peak obtained for the oxidation of the thiocyanate ion is considerably better defined at the glassy carbon electrode than at the platinum electrode[7]. For a totally irreversible electrode reaction at the stationary electrode the fundamental equation relating the peak potential to the scan rate is given[8–10] by

$$i_p = 3.01 \cdot 10^5 \, n(\alpha n_a)^{\frac{1}{2}} A D^{\frac{1}{2}} C_0 V^{\frac{1}{2}} \tag{1}$$

A plot of i_p *versus* $V^{\frac{1}{2}}$ should produce a straight line. Figure 2 shows these data for potassium nitrate and chloride as supporting electrolytes. Data for the

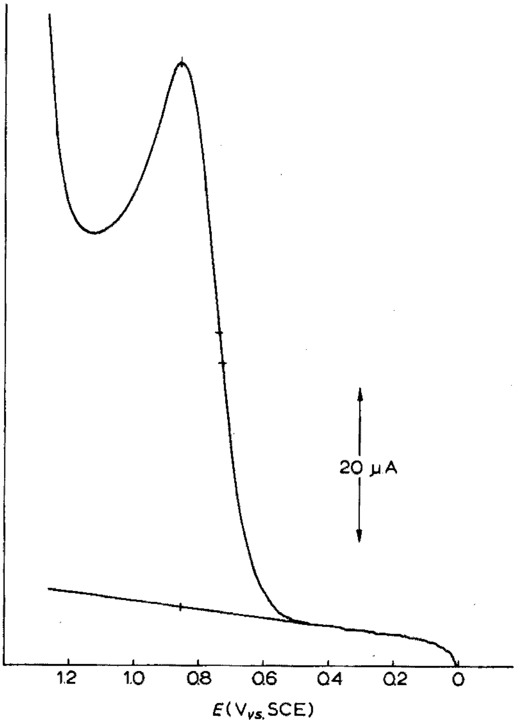

Fig. 1. Peak voltammogram for a $4.86 \cdot 10^{-3}$ *M* SCN$^-$ solution in 0.1 *M* KCl, 0.02 V s^{-1}, 25°.

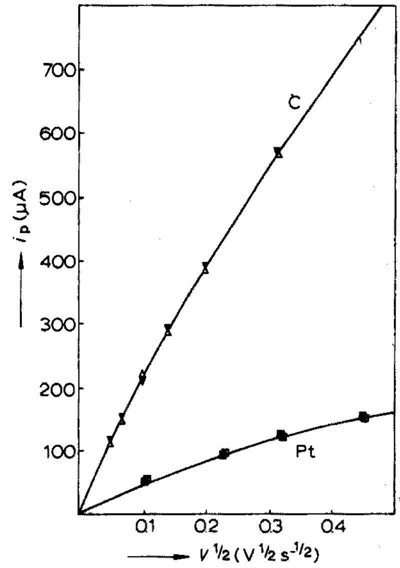

Fig. 2. Peak current *vs.* scan rate, $4.86 \cdot 10^{-3}$ *M* SCN$^-$, 25°; glassy carbon electrode in: (△) 0.1 *M* KCl, (▼) 0.1 *M* KNO$_3$; platinum electrode in: (■) 0.1 *M* KCl, (□) 0.1 *M* KNO$_3$.

platinum electrode are included for comparison. The curve obtained for the glassy carbon electrode more closely approximates a straight line.

The peak current showed a linear dependence on the thiocyanate ion concentration (Fig. 3). A faster scan rate and higher concentrations were necessary to obtain reproducible results with the platinum electrode than with the glassy carbon electrode. In addition, a wider range of concentrations and scan rates may be investigated with the glassy carbon electrode. Coupled with the fact that the oxidation peak is well-defined, the glassy carbon electrode is preferred for analytical purposes.

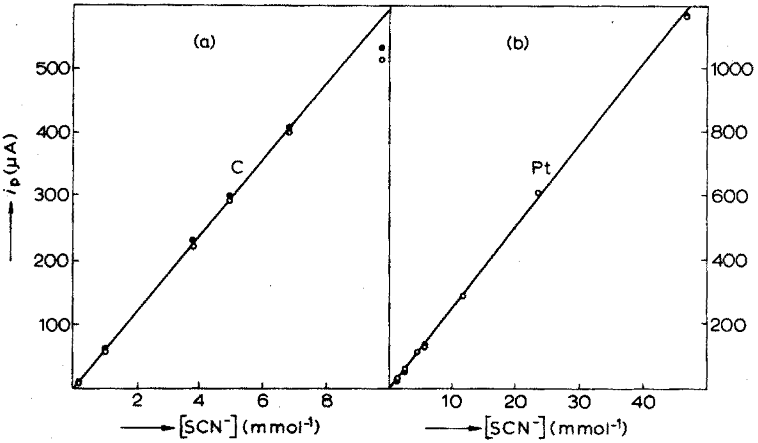

Fig. 3. Peak current *vs.* concentration, at 25°, for (a) glassy carbon electrode, scan rate = 0.02 V s^{-1}, in (○) 0.1 *M* KCl and (●) 0.1 *M* KNO_3; (b) platinum electrode, scan rate = 0.1 V s^{-1}, in (○) 0.1 *M* KCl and (●) 0.1 *M* KNO_3.

For the determination of the kinetic parameters a scan rate was chosen from the straight-line portion of the graph shown in Fig. 2. A scan rate of 0.1 V s^{-1} was used for the platinum electrode, and 0.02 V s^{-1} for the glassy carbon electrode. The product αn_a (see above) was evaluated from the slopes of the peak voltammograms. If the quantity $E_p - E_{p/2}$ is independent of the scan rate V, the electrode process can be assumed to fit the totally irreversible situation. The data in Table I show that the quantity $E_p - E_{p/2}$ is very nearly independent of the scan rate. The equation used to evaluate αn_a has been given[8–10], and for the anodic process the following relationship exists:

$$E_p - E_{p/2} = N \frac{RT}{\alpha n_a F} \tag{2}$$

The constant $N = 1.85$ for the totally irreversible reaction. The values calculated for αn_a from eqn. (2) are also shown in Table I.

The values for the diffusion coefficient of the thiocyanate ion were determined from eqn. (1). The diffusion coefficients obtained for the glassy carbon electrode are *ca.* 4-fold those for the platinum electrode. This discrepancy may be explained by the fact that during anodic polarization of the platinum, stepwise formation of oxide films occurs[11,12], at a potential close to that for the

TABLE I

TRANSFER COEFFICIENTS AND DIFFUSION COEFFICIENTS AT 25°

[SCN⁻] *(mM l⁻¹)*	i_p *(μA)*	$E_p - E_{p/2}$	αn_a	*D* *(cm² s⁻¹ · 10⁶)*	*[SCN⁻]* *(mM l⁻¹)*	i_p *(μA)*	$E_p - E_{p/2}$	αn_a	*D* *(cm² s⁻¹ · 10⁶)*
Pt electrode; scan rate, 0.1 V s⁻¹					*Glassy C electrode; scan rate, 0.02 V s⁻¹*				
Supporting electrolyte, 0.1 *M* KCl					Supporting electrolyte, 0.1 *M* KCl				
46.8	1173	0.149	0.320	3.09	9.71	535	0.120	0.397	11.0
23.4	607	0.105	0.453	3.31	6.80	396	0.110	0.433	12.3
11.7	286	0.090	0.528	2.94	4.86	280	0.110	0.433	12.0
5.85	135	0.110	0.433	2.62	3.88	221	0.100	0.476	11.8
4.68	125	0.105	0.453	3.51	0.971	60.5	0.090	0.528	14.1
2.34	59.2	0.110	0.433	3.15	0.0971	6.8	0.110	0.433	17.8
1.17	30.9	0.110	0.433	3.43	0.0486	3.3	0.090	0.528	16.7
			0.436 ± 0.061	3.15 ± 0.31				0.461 ± 0.051	13.7 ± 2.6
Supporting electrolyte, 0.1 *M* KNO_3					Supporting electrolyte, 0.1 *M* KNO_3				
46.8	1088	0.143	0.333	2.73	9.71	570	0.100	0.476	11.0
23.4	608	0.100	0.476	3.41	6.80	418	0.110	0.433	12.0
11.7	286	0.110	0.433	3.02	4.86	300	0.100	0.476	12.1
5.85	139	0.100	0.476	2.85	3.88	230	0.095	0.501	11.2
4.68	123	0.105	0.453	3.49	0.971	59.1	0.080	0.595	11.8
2.34	47.9	0.110	0.433	2.12	0.0971	7.2	0.080	0.595	17.5
1.17	21.0	0.130	0.366	1.63	0.0486	3.6	0.080	0.595	17.5
			0.424 ± 0.055	2.75 ± 0.67				0.524 ± 0.069	13.3 ± 2.9

thiocyanate oxidation. If these films render the electrode partially inert, a lower peak current would result, and thus a lower calculated value for the diffusion coefficient. Moreover, a small amount of a polymeric substance is formed[4,7], which adheres to the electrode, again resulting in a decreased electroactive surface area, and inaccurate value for i_p.

It appears that the glassy carbon electrode is considerably less involved in the electrochemical process than is the platinum electrode, and the kinetic parameters for the reaction at the carbon electrode were calculated. Vetter[13] has shown the relationship, at a constant potential, between the current density, the concentration of the electroactive species, and the electrochemical reaction order z: $z = \Delta \log i / \Delta \log c$.

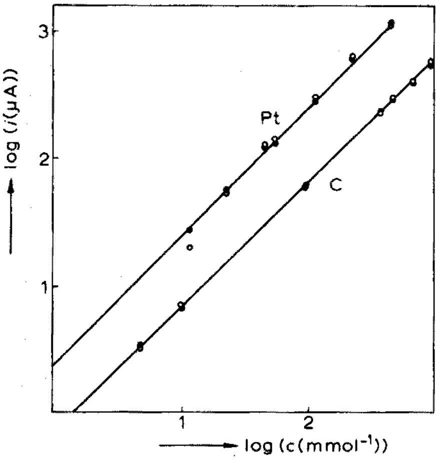

Fig. 4. Effect of concentration on current in a stirred solution for glassy carbon electrode, 21°, $[SCN^-] \cdot 10^5$: (○) 0.1 *M* KCl, potential 0.55 V (N.H.E.); (●) 0.1 *M* KNO_3, potential 0.62 V (N.H.E.). For the platinum electrode, 25°, $[SCN^-] \cdot 10^4$: (○) 0.1 *M* KCl, potential 0.52 V (N.H.E.); (●) 0.1 *M* KNO_3, potential 0.52 V (N.H.E.).

To evaluate z, the rate of the electron-transfer reaction must be the limiting step in the overall electron-transfer sequence which is accomplished by efficiently stirring the solution. Figure 4 shows the plots for log *i versus* log *c*. The slopes of these lines are close to unity in both instances, and it must be concluded that the initial step of the electrochemical oxidation involves the removal of one electron from one thiocyanate ion. Moreover, the product αn_a varies between 0.436 and 0.524, depending on the electrode material and supporting electrolyte; the transfer coefficient for slow electrochemical reaction steps usually falls within the range 0.4–0.6, thus the value of 1 for n_a seems indicated. These data disagree with Gauguin's conclusion that the reaction proceeds by the transfer of one electron each from two thiocyanate ions simultaneously, followed by a dimerization or dismutation process. The rate constant for the irreversible electron transfer may be calculated from the peak potential[8–10]

$$E_p = \frac{RT}{\alpha n_a F} 0.78 - \ln \frac{k_0 f}{D^{\frac{1}{2}}} + 0.5 \ln \frac{\alpha n_a F V}{RT}$$

where k_0 is the rate constant in cm s^{-1}, f is the activity coefficient of the oxidized species, V is the scan rate in V s^{-1}, and the other parameters have their usual significance. These data are shown in Table II. The temperature-dependence of the peak potential could, for the range 2–45°, entirely be explained by changes in the values for αn_a and the diffusion coefficient (within the limits of accuracy of the measurements). At temperatures above 45° the peak current was not reproducible.

TABLE II

RATE CONSTANTS WITH THE GLASSY CARBON ELECTRODE AT 25°

Scan rate ($V\ s^{-1}$)	*KSCN ($mM\ l^{-1}$)*	*Supporting electrolyte, 0.1 M KCl*[a]		*Supporting electrolyte, 0.1 M KNO_3*[c]	
		E_p (V)	*log k_0 ($cm\ s^{-1}$)*	*E_p (V)*	*log k_0 ($cm\ s^{-1}$)*
0.02	9.71	0.59	6.98	0.63	7.89
0.02	6.80	0.57	6.82	0.63	7.89
0.02	4.86	0.57	6.82	0.62	7.79
0.02	3.88	0.61	7.14	0.63	7.89
0.02	0.971	0.60	7.06	0.62	7.79
0.02	0.0971	0.58	6.90	0.53	6.99
0.02	0.0486	0.58	6.90[b]	0.53	6.99
0.002	4.68	0.56	7.19	0.62	8.29
0.004	4.68	0.56	7.04	0.63	8.24
0.010	4.68	0.56	6.84	0.63	8.04
0.020	4.68	0.57	6.77	0.63	7.89
0.040	4.68	0.57	6.62	0.65	7.90
0.100	4.68	0.64	6.96	0.68	7.98
0.200	4.68	0.68	7.13	0.70	8.00
			6.94 ± 0.15		7.83 ± 0.38

[a] $D=1.37\cdot10^{-5}$ $cm^2\ s^{-1}$; $\alpha n_a=0.461$. [b] The same E_p and log k_0 values were obtained for 0.0243 in M KSCN l^{-1}. [c] $D=1.38\cdot10^{-5}$ $cm^2\ s^{-1}$; $\alpha n_a=0.524$.

The products of the anodic oxidation of the thiocyanate ion were confirmed to be cyanide, sulfate, and minute quantities of an assumed polymeric substance at the platinum electrode. This compound was insoluble in CS_2, CCl_4, methanol, benzene and water, and decomposed in concentrated sulfuric acid. The infrared spectrum in a KBr pellet showed only one strong absorption band at 1975 cm^{-1}, which fell within the range for the cyanide stretching band. Evidence for sulfide or disulfide linkages or for rhombic S_8 could not be found. The results of the mass spectrum for the compound are shown in Table III. Comparison with the formulae ($HN_3S_3C_3 \cdot H_2O$ and $HN_3S_3C_3$) suggested by Rius and Terol[4] show little agreement. An empirical formula fitting the mass spectral data is $C_6N_4S_4$. A more plausible explanation appears to be that the substance is largely composed of elemental sulfur, especially considering the isotopic distribution of sulfur in S_8.

TABLE III

MASS SPECTRUM OF UNKNOWN COMPOUND

M/e	*Relative abundance*			*M/e*	*Relative abundance*		
	Unknown	S_8	*S*		*Unknown*	S_8	*S*
32	—	14	—	161	—	1	—
34	—	3	—	162	10	5	—
44	80	2	CS–CO_2	163	8	1	—
64	100	100	S_2	192	34	17	S_6 $N_3C_5S_3 \cdot H_2O$
66	17	10	—	194	10	15	—
76	10	8	CS_2	223	8	—	—
96	—	15	S_3	224	7	2	S_7
98	—	2	—	226	—	1	—
128	80	35	S_4	256	92	32	S_8 $N_4C_6S_4$
129	12	2	—	257	6	2	—
130	16	6	—	258	30	12	—
160	56	26	S_5	259	2	1	—
				260	5	2	—

SUMMARY

The kinetic parameters for the irreversible oxidation of the thiocyanate ion at the glassy carbon electrode have been determined. The rate-controlling step in the overall sequence was found to be the removal of the first electron from the thiocyanate ion.

RÉSUMÉ

On a déterminé les paramètres cinétiques de l'oxydation irréversible, à l'électrode carbone vitreux, de l'ion thiocyanate. La vitesse de la réaction est contrôlée par la vitesse de l'enlèvement du premier électron de l'ion thiocyanate.

ZUSAMMENFASSUNG

Die kinetischen Parameter für die irreversible Oxidation des Thiocyanations an der glasartigen Kohleelektrode wurden ermittelt. Der geschwindigkeitsbestimmende Schritt beim Gesamtvorgang ist die Entfernung des ersten Elektrons vom Thiocyanation.

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DETERMINATION OF PROTEINS BY AMPEROMETRIC TITRATION WITH 12-PHOSPHOTUNGSTIC ACID AT ROTATING GOLD ELECTRODES

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The determination of proteins is of major importance in biochemistry and clinical chemistry. There are very few high-precision methods for total protein; the most precise and generally acknowledged reference method is Kjeldahl nitrogen analysis[1]. A number of quite sensitive but relatively inselective colorimetric methods are available[2].

12-Phosphotungstic acid has been used for many years to stain proteins[3] and has been introduced as a titrant for proteins with thermometric end-point location[4]. Unfortunately, thermometric detection is not entirely satisfactory in that rather small temperature changes accompany the reaction and the titration curves are encumbered by the change in heat production caused by stirring as the sample's viscosity varies during the course of titration. In the thermometric titration studies[4], it was shown that purified proteins, such as bovine serum albumin (BSA), react with 12-phosphotungstic acid in 0.1 *M* hydrochloric acid with a stoichiometry dictated by the number of basic amino acids residues plus the number of N-terminal chains per molecule of protein. For samples of human serum, the method correlates quite well with both Kjeldahl nitrogen analysis and biuret colorimetry. It was also shown[4] that the reaction stoichiometry is independent of protein concentration in the range 2–10 g l^{-1} provided that the rate of addition of titrant is sufficiently low. No interference from free amino acids or the inorganic cations commonly encountered in serum was discovered.

Since 12-phosphotungstic acid is easily reduced at the dropping mercury electrode (in 0.1 *M* sulfuric acid it is reduced in three steps whose half-wave potentials are −0.218, −0.448 and −0.895 V *vs.* SCE[5]), an attempt was made to use the reagent in conjunction with amperometric end-point location. Amperometry has been neglected as a clinical tool, owing to inherent difficulties in deaeration of sample solutions (foaming, loss of sample, etc.) and the adsorptive behavior of proteins on electrode surfaces. As will be shown, the presence of oxygen in the sample need not be a serious analytical problem, even if it is electro-reduced at the potential used for detection of the end point. The high cathodic overpotential of water on a gold electrode permits continuous amperometric titrations to be carried out at relatively negative potential (−0.25 V· *vs.* SCE) even in strongly acidic media (*e.g.* 1 *M* hydrochloric acid). The use of a solid electrode circumvents a major problem encountered with polarographic analyses in proteinaceous media, *i.e.* the distortion of the current–time curves and concomitant complex wave shape.

EXPERIMENTAL

Apparatus

Amperometric titrations were carried out with a stabilized Heath Polarographic Module Model EUA-19-2. The rotating gold electrode, constructed in this laboratory, was mounted in a synchronous rotator (Sargent-Welch Scientific Co., Skokie, Ill.). The current–time curves were monitored on a Sargent (Model SR) recorder. A saturated calomel electrode (Fisher Scientific Co.) was used as the reference electrode and a platinum wire as the counter electrode in the three-electrode polarograph.

Titrant solutions were added with a syringe drive pump (Sage Instruments, Cambridge, Mass., Model 234-3). A Hamilton 1-ml gas-tight syringe Model 1001-LT was used in all titrations in order to prevent seepage of the titrant and subsequent evaporation on the syringe piston. The rate of titrant addition was $4.372 \cdot 10^{-2}$ ml min^{-1} of 0.161 N 12-phosphotungstic acid; this is well below the maximum addition rate suggested by Smith and Carr[4]. A syringe microburet (Micro-Metric Instruments, Cleveland, Ohio, Model No. SB2) was used to dispense aliquots of albumin, sera and cesium solutions for the biuret and amperometric analyses. A Fisher Scientific magnetic stirrer with a Teflon-coated stir-bar was used to mix the solution during the titration.

Biuret analyses were performed with a Spectronic 505 spectrophotometer. A clinical centrifuge (International Equipment Company, Needham, Mass., Serial 248) was used to spin down both blood cells and fibrin to obtain the sera. The centrifuge was also used to remove insoluble matter present in freshly prepared phosphotungstic acid solutions.

Reagents

Bovine serum albumin. (Fraction V, powder; Schwarz/Mann, Orangeburg, N.Y.) was specified in the maker's certificate of analysis as 97% albumin by weight, the remainder being globulins. This material was desiccated, refrigerated and used without further purification.

Stock solutions of bovine serum albumin were prepared by dissolving the desired quantity of protein in cold water by means of a cold-plate stirrer (Stir Kool, Model SK-12, Thermoelectronics Unlimited, Wilmington, Del.). The solution was then quantitatively transferred to a volumetric flask, allowed to warm to room temperature and diluted to volume. In general, the stock solutions contained 3 g of albumin per 100 ml of water. The solution was stored in a refrigerator and allowed to warm to room temperature before use. These solutions were found to be stable up to two weeks. Generally, after two weeks, insoluble material was noted in the solutions and they were discarded.

12-Phosphotungstic acid solution. This was prepared by weighing the desired amount of material (Baker Analyzed reagent grade), dissolving it in water and diluting to volume. The cloudy solution which resulted was unsuitable for use and was cleared by centrifugation and decantation of the supernate. The titer of the solution against cesium chloride was stable up to a month or more even though a slight blue color developed after about two weeks.

Cesium chloride. This (99.96% Fisher Scientific) was used for the standardization of the titrant solutions; the cesium chloride was dried at 110° for 24 h and desiccated before use.

All other chemicals employed were reagent grade and used without further purification. All solutions were prepared with doubly deionized water.

Serum samples

The serum samples were prepared from blood specimens collected the previous day. Cellular constituents were centrifuged. The plasma was removed and allowed to clot by the addition of calcium chloride and centrifuged again to remove fibrin. The sera were decanted and stored at 4° until required. Approximately 2 ml of serum were available from each sample. Human serum albumin and globulin protein fractions were prepared from a pool of sera from 25 individuals by the standard procedure of Wolfson and Cohn[6] as described by Henry[7]: 950 ml of a 28 w/v% sodium sulfite solution were added to 50 ml of serum, followed by the addition of 300 ml of ether. After mixing and allowing the phases to separate, the solution was centrifuged for 10 min. The globulin protein fraction remained as a pellet on the top of the solution and was easily removed. This material was dialyzed against 0.1 *M* sodium chloride for 48 h. After dialysis, all insoluble protein was removed, and the solution refrigerated.

The remaining albumin solution was dialyzed against water for 24 h. This fraction was then salt-fractionated by adding 560 g of ammonium sulfate to each liter of albumin solution[7]. The resulting phases were separated by centrifugation and the protein removed. The isolated albumin was dialyzed against water for 48 h. All insoluble matter was removed and the protein solution was refrigerated. Although more rigorous procedures are available for isolation of the protein fractions of serum and purified commercial materials are available, our principal interest was the dependence of the titration stoichiometry upon the broad classes of serum proteins, *i.e.* albumins and globulins. Therefore human serum fractionated as described above was deemed more suitable than highly purified commercial proteins.

Procedure

Add an appropriate amount of the serum, protein, or cesium chloride solution to the amperometric tiration cell, followed by 40 ml of 1.0 *M* hydrochloric acid. Insert the gold working electrode, the reference electrode, the platinum anode and a Teflon-coated magnetic stirring bar and stir the sample. The best titration curves are obtained when the stirring is extremely vigorous.

Fill the buret with titrant and introduce an air gap of known volume into the tip of the titrant delivery line; the air bubble serves to prohibit contact between the titrant and sample before analysis. Only then, apply a potential of −0.25 V *vs.* SCE to the gold electrode and start rotation. Start the recorder and engage the syringe pump motor at a predetermined point on the chart paper.

Stop the titration when the post-titration region is long enough to provide satisfactory precision and accuracy in locating the end-point[8]. Turn off the recorder and break electrical contact to the cell.

Clean both the cell and gold electrode by rinsing with copious quantities of alcoholic potassium hydroxide solution. Scrupulous cleaning is mandatory in order to remove all traces of adsorbed proteinaceous material from the electrode surface. Adsorbed material on the electrode surface lowers the observed cathodic current, obscures the end-point extrapolation, and thereby decreases the precision and accuracy of the method.

RESULTS AND DISCUSSION

Electroduction of 12-phosphotungstic acid at a rotated gold electrode

Figure 1 shows a typical voltammogram of 12-phosphotungstic acid in 1.0 *M* hydrochloric acid. In contrast to the polarographic waves observed at a DME[5], only one wave is observed at the gold electrode. The half-wave potential of this cathodic process is −0.16 V *vs.* SCE, in reasonable agreement with the value of the first wave at −0.22 V in 0.1 *M* sulfuric acid reported by Kennedy[5]. It should be noted from Fig. 1 that reduction of solvent starts at −0.4 V, the region of the second polarographic 12-phosphotungstic acid wave[5]. It is most likely that the second reduction wave of 12-phosphotungstic acid is hidden by the larger solvent reduction current and therefore not visible. The polarographic wave at −0.70 V is, similarly, too negative to be observed on the gold electrode.

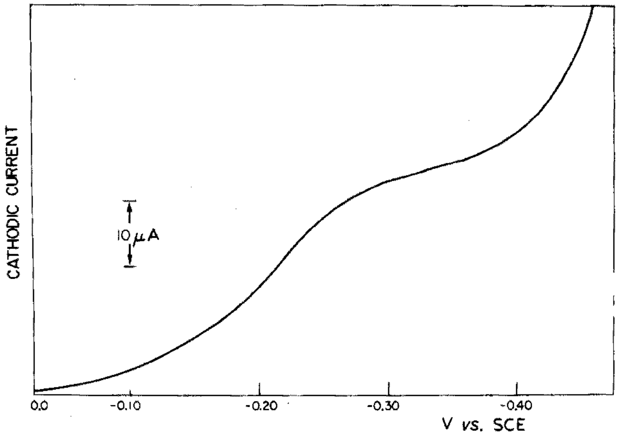

Fig. 1. Voltammogram on a rotating gold electrode of an oxygen-free $1.95 \cdot 10^{-3}$ *N* solution of 12-phosphotungstic acid at pH 0.0.

The effect of oxygen on the reduction of 12-phosphotungstic acid is shown in Fig. 2. Curve *a* is a voltammogram of an air-saturated solution of 1.0 *M* hydrochloric acid; the half-wave potential of the oxygen reduction wave is −0.22 V *vs.* SCE. Curve b is a voltammogram of 12-phosphotungstic acid in deaerated 1 *M* hydrochloric acid. The proximity of the half-wave potentials of the 12-phosphotungstic acid and oxygen reduction waves might indicate that the presence of oxygen would completely obscure the end-point; this is not the case. At the protein concentrations found in serum, a rather small excess of the electroactive titrant is readily discerned even in the presence of oxygen.

An additional complication arises from the presence of protein in solution. Proteins are strong surfactants which adsorb tenaciously to electrode surfaces[9] and interfere with mass transport and heterogeneous electron transfer[10]. Normal human

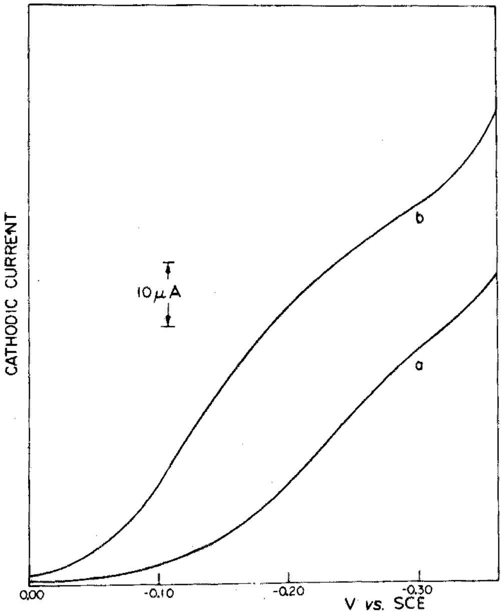

Fig. 2. Effect of oxygen on the voltammetry of 12-phosphotungstic acid at a rotating gold electrode; (a) air-saturated solution of 40 ml 1.0 *M* HCl, (b) 0.25 ml of 0.161 *N* phosphotungstic acid in deaerated HCl solution.

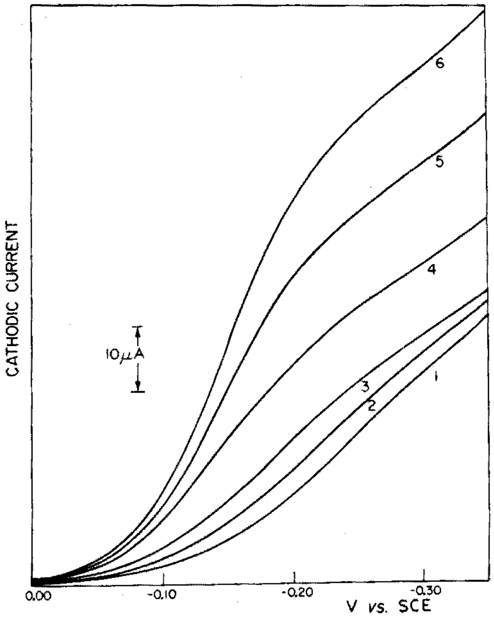

Fig. 3. Rotating gold electrode voltammograms during a titration of serum with 0.161 *N* 12-phosphotungstic acid (1.0 ml of serum, 40 ml of 1.0 *M* HCl; eq. pt. at 0.51 ml of 0.161 *M* 12-phosphotungstic acid). (1) Air-saturated serum solution, (2) 0.25 ml, (3) 0.50 ml, (4) 0.75 ml, (5) 1.00 ml and (6) 1.25 ml of titrant added.

serum contains about 6.4–8.3 g of protein per 100 ml[7]. In this work a 1-ml aliquot of serum was taken and diluted to 40 ml; the protein concentration in the diluted sample is about 2 mg ml^{-1}. Previous electrochemical studies of heme proteins by Betso *et al.*[11] have shown that protein concentrations as high as 4 mg ml^{-1} do not completely inhibit electron transfer, but allow reduction to occur. Figure 3 shows that 12-phosphotungstic acid is reduced even in the presence of serum. The curves presented in Fig. 3 are voltammograms obtained at various stages in the titration of serum. After the equivalence point, it is evident that the reduction current of 12-phosphotungstic acid is detectable and increases with increasing volume of added titrant. Similar voltammograms were obtained for the titration of bovine serum albumin samples at the same protein concentration.

Therefore, the 12-phosphotungstic acid concentration in the presence of oxygen can be monitored during a titration by poising the electrode at a potential on the current plateau of the 12-phosphotungstic acid reduction wave. A potential of −0.25 V *vs.* SCE was applied to the rotating gold electrode in all titrations.

Standardization of solution

Smith and Carr[4] studied the 12-phosphotungstic acid titration system and found that the negatively charged 12-phosphotungstic ion reacts quantitatively with the protonated sites on the protein molecule forming a precipitate, the sites involved being the protonated basic amino acid groups and the N-terminal groups of the polypeptide chain. At very low pH, less than pH2 for bovine serum albumin[12, 13], all the basic amino acid groups and N-terminal chain ends are protonated and titratable. The 12-phosphotungstic acid stoichiometry is the sum of all these sites. Smith and Carr[4] found that bovine serum albumin has an apparent stoichiometry of 144 moles per 100,000 g which is in good agreement with the amino acid analysis value of 148 basic residues per 100,000 g[12, 14]. Standardization of the 12-phosphotungstic acid involves determining the number of equivalents of 12-phosphotungstic acid per volume of titrant which are capable of reacting with the positive charge sites on the protein. Cesium chloride is suitable for this standardization. It has been shown[4] that the reaction of cesium(I) involves three cesium(I) ions and 1 molecule of 12-phosphotungstic acid. The reaction is stoichiometric at low pH and the precipitate formed is very insoluble. The reaction of 12-phosphotungstic acid with cesium(I) is similar to that of proteins in that both reactions involve precipitation of a positively charged ion by the negatively charged 12-phosphotungstic acid moiety. However, the analogy is not complete because the cesium precipitation involves only one molecule of 12-phosphotungstic acid whereas the protein precipitation involves multiple molecules of 12-phosphotungstic acid, *i.e.* $n/3$ ions, where n is the number of protonated residues on the protein.

Table I shows the results of the standardization of 12-phosphotungstic acid against cesium chloride. It should be noted here that the cesium amperometric titration was carried out in the presence of oxygen with no apparent adverse effects. The high precision of the standardization (+0.1–0.2%) testifies to the absence of interference by oxygen, as does the invariance of the 12-phosphotungstic acid normality determined in an amperometric titration of air-saturated and deaerated cesium chloride solutions.

TABLE I

PRECISION OF THE STANDARDIZATION AND ANALYSIS PROCEDURES

Amperometric titration of cesium chloride with 12-phosphotungstic acid[a]		*Titration of pooled sera with 12-phosphotungstic acid*[c]	
Sample no.	*Calculated titrant normality*[b]	*Sample no.*	*Protein determined (g)*[e,f]
1	0.1605	1	0.07352
2	0.1604	2[d]	0.07352
3	0.1609	3	0.07308
4	0.1603	4	0.07321
		5[d]	0.07345
		6	0.07339

[a] For 1.00 ml of 92.03 m*M* cesium chloride in 40 ml of 1.0 *M* hydrochloric acid. Solutions were not deaerated.

[b] Coefficient of variation, 0.16%.

[c] For 1.00 ml of sera in 40 ml of 1.0 *M* hydrochloric acid, titrant concentration 0.1604 *N*. Solutions were not deaerated.

[d] Electrode stationary, solution stirred vigorously.

[e] Coefficient of variation 0.24%.

[f] Grams of protein calculated from 112 moles of basic amino acid residues per 10^5 g of serum protein.

Titrations of proteins

In order to check the validity of the analysis, solutions with a known concentration of bovine serum albumin were prepared and titrated amperometrically with 12-phosphotungstic acid. Bovine serum albumin was chosen as a model protein because it can be obtained in high purity, it is similar to human serum albumin, the number of protonated sites per g of protein is known and has been extensively studied as a function of pH, and it is available in large quantities. Tanford[12] studied by acid–base titration the number of protonation sites on the protein as a function of pH and found that there are 148 moles of basic amino acid groups per 10^5 g of the albumin. At pH below 2, all the basic amino acid groups are protonated and there are 148 moles of positively charged sites per 10^5 g of albumin.

Table II presents the results of the amperometric titrations of bovine serum albumin. The samples were not deaerated before titration. Deaeration via a gas dispersion tube or a simple capillary bubbler caused extensive foaming and loss of sample; addition of defoaming agents such as isoamyl alcohol helped but did not solve the problem. Consequently, the titrations were necessarily carried out in the presence of oxygen but without any apparent deleterious effect on the precision of analysis. The results in Table II suggest the presence of two unexpected phenomena. First, there is an effect of pH on the measured reaction stoichiometry; an F-test showed that this effect is statistically significant at the 95% confidence level. The amperometric titration curves at several acid concentrations are shown in Fig. 4. In dilute acid (pH *ca.* 1), the titration curve before the end-point is not linear. Initially, the current rises and passes through a very shallow maximum before the end-point is reached. It is possible that this non-linearity introduces a determinate error into the extrapolated end-point thereby producing the apparent

TABLE II

REACTION STOICHIOMETRY AS A FUNCTION OF pH AND BOVINE SERUM ALBUMIN CONCENTRATION

Acid concentration[a] *(M)*	*Weight albumin taken* $(\cdot 10^2\ g)$	*MBA*[b]	*Weight albumin found*[c] $(\cdot 10^2\ g)$	*%Range*[d]
0.15	6.122	130.0	5.360	0.6
0.15	8.746	130.6	7.693	1.7
0.30	8.746	133.3	7.855	0.5
1.0	8.746	136.4	8.033	0.3
1.0	7.434	136.6	6.841	0.2
1.0	6.122	136.9	5.642	0.2
1.0	4.373	137.1	4.037	0.3

[a] All samples were contained in 40 ml of the indicated concentration of hydrochloric acid.

[b] Moles of basic amino acid residues per 10^5 g of protein, determined from standardization of the titrant against cesium chloride.

[c] Calculated using 148 MBA for bovine serum albumin and a titrant concentration of 0.1770 *N*.

[d] Based on duplicate measurements.

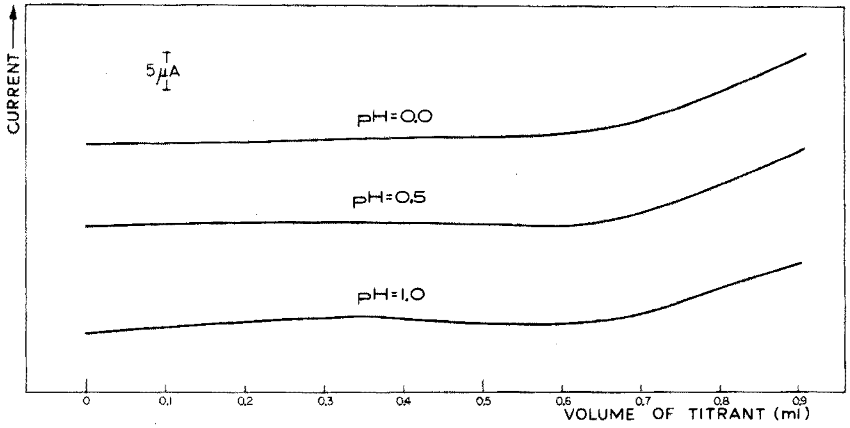

Fig. 4. Amperometric titration curves of human serum with 12-phosphotungstic acid as a function of hydrochloric acid concentration.

decrease in stoichiometry with decrease in acidity. Since the titrations are carried out in oxygen, one cannot extrapolate the post titration curve to zero current and eliminate this effect. Titration curves of cesium chloride did not exhibit this phenomenon before the end-point. A second factor in relation to this observation is that the protein precipitate settles very slowly in dilute acid (0.1–0.15 *M* hydrochloric acid) but at pH 0 the precipitate begins to settle as soon as stirring ceases. The ill-defined pre-end-point region may be related to adsorption of the protein and/or the viscous precipitate at the electrode surface.

The concentration of bovine serum albumin has an apparent effect on stoichiometry, but is not statistically significant at the 90% confidence level. In the thermometric titration of this albumin with 12-phosphotungstic acid no statistically significant effect of protein concentration was observed[4]. Both direct amino acid analysis[14] and acid–base titration studies[12] of bovine serum albumin indicate the presence of 147–148 moles of reactive basic amino acid groups per 10^5 g of protein. Extrapolation of the albumin data obtained in 1 *M* hydrochloric acid (see Table II) to zero protein concentration indicates a stoichiometry of 137.8 ± 0.3 moles of reactive groups per 10^5 g of protein. Since no correction was made in this work for the purity of the protein (97% according to the supplier) or its water content, this value of 137.8 seems to be in reasonable agreement with the literature value for the number of positive charge sites.

In order to assess the capability of the amperometric titration for the determination of homogeneous protein fractions, *i.e.* mixtures which have uniform relative compositions, and of serum samples from various individuals, the data summarized in Fig. 5 curves a and b, and those shown in Fig. 6 were obtained. Figure 5 represents the results for the albumin (curve a) and globulin (curve b) fractions of pooled serum from 25 individuals. The fractions were prepared as described above. Since the biuret analysis is not precise (2–3%), a stock albumin and globulin solution was prepared and assayed by biuret colorimetry. This stock solution was diluted precisely to a desired level and assayed amperometrically. Consequently, the relative concentration of protein is known quite precisely (0.2%). The classical conversion factor of 2.77 dl g^{-1} cm^{-1} was used in all biuret determinations reported here. From the least-squares slope, an average stoichiometry of 114 ± 0.7 and 83.1 ± 1.6 equivalents per 100,000 g was obtained for the albumin and globulin fractions, respectively. Both of these values are considerably below the literature values for the basic amino acid content of the materials which are 148 and 105 equivalents per 100,000 g for the albumin[14] and globulin[16] fractions, respectively. This discrepancy may reflect an error in the biuret conversion factor but is more likely due to the binding of various organic materials at or near the basic amino acid residues.

The albumin fraction was gold in color, suggesting the presence of bound bilirubin. Albumin is known to bind bilirubin[17], free fatty acids and lipids[18]

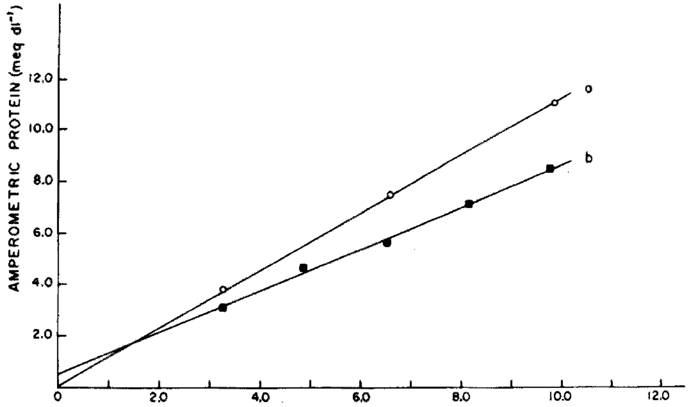

Fig. 5. Correlation of equivalents found *vs.* weight of protein taken for fractionated human serum. (a) Albumin fraction, (b) globulin fraction.

quite strongly. Bilirubin interferes with the dye-binding procedure for albumin with 2-(4′-hydroxyazobenzene)-benzoic acid[17]; this method is similar to the present one in that the essential reaction is between positively charged sites on the protein and an anion.

It is evident from the linearity of the two curves, in Fig. 5, the correlation coefficients being greater than 0.999 at the 99% confidence interval, that homogeneous mixtures of proteins may be determined by amperometric titration or by biuret colorimetry, and comparable results obtained, once a standard curve for the samples in question has been established.

Since the albumin and globulin fractions have distinctly different stoichiometries, it is evident that a series of samples of proteins each containing a different relative amount of albumin and globulin (or any other proteins) cannot correlate as closely with the biuret procedure as will a homogeneous series of samples. The results shown in Fig. 6 demonstrate this expectation. The correlation coefficient in this case is only 0.973 at the 99% confidence level, despite the fact that many more points were used to establish the correlation than for either curve of Fig. 5. The least-squares best line indicates that the stoichiometry, as derived from the slope of the line, is 75.3 ± 4 equivalents per 100,000 g of protein and that a statistically significant intercept of 2.77 ± 0.3 equivalents per dl is obtained. This correlation curve has several additional features. First, several samples lie distinctly off the line despite the fact that all samples were run in duplicate. Secondly there appears to be a definite decrease in slope when the protein concentration as measured by biuret colorimetry exceeds *ca.* 8.0 g dl^{-1}. This is indicated by a very non-random (*i.e.* systematic) variation in the sign and magnitude of the residuals.

The non-zero intercept evident in Fig. 6 suggests the presence of a biuret-inactive *i.e.* non-proteinaceous but precipitatable, material in the test sera. As shown previously, the free amino acids and common inorganic cations are not responsible for the intercept[4]. There are no other non-proteinaceous (*i.e.* biuret-inactive) materials present in serum at a level of *ca.* 20–30 meq l^{-1} which precipitate 12-phosphotungstic acid. A second factor which mitigates against the presence of a major interference is the high correlation between the biuret and amperometric deter-

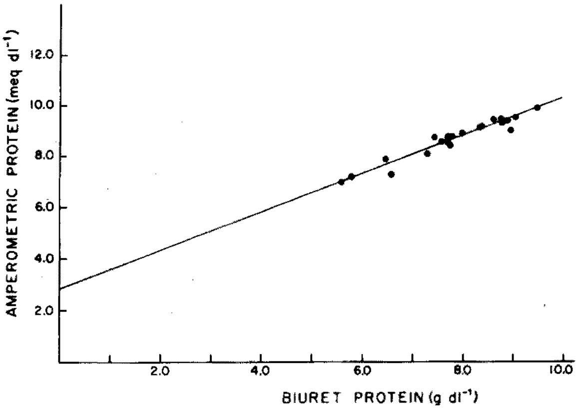

Fig. 6. Correlation of equivalents of protein found by amperometric titration with weight found by biuret for 23 individual sera.

ninations. The intercept represents *ca.* 20% of the total equivalents of protein found. ntuitively, any variation in such a constituent should produce a very large variance f the fit, yet the correlation coefficient indicates that over 97% of the total ariation of the Y axis (amperometric) data is due to the change in the X axis biuret) data. The non-zero intercept of Fig. 6 could also be due to the presence of amples with abnormal albumin to globulin (A/G) ratios. In order to achieve a erfect match between the biuret and amperometric methods, it is necessary that the iuret absorbance per g of protein and the number of equivalents per g of protein e independent of protein type. The report of Peters[19] indicates that the first ondition is essentially correct but it is evident from the present data that the econd condition is not valid for the two major protein fractions of human serum. difference of more than 25 equivalents/10^5 g exists between the albumin and lobulin fractions. Therefore, a positive intercept would result if the data set used to stablish Fig. 6 contained samples with either high A/G ratios and low total rotein or low A/G ratios and high total protein. There are many disease states vhich bring about this situation[20].

In the absence of electrophoretic or other data on the A/G ratios of these amples, an attempt was made to exclude subnormal samples by deleting all data utside the normal range[7] of 6.3–8.4 g dl^{-1} from the least-squares analysis. The verage stoichiometry increased to 86.1 equivalents/10^5 g, and the intercept de-reased to 1.9 meq dl^{-1}. The direction of both of these changes is in accord with he presence of samples having abnormal A/G ratios in the original data set.

Despite the above discussion, it is not yet certain that serum contains no naterials capable of contributing at least part of the above intercept. Further work levoted to developing the technique for the determination of total serum protein is in rogress. The nature of the intercept will be elucidated by application of various eparation methods to serum with the goal of eliminating or minimizing the inter-ept, if it is due to an interference. In addition, a large number of sera will be xamined electrophoretically to determine whether abnormal A/G ratios are respon-ible for the intercept. In its present state of development, the amperometric echnique may be the method of choice for the rapid (5 min) and precise (0.3%) letermination of protein preparations consisting predominantly of a single protein omponent or to samples containing mixtures of proteins in fixed relative amounts.

This work was supported by grant GM17913 from the National Institute f Health.

UMMARY

Amperometric titration of various protein fractions and human serum with 2-phosphotungstic acid at the rotating gold electrode is described. The titrant is lectroreducible and its concentration may be monitored by poising an ampero-netric indicator electrode at a potential of −0.25 V *vs.* SCE. In 1.0 *M* hydrochloric cid, well defined titration curves of proteins may be obtained in the presence of air-aturated solutions. The relative standard deviation of replicate samples is 0.2–0.3% n the range 2–4 g of protein per l in the sample solution. The technique is prin-ipally applicable to homogeneous mixtures of proteins.

RÉSUMÉ

Un titrage ampérométrique avec électrode d'or tournante est décrit pour le dosage de diverses fractions de protéïne et de sérum humains, au moyen d'acide 12-phosphotungstique. Le titrant est électroréductible; sa concentration peut être contrôlée en maintenant l'électrode ampérométrique indicatrice à un potentiel de −0.25 V *vs.* ECS. En milieu acide chlorhydrique 1.0 *M*, des courbes de titrage bien définies de protéïnes peuvent être obtenues, en présence de solutions saturées d'air. La déviation standard relative est de 0.2–0.3% pour des concentrations de l'ordre de 2–4 g de protéïne par litre. Cette technique est principalement applicable à des mélanges homogènes de protéïnes.

ZUSAMMENFASSUNG

Es wird die amperometrische Titration von verschiedenen Proteinfraktionen und menschlichem Serum mit 12-Phosphorwolframsäure an der rotierenden Goldelektrode beschrieben. Das Reagenz ist elektrochemisch reduzierbar, und dessen Konzentration kann kontrolliert werden, indem eine amperometrische Indikatorelektrode auf einem Potential von −0.25 V gegen ges. Kalomelelektrode gehalten wird. In 1.0 *M* Salzsäure können gut ausgebildete Titrationskurven von Proteinen in Gegenwart luftgesättigter Lösungen erhalten werden. Die relative Standardabweichung im Bereich 2–4 g Protein pro 1 Probelösung ist 0.2–0.3%. Das Verfahren ist prinzipiell auf homogene Gemische von Proteinen anwendbar.

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POTENTIOMETRIC STUDIES ON ORGANIC COMPOUNDS CONTAINING SULPHUR WITH A SULPHIDE ION-SELECTIVE MEMBRANE ELECTRODE

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Some early potentiometric studies of sulphur compounds, such as thiourea and thioacetamide, have already been reported[1, 2]. In these publications, methods were described for the determination of the compounds mentioned and the reaction steps proceeding during potentiometric titrations were established.

In the present paper, an account is given of a continuation of this work, and similar studies made on phenylthiourea and N,N-diphenylthiourea.

EXPERIMENTAL

E.m.f. measurements were carried out with a Radelkis Precision pH meter type OP-205 (Radelkis, Budapest, Hungary).

A Radelkis sulphide ion-selective electrode (Type OP-S-711) was used as indicator electrode and a saturated calomel electrode as reference electrode. The reference electrode was connected with the solution to be titrated by a 0.1 *M* potassium nitrate agar salt bridge.

I.r. spectra were recorded by Zeiss UR-10 and UR-20 (Karl Zeiss, Jena) instruments.

All reagents used were of analytical grade. The stock solutions were prepared with ethanol, as the compounds studied are poorly soluble in water. The phenylthiourea stock solution was 0.1 *M*, while the N,N-diphenylthiourea solution was 0.05 *M*. The standard solutions were prepared from the stock solutions by dilution with distilled water.

RESULTS AND DISCUSSION

Potentiometric titrations of phenylthiourea and N,N-diphenylthiourea were carried out in alkaline, neutral and acidic (nitric) media.

Titrations in alkaline media

In Figs. 1 and 2 the potentiometric titration curves of 10^{-1}–10^{-4} *M* phenylthiourea and N,N-diphenylthiourea in 1 *M* sodium hydroxide are shown. Figures 3 and 4 show the same titrations in 0.1 *M* sodium hydroxide. As can be seen from Figs. 1 and 2, both phenylthiourea and N,N-diphenylthiourea can be titrated with standard silver nitrate solution with high accuracy in the presence

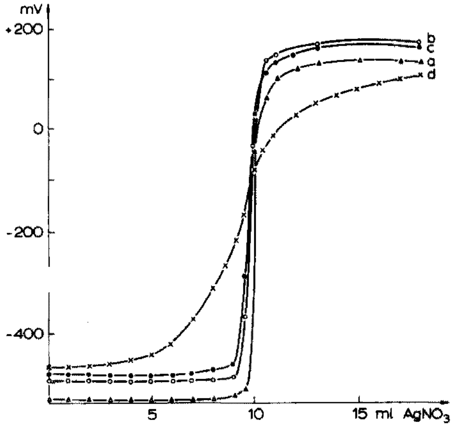


Fig. 1. Potentiometric titration curves of 10^{-1}–10^{-4} M phenylthiourea solutions in 1 M NaOH. (a) 10^{-1} M; (b) 10^{-2} M; (c) 10^{-3} M; (d) 10^{-4} M.

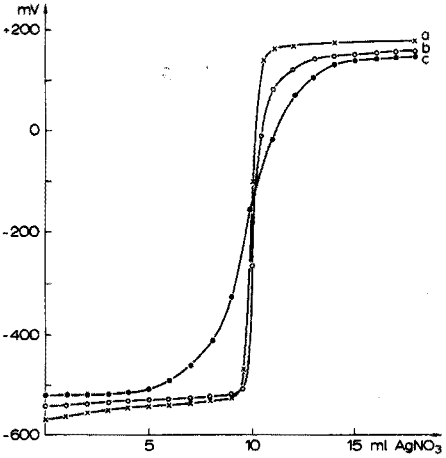


Fig. 2. Potentiometric titration curves of $5 \cdot 10^{-2}$–10^{-3} M N,N-diphenylthiourea solutions in 1 M NaOH. (a) $5 \cdot 10^{-2}$ M; (b) 10^{-2} M; (c) 10^{-3} M.

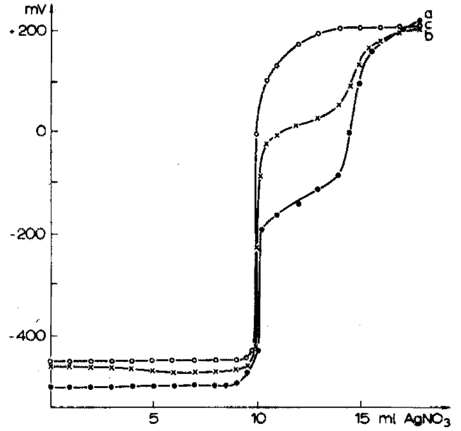


Fig. 3. Potentiometric titration curves of 10^{-1}–10^{-3} M phenylthiourea solutions in 0.1 M NaOH. (a) 10^{-1} M; (b) 10^{-2} M; (c) 10^{-3} M.

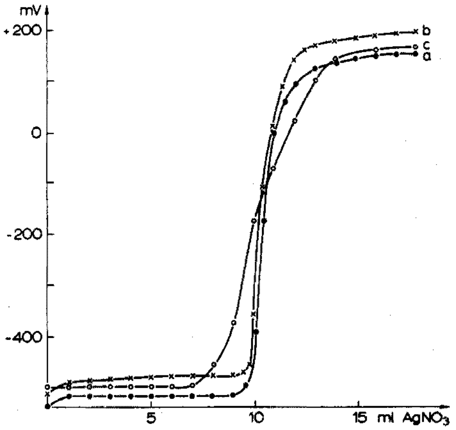


Fig. 4. Potentiometric titration curves of $5 \cdot 10^{-2}$–10^{-3} M N,N-diphenylthiourea solutions in 0.1 M NaOH. (a) $5 \cdot 10^{-2}$ M; (b) 10^{-2} M; (c) 10^{-3} M.

of 1 M sodium hydroxide, in the concentration range 10^{-1}–10^{-3} M. The changes which occur during the titration can be sensitively followed by the sulphide ion-selective electrode. The determination of 10^{-4} M phenylthiourea takes a longer time, as the establishment of the equilibrium potential at the electrode is slower at lower concentrations. Nevertheless, the titration curve can be readily evaluated even in this case.

In the presence of 1 M sodium hydroxide, one potential jump occurs on the titration curves of both compounds studied, at a volume of titrant corresponding to the stoichiometric reaction with silver nitrate. On the potentiometric titration curves of phenylthiourea in the presence of only 0.1 *M* sodium hydroxide (Fig. 3), two potential jumps can be observed, the first being applicable to the determination of phenylthiourea with adequate accuracy in the concentration range 10^{-1}–10^{-3} *M*. At a concentration of 10^{-3} *M*, the second potential jump does not occur, owing to the relatively large amount of alkali concentration compared to the amount of the phenylthiourea determined. N,N-diphenylthiourea can be determined with high accuracy in the presence of 0.1 *M* sodium hydroxide in the concentration range $5 \cdot 10^{-2}$–10^{-3} *M* (Fig. 4).

In solutions containing sodium hydroxide concentrations less than 0.1 *M*, the position of the potential jump changes with change in the alkali concentration in the case of both compounds investigated. Thus, neither of the compounds can be determined by this method under these conditions.

As shown by the titration curves recorded in the presence of 0.1 *M* and 1 *M* sodium hydroxide, the equivalent weight of both phenylthiourea and N,N-diphenylthiourea is half the molecular weight.

Titrations in acidic media

In Fig. 5, the titration curves of phenylthiourea in the presence of 0.01 and 1 *M* nitric acid are presented, while Fig. 6 shows those of N,N-diphenylthiourea in 0.1 and 1 *M* nitric acid. The reactions of phenylthiourea and N,N-diphenylthiourea with silver nitrate are similar to each other also in nitric acid medium, but quite different from those proceeding in alkaline medium. In nitric acid solution two organic molecules react with only one silver nitrate molecule.

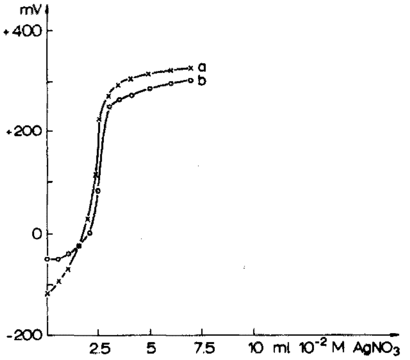


Fig. 5. Potentiometric titration curves of 10^{-2} *M* phenylthiourea in nitric acid. (a) 0.01 *M* HNO_3; (b) 1 *M* HNO_3.

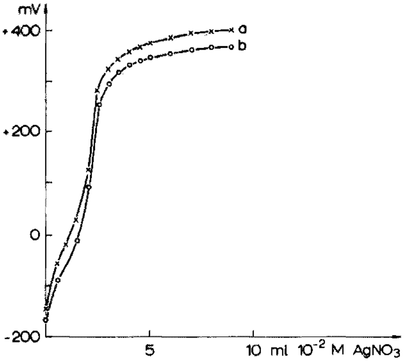


Fig. 6. Potentiometric titration curves of 10^{-2} *M* N,N-diphenylthiourea in nitric acid. (a) 0.1 *M* HNO_3; (b) 1 *M* HNO_3.

IDENTIFICATION OF THE PRODUCTS OF TITRATION

Reactions of phenylthiourea

During the titration of phenylthiourea with standard silver nitrate solution in the presence of 0.1 *M* sodium hydroxide, two potential jumps occur on the potentiometric titration curve. Up to the first potential jump, one molecule of the organic substance reacts with two molecules of silver nitrate to form a black precipitate. The precipitate was filtered, analysed and found to be silver sulphide.

The i.r. spectrum of the solid residue obtained from the filtrate after an extraction with ether and evaporation to dryness was recorded (Fig. 7). The filtrate obtained in a parallel experiment was titrated further. It reacted with one mole of silver nitrate to give a light, greyish precipitate. The precipitate was investigated by i.r. spectroscopy, with the result shown in Fig. 8.

In the i.r. spectrum shown in Fig. 7, two bands appear in the range between 3100 and 3500 cm^{-1}. The broad band at 3155 cm^{-1} is due to the stretching vibration of the N–H group which is hydrogen-bonded with the $-C{\equiv}N$ group. The band at 3435 cm^{-1} can be interpreted as the stretching vibration of the free N–H group. There is a strong band in the spectrum at 2225 cm^{-1}, which is caused by the stretching vibration of the $-C{\equiv}N$ triple bond. It should be noted that this band is highly characteristic, and always appears in a narrow frequency interval, because its position is only slightly influenced by various substituents[3]. This allows the $-C{\equiv}N$ group to be readily identified in organic compounds. The sharp band at 1608 cm^{-1} is due to the stretching vibration of the C=C bond in the aromatic ring, and that at 3020 cm^{-1} to the stretching vibration of the aromatic C–H bond. On this basis, the compound can be identified as phenylcyanamide.

When phenylcyanamide is titrated further with silver nitrate (see Fig. 8), the product obtained shows an i.r. spectrum which does not exhibit the band due to the stretching vibration of the N–H bond which is characteristic of phenylcyanamide (see Figs. 7 and 8). This can be accounted for by the replacement of the hydrogen in the imino group by silver.

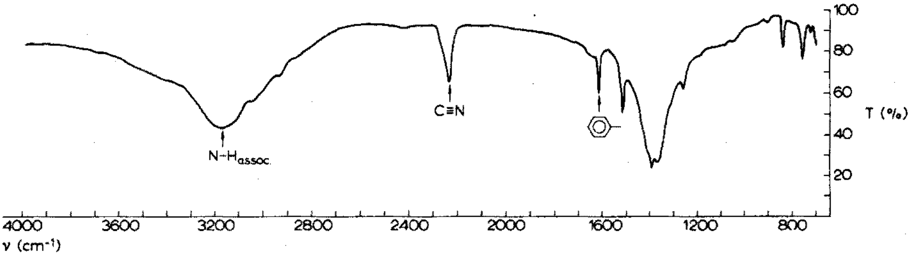


Fig. 7. I.r. spectrum of phenylcyanamide.

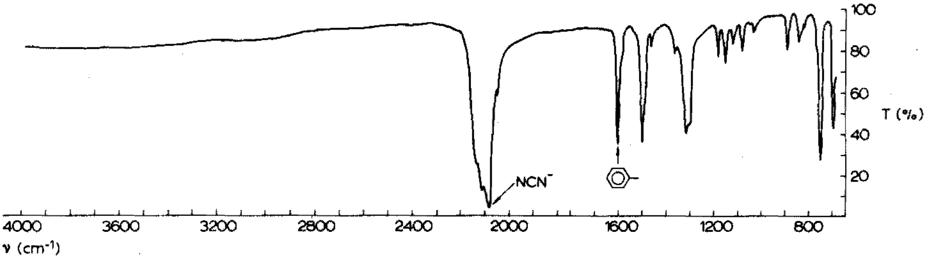
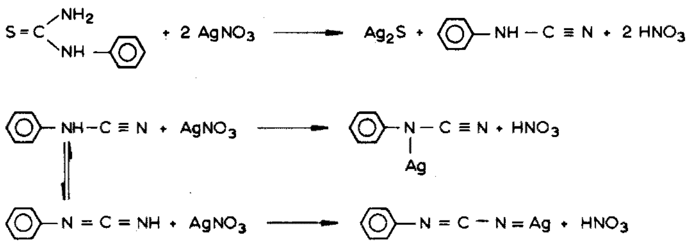


Fig. 8. I.r. spectrum of silver–phenylcyanamide precipitate.

It has already been stated on the basis of spectroscopic studies[1] that cyanamide forms a salt with silver. With phenylcyanamide the tendency to salt formation is much greater, because the phenyl group, being an electron acceptor, enhances the acidic character of the hydrogen in the imino group. In the spectrum of silver–phenylcyanamide, the stretching vibration of the $-C\equiv N$ triple bond appears at 2090 cm^{-1}, the band being shifted to wave numbers smaller by 135 cm^{-1}, compared with the band of free phenylcyanamide, and becoming closer to the stretching vibration of carbodiimide, which appears[4] at 2100 cm^{-1}.

It can be considered that phenylcyanamide (eqn. 1b), reacts with silver nitrate in the form of the corresponding carbodiimide (eqn. 1c). The results support the following reaction mechanism between phenylthiourea and silver nitrate in 0.1 *M* sodium hydroxide:

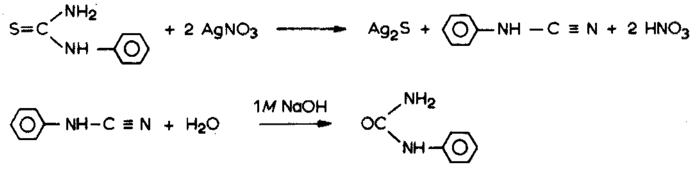


(1a)

(1b)

(1c)

In 1 *M* sodium hydroxide only one potential jump can be observed on the titration curve of phenylthiourea at a position corresponding to the reaction with two equivalents of silver nitrate. The product of reaction is also silver sulphide, but the by-product does not react with silver nitrate. This by-product was extracted from the filtrate with ether, and its i.r. spectrum was taken, (Fig. 9). The band in the spectrum at 1658 cm^{-1} is characteristic of the carbonyl group. This spectrum is almost completely identical with that of phenylurea; hence it can be assumed that the compound obtained was phenylurea. Presumably, phenylcyanamide is also formed here in the first step, in addition to the silver sulphide precipitate, but it undergoes hydrolysis in the strongly alkaline medium and gives phenylurea, according to the following scheme:



(2a)

(2b)

In the presence of nitric acid, two molecules of phenylthiourea react with one molecule of silver nitrate only, to form a white precipitate (Fig. 10) which is slightly soluble in acid, but is readily soluble in ammonia solution. Simultaneously with dissolution, silver sulphide is precipitated. After the precipitate has been removed, the filtrate (Fig. 11) can be titrated further with silver nitrate, and silver sulphide is formed during the titration. Again, the filtrate can be titrated with silver nitrate; after the consumption of one mole of silver nitrate, silver phenylcyanamide (Fig. 8) precipitate is obtained.

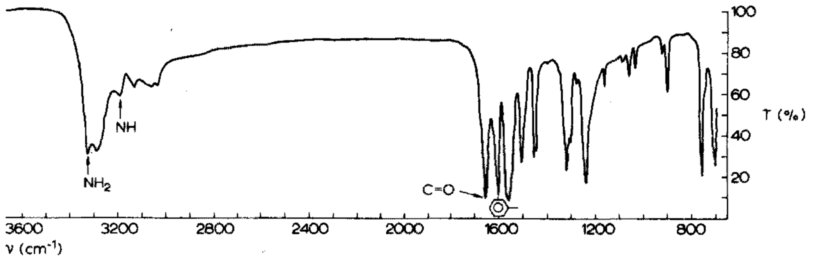


Fig. 9. I.r. spectrum of phenylurea formed during the titration of phenylthiourea in 1 *M* NaOH.

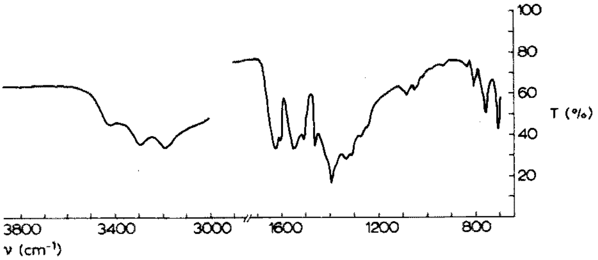


Fig. 10. I.r. spectrum of silver–1,5-diphenyl-2,4-dithiobiuret.

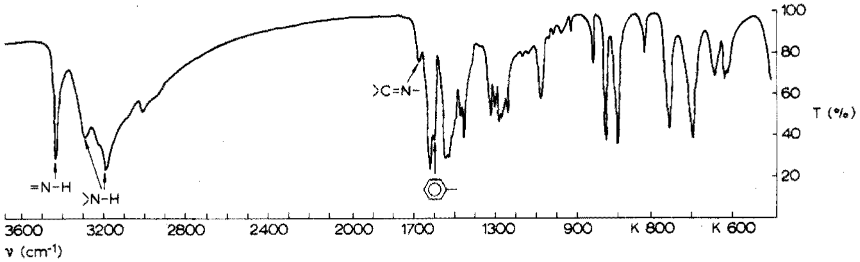
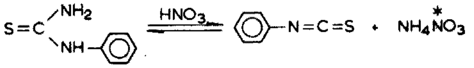
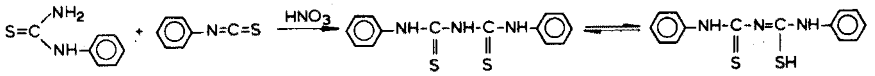


Fig. 11. I.r. spectrum of 1,5-diphenyl-2-guanidino-4-thiobiuret.

The reactions can be described by the following equations:



(3a



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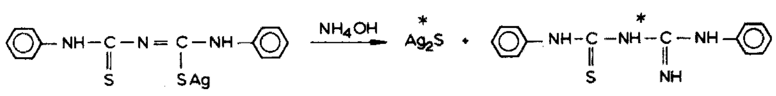
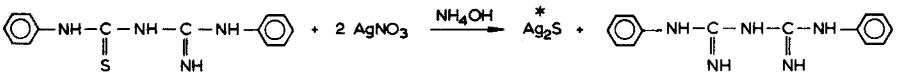
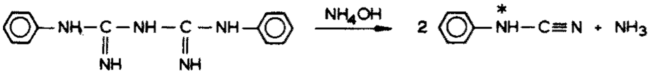


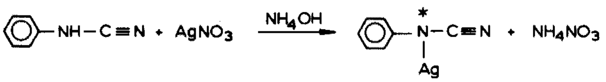
(4)



(5)



(6a)



(6b)

The products marked by an asterisk in the above reaction equations were identified by elemental analysis and u.v. or i.r. spectroscopy.

The i.r. spectrum of the precipitate formed in reaction (3c) markedly differs from that of the starting material, phenylthiourea (Fig. 10). This suggests that phenylthiourea has undergone a greater change than expected in the case of the formation of the usual phenylthiourea–silver nitrate complex. The u.v. spectra provided evidence for the presence of ammonium nitrate in the nitric acid solution of phenylthiourea (Fig. 12). This can be accounted for by the above reaction (3a). The isothiocyanate formed in this reaction shows a characteristic infrared absorption at 2100 cm^{-1}. Isothiocyanate reacts with phenylthiourea to form 1,5-diphenyl-2,4-dithiobiuret (reaction 3b). Dithiobiuret and its derivatives are known to be weak acids[5, 6] which may form 1:1 complexes with silver ions[7]. According to the present studies, the 1,5-diphenyl-2,4-dithiobiuret formed a 1:1 complex with silver nitrate (reaction 3c).

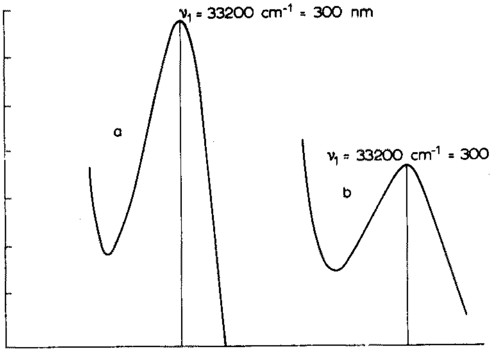


Fig. 12. U.v. spectrum of (a) pure NH_4NO_3; (b) NH_4NO_3 in solution.

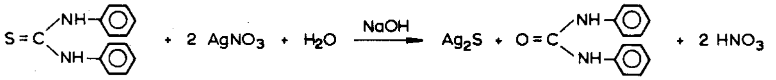
The product formed in the above reaction (4) was studied by i.r. spectroscopy (Fig. 11). The stretching vibration of the C=S group appears[8] in the spectrum at 1065 cm^{-1}. The band at 1670 cm^{-1} can be interpreted as the stretching vibration of the C=N group. It is known from the literature that the C=N stretching vibrations of this group in various guanidines appear in this spectral range[9, 10] The narrow and intense band at 3430 cm^{-1} is due to the stretching vibrations of the =N–H group.

On titration, the guanidino-thiobiuret formed reacts with two moles of silver nitrate, and the sulphur is replaced further by an imino group to give 1,5-diphenylbiguanide (reaction 5). However, this compound could not be separated from the solution; when it was titrated further with silver nitrate, the product obtained showed an i.r. spectrum identical with that of silver phenylcyanamide. This may be explained by assuming that 1,5-diphenylbiguanide decomposes to give phenylcyanamide which reacts with silver nitrate (reactions 6a and 6b).

The process of the titration of 1,5-diphenylguanide also supports this assumption. The time of reaction depends on the degree of decomposition of the compounds. The decomposition of guanides of this type has been described in the literature[11, 12]

Reactions of N,N-diphenylthiourea

With regard to the titration curve of N,N-diphenylthiourea with standard silver nitrate solution, only one potential jump can be observed in distilled water, or in 0.1 *M* and 1 *M* sodium hydroxide. Here, no phenylcyanamide can be formed, because of the presence of the second phenyl group. The reaction is the same in all three cases, involving one molecule of N,N-diphenylthiourea and two molecules of silver nitrate, and giving silver sulphide and diphenylurea:



(7)

The i.r. spectrum of diphenylurea obtained from the filtrate of silver sulphide by extraction is presented in Fig. 13.

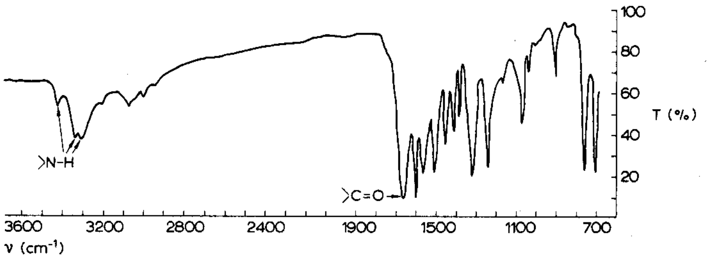


Fig. 13. I.r. spectrum of diphenylurea formed during the titration of N,N-diphenylthiourea in alkaline medium.

In nitric acid medium, the reaction of N,N-diphenylthiourea with silver nitrate proceeds in a similar way to that of phenylthiourea, *i.e.*, two molecules of N,N-diphenylthiourea react with one molecule of silver nitrate to form a white precipitate. The mechanism of this reaction will be dealt with at a later date.

SUMMARY

Phenylthiourea and N,N-diphenylthiourea can be determined in the concentration range 10^{-1}–10^{-3} *M* by potentiometric titration with silver nitrate solution with a sulphide ion-selective membrane electrode. The influence of the alkali and acid concentration on the course of the reactions with the silver nitrate titrant was studied. When phenylthiourea is titrated in the presence of 0.1 *M* sodium hydroxide, silver sulphide is precipitated, and the phenylcyanamide formed simultaneously reacts further with silver nitrate to form a silver phenylcyanamide precipitate. When N,N-diphenylthiourea is titrated under similar conditions, silver sulphide is again formed, but no cyanamide-type compound can be formed owing to the presence of the second phenyl group. In 1 *M* sodium hydroxide both compounds studied react with two equivalents of silver to give silver sulphide and phenylurea or diphenylurea, respectively. In the presence of nitric acid both compounds react similarly with silver nitrate. Two molecules of the compounds react with one molecule of silver nitrate to give a white precipitate.

RÉSUMÉ

La phénylthiourée et la N,N-diphénylthiourée peuvent être dosées, en concentration de l'ordre de 10^{-1} à 10^{-3} *M*, par titrage potentiométrique au moyen de nitrate d'argent; on utilise une électrode à membrane sélective à ion sulfure. On examine l'influence de la concentration en base et en acide sur le cours des réactions avec le nitrate d'argent. Il peut se former du sulfure d'argent, de l'argent–phénylcyanamide, de la phénylurée et de la diphénylurée suivant les concentrations en hydroxyde de sodium. En présence d'acide nitrique, les deux composés ont un comportement identique avec le nitrate d'argent. Deux molécules de ces composés réagissent avec une molécule de nitrate d'argent pour donner un précipité blanc.

ZUSAMMENFASSUNG

Phenylthioharnstoff und N,N-Diphenylthioharnstoff können im Konzentrationsbereich 10^{-1}–10^{-3} *M* durch potentiometrische Titration mit Silbernitratlösung unter Verwendung einer sulfidionen-selektiven Membranelektrode bestimmt werden. Der Einfluss der Alkali- und Säurekonzentration auf den Ablauf der Reaktionen mit der Silbernitrat-Masslösung wurde untersucht. Wenn Phenylthioharnstoff in Gegenwart von 0.1 *M* Natriumhydroxid titriert wird, wird Silbersulfid gefällt, und das gleichzeitig entstandene Phenylcyanamid reagiert mit Silbernitrat weiter unter Bildung eines Silber–Phenylcyanamid-Niederschlages. Bei der Titration von N,N-Diphenylthioharnstoff unter ähnlichen Bedingungen wird wiederum Silbersulfid gebildet, jedoch kann wegen der Gegenwart der zweiten Phenylgruppe keine Verbindung vom Cyanamid-Typ entstehen. In 1 *M* Natriumhydroxid reagieren beide untersuchten Verbindungen mit zwei Äquivalenten Silber unter Bildung von Silbersulfid und Phenylharnstoff bzw. Diphenylharnstoff. In Gegenwart von Salpetersäure reagieren beide Verbindungen mit Silbernitrat ähnlich. Zwei Moleküle der Verbindungen reagieren mit einem Molekül Silbernitrat unter Bildung eines weissen Niederschlages.

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AMPEROMETRIC ENZYME ELECTRODES

PART II. AMINO ACID OXIDASE

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Enzyme electrodes based on the continuous amperometric detection of hydrogen peroxide are not limited to the glucose oxidase system described earlier[1,2]. Other oxygen oxidoreductases have been isolated which catalyze the oxidation of a specific substrate, utilize oxygen, and form hydrogen peroxide. L-Amino acid oxidase (E.C. 1.4.3.2) is another such enzyme.

Several methods have been developed for the measurement of amino acids[3,4], but most either lack good sensitivity or require long analysis times. Amino acids have been measured directly by their absorbance or fluorescence in the ultraviolet[5–7] and by direct reaction with colorimetric reagents such as ninhydrin[8] and p-dimethylaminobenzaldehyde[9]. Several enzymatic methods use the enzymes L-amino acid oxidase and peroxidase. o-Dianisidine[10], scopoletin[11] and homovanillic acid[12] have been used in such systems. Guilbault and Hrabankova[13] have described an enzyme electrode based on an ammonium ion-selective electrode which responds to L-amino acids, but there were serious interferences from monovalent cations, especially Na^+, K^+, and H^+, which give a response at the cation electrode.

L-Amino acid oxidase catalyzes the oxidative deamination of a number of L-amino acids according to the reaction

$$RCHNH_2COOH + O_2 + H_2O \rightarrow RCOCOOH + NH_3 + H_2O_2 \qquad (1)$$

It is thus possible to develop enzyme electrodes for the measurement of certain L-amino acids by utilizing L-amino acid oxidase and measuring the hydrogen peroxide produced amperometrically.

This paper describes an enzyme electrode for the measurement of several L-amino acids which consists of covalently insolubilized L-amino acid oxidase held over a platinum sensor by means of cellophane and an "O" ring.

EXPERIMENTAL

Preparation of covalently bound L-amino acid oxidase

Enzacryl AA (Aldrich Chemical Co.) (200 mg) was placed into a 50-ml plastic centrifuge tube and stirred magnetically overnight with hydrochloric acid (2 *M*, 20 ml). It was then cooled to 0° in an ice bath and an ice-cold sodium nitrite solution (4%, 8 ml) was added. The mixture was stirred for 15 min, then washed four times with phosphate buffer (0.1 *M*, pH 7.8) at 0° by centrifugation and decantation. To the diazonium salt formed was added a phosphate buffer solution (0.1 *M*, pH 6.8, 2 ml) containing 80 mg of L-amino acid oxidase (Sigma Chemical Co.; E.C. 1.4.3.2; Type I, from *Crotalus adamenteus* venom). The mixture was stirred at 0° for up to 48 h. The bound enzyme was then washed several times with phosphate buffer (0.1 *M*, pH 6.8) and stored under refrigeration.

Preparation of enzyme electrode

A thin layer of insolubilized enzyme was trapped between a Beckman 39273 platinum inlay electrode and a layer of cellophane (Will Scientific, Inc.) secured with a rubber "O" ring. The gel layer was made uniformly thin by pressing the layer lightly on a glass plate.

Apparatus

A Heath polarograph module (Model EUA-19-2) combined with a Heath operational amplifier system (Model EUW-19A) was used as a two-electrode polarograph at a constant potential of 0.35 V (*vs.* S.C.E.). A side-arm saturated calomel electrode was placed in contact with the enzyme electrode just below the "O" ring to allow application of a potential when the electrodes were out of solution for the purpose of pretreatment.

Reagents

All buffers were made at an ionic strength of 0.1 with reagent-grade chemicals. L-Amino acid solutions were prepared daily with reagent-grade chemicals (Sigma Chemical Co.). All water was doubly distilled in glass.

Procedures

Pretreatment of electrodes. Enzyme electrodes were pretreated before use by applying a potential of 0.35 V (*vs.* S.C.E.) until the anodic current decayed to a low value. After each run the electrodes were washed in a stirred phosphate buffer solution (pH 7.8) until the current decayed to a low value, which took 30–60 s, and which indicated the removal of unreacted substrate and the reaction products.

Effect of L-amino acid concentrations. The electrode, poised at 0.35 V (*vs.* S.C.E.), was placed in stirred 3-ml solutions of phosphate buffer (pH 7.8) containing various concentrations of L-amino acids. The acids used were L-cysteine, L-leucine, L-tryptophan, L-phenylalanine, L-tyrosine, L-methionine, L-alanine, and glycine. The initial reaction rate and steady-state current at 1 min were measured.

Effect of pH. Solutions (10 ml) containing 10 mg % of L-phenylalanine were used in the following buffers: acetate (pH 5.4 and 5.8), phosphate (pH 6.0, 6.6, 7.0, 7.4), and tris(hydroxymethyl)aminomethane-HCl (pH 8.0, 8.4, and 8.8). The solutions were stirred and the electrode potential was set at 0.6 V (*vs.* S.C.E.).

RESULTS AND DISCUSSION

Electrode response

The initial reaction rate within 12 s and the steady-state current at 1 min were used as measures of the initial amino acid concentration. Typical calibration curves for L-amino acid electrodes are shown in Figs. 1 and 2 for the reaction

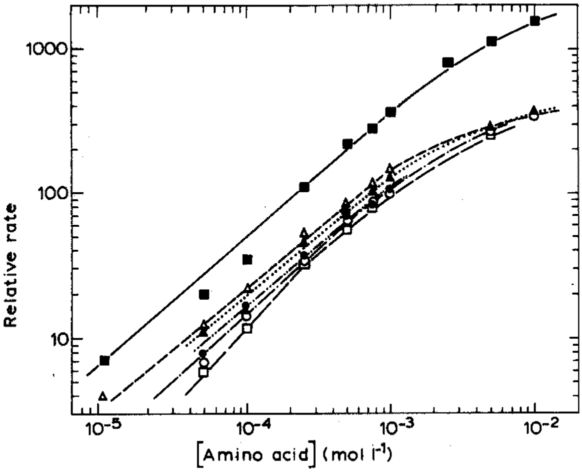


Fig. 1. Calibration curves for the L-amino acid oxidase electrode by the reaction rate method. (—— ■) Cysteine; (...... ▲) leucine; (· — · — · — ●) tyrosine; (· — · — ○) phenylalanine; (—— □) tryptophan; (------ △) methionine.

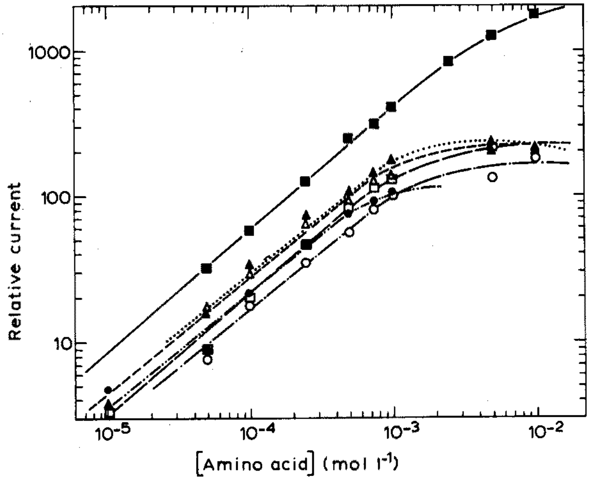


Fig. 2. Calibration curves for the L-amino acid oxidase electrode by the steady-state current method. (—— ■) Cysteine; (······ ▲) leucine; (—· · —· · —· · ●) tyrosine; (—·— · ○) phenylalanine; (—— □) tryptophan; (······ △) methionine.

rate and steady-state current methods, respectively. Because the Michaelis constant for L-amino acid oxidase is very low, *ca.* $1 \cdot 10^{-3}$ *M* in solubilized systems[14], linearity is observed only at concentrations below $1 \cdot 10^{-4}$–$5 \cdot 10^{-4}$ *M*, depending on the substrate. The response to L-cysteine is greater than that to other amino acids because L-cysteine is oxidized at platinum electrodes at potentials as low as 0.35 V (*vs.* S.C.E.). The observed signal is due to a combination of the responses to cysteine and to hydrogen peroxide oxidation. The responses to L-alanine and glycine were negligible.

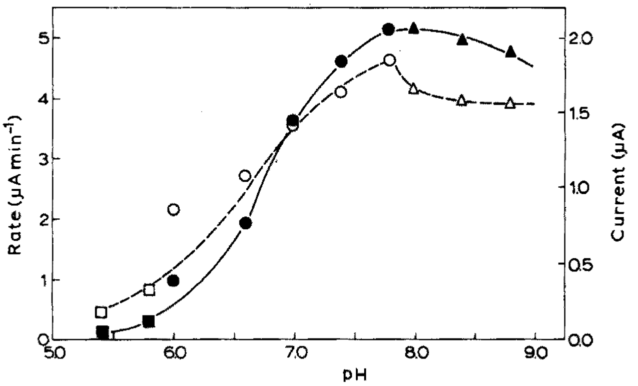


Fig. 3. Effect of pH on L-amino acid oxidase electrode response. (■ □) Acetate buffer; (● ○) phosphate buffer; (▲ △) tris–HCl buffer; (——) reaction rate method; (------) steady-state current method.

Effect of pH

The effect of pH on the response of L-amino acid oxidase electrodes to 10 mg% solutions of L-phenylalanine is shown in Fig. 3. The optimal pH was 7.8 for both the reaction rate method and the steady-state current method. Solubilized enzyme from moccasin venom has a broad maximum at pH 7.0–7.5 with a sharp decline on either side[14]. The optimal pH values for the solubilized enzyme from *Vipera aspis*[15] and *Crotalus adamanteus*[16] venoms were *ca.* 7.2 and 7.5, respectively. In those studies L-leucine was used as the substrate. The enzyme from *Crotalus adamanteus* venom covalently coupled to porous glass has a maximal response at a pH of *ca.* 7.8 for the substrates L-leucine, L-isoleucine, and L-phenylalanine[17]. The shift of *ca.* 0.3 pH units to the more basic region is similar to results obtained here.

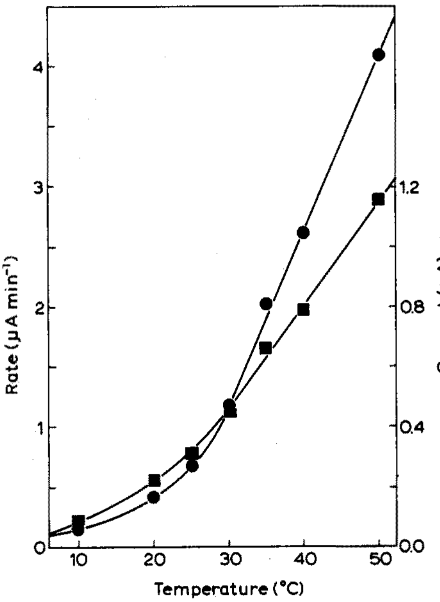
Effect of temperature

The effect of temperature on the response was studied from 10° to 50° for stirred 25-ml aliquots of $1 \cdot 10^{-3}$ *M* L-phenylalanine solution in phosphate buffer pH 7.8. Results are shown in Figs. 4 and 5 for response *vs.* T and for Arrhenius and van't Hoff plots, respectively. The curves in Fig. 5 are not single straight lines as would be expected from the Arrhenius and van't Hoff equations. Instead, they appear to be two straight lines meeting at an angle at a point where $1/T$ corresponds to a temperature of 35°. A number of explanations for such behavior has been given by Dixon and Webb[18]. In this case it is due to two temperature-dependent conformations of the enzyme[19].

Reversible inactivation of L-amino acid oxidase occurs in all species of *Crotalidae* venoms and in many *Viperidae* venoms, but only in some *Elapidae* venoms[20]. The enzyme used in this study was from *Crotalus adamanteus*, which is a member of the *Crotalidae* family. The rates of inactivation and reactivation are strongly temperature-dependent and are associated with conformational changes at the active center of the enzyme and changes in the mode of binding of flavine adenine dinucleotide to the protein[21].

Selectivity

L-Amino acid oxidases are specific for L-amino acids, but not very selective



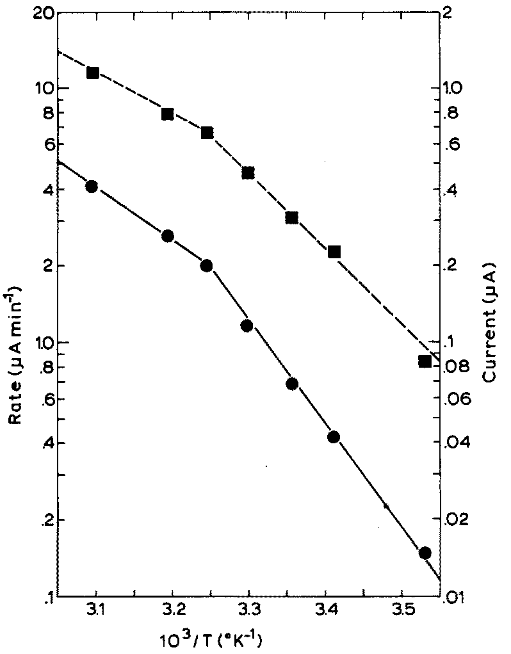


Fig. 4. Effect of temperature on L-amino acid oxidase electrode response. (●) Reaction rate method; (■) steady-state current method.

Fig. 5. Arrhenius and Van't Hoff plots for L-amino acid oxidase electrodes. (●) Reaction rate method; (■) steady-state current method.

for the side chain. As a result, they act on a considerable number of L-amino acids. Zeller *et al.*^{22,23} have shown that the enzymes from different sources, even different species of poisonous snakes, have differences in selectivity. The response to various substrates has been studied with L-amino acid oxidases of eastern Diamond-back Rattlesnake (*Crotalus adamanteus*) venom^{11,12,17}, moccasin (*Agkistrodon piscivorus*) venom^{14}, Asp Viper (*Vipera aspis*) venom^{22}, and rat kidney^{24}

In general, it has been found that D-amino acids are not acted upon by L-amino acid oxidases. Other amino acids with little or no activity are glycine and L-isomers of alanine, serine, threonine, asparagine, valine, glutamine, and lysine. Substrates with good activity are the L-isomers of leucine, phenylalanine, methionine, tryptophan, tyrosine, and cysteine.

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SUMMARY

An amperometric method based on an L-amino acid oxidase electrode has been developed for the determination of several L-amino acids. The time of measurements is less than 12 s if a kinetic method is used, and 1 min if a steady-state method is used. The only reagent required is a phosphate buffer solution.

RÉSUMÉ

Une méthode ampérométrique, basée sur l'utilisation d'une électrode acide aminé L-oxydase est proposée pour le dosage de plusieurs acides aminés L. La durée des mesures est inférieure à 12 s si l'on utilise une méthode cinétique, et autrement d'une minute. Le seul réactif nécessaire est une solution tampon au phosphate.

ZUSAMMENFASSUNG

Für die Bestimmung von verschiedenen L-Aminosäuren wurde eine amperometrische Methode unter Verwendung einer L-Aminosäureoxidase-Elektrode entwickelt. Die Messzeit beträgt weniger als 12 s bei Anwendung einer kinetischen Methode und 1 min bei Anwendung einer stationären Methode. Als einziges Reagenz ist eine Phosphatpuffer-Lösung erforderlich.

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AMPEROMETRIC ENZYME ELECTRODES

PART III. ALCOHOL OXIDASE

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Amperometric enzyme electrodes have been described based on glucose oxidase[1,2] and L-amino acid oxidase[3]. These electrodes are based on the amperometric monitoring of the hydrogen peroxide produced by the enzymatic reactions. A rapid method for the measurement of ethanol has been developed based on this technique.

Alcohols have been determined by gas chromatography[4,5], spectrophotometric analysis of complexation with ammonium nitrate[6] or vanadium oxinate[7], and back-titration of excess of oxidants such as xenon trioxide[8] or cerium(IV) sulfate[9]. The desire for a quick, simple and reliable method for the measurement of ethanol in blood has led to the development of enzymatic methods which utilize alcohol dehydrogenase[10–12] or alcohol oxidase[13].

Alcohol oxidase catalyzes the oxidation of lower primary aliphatic alcohols according to the reaction

$$RCH_2OH + O_2 \rightarrow RCHO + H_2O_2 \qquad (1)$$

This reaction was coupled with amperometric monitoring of the hydrogen peroxide formed. Because the activity of the available enzyme was low, the enzyme insolubilized by methods similar to those used for glucose oxidase[2] had an activity too low to be useful in enzyme electrodes. For this reason, solutions of alcohol oxidase were used in the measurements.

EXPERIMENTAL

Apparatus

A Heath polarograph module (Model EUA-19-2) combined with a Heath operational amplifier system (Model EUW-19A) was used as a two-electrode polarograph at a constant potential of 0.35 V (*vs.* S.C.E.). The working electrode was a platinum inlay electrode (Beckman Model 39273).

Reagents

All buffers were made at an ionic strength of 0.1. All chemicals were reagent grade. The alcohol oxidase was from the mycelium of a Basidiomycete and was a gift of Wyeth Laboratories.

Procedures

Pretreatment of electrode. Pretreatment of the platinum electrode consisted of immersion in a dichromate–sulfuric acid cleaning solution for several seconds each morning it was to be used. It was then rinsed several times with doubly distilled water and immersed in phosphate buffer pH 7.8. A potential of −0.2 V (*vs.* S.C.E.) was applied until the cathodic current decayed close to zero. Then the potential was moved to +0.05 V until the current decayed to zero. The electrode was pretreated before each use by placing in the alcohol solution to be measured and applying a potential of 0.35 V (*vs.* S.C.E.) until the current decayed to a low value.

Measurement of alcohols. The electrodes were placed in a stirred 1-ml solution of the alcohol in phosphate buffer pH 7.8. A potential of 0.35 V (*vs.* S.C.E.) was applied until the current decayed to a low value. Then 0.1 ml of alcohol oxidase (10 mg ml^{-1} of phosphate buffer) was added, and the initial reaction rate was measured.

Effect of pH. Solutions (1 ml) containing 10 mg of ethanol/100 ml were used in the following buffers: acetate (pH 5.4 and 5.8), phosphate (pH 6.0, 6.6, 7.0, 7.4, and 7.8), tris(hydroxymethyl)aminomethane–HCl (pH 8.4), and glycine (pH 8.8, 9.4, and 10.0). The electrode was poised at a potential of 0.6 V (*vs.* S.C.E.). After the current decayed to a low value, 0.1 ml of alcohol oxidase solution (10 mg ml^{-1}) was added and the initial reaction rate was followed.

RESULTS AND DISCUSSION

Measurement of ethanol

A typical calibration curve based on the initial rate of reaction as a measure of ethanol concentration is shown in Fig. 1. In Table I the concentrations found and relative errors calculated by linear least-squares analysis are given for points on the linear portion of the curve. The average relative error was 3.2% in the 0.5–7.5 mg/100 ml range. The Michaelis constant for ethanol as a substrate was determined to be 46 mg/100 ml (10 m*M*) by Janssen and Ruelius[14] and 8.4 mg/100 ml (1.82 m*M*) by Guilbault and Sadar[13]. The linearity at con-

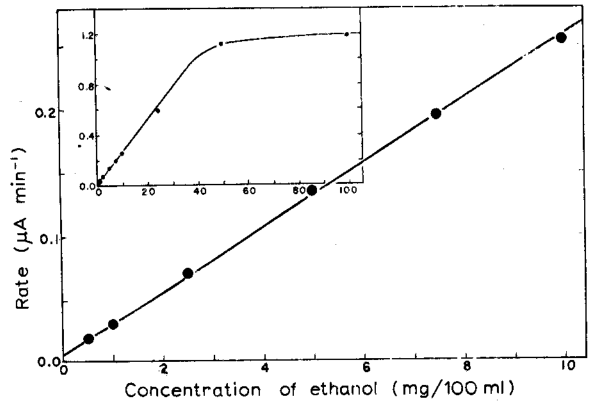

Fig. 1. Calibration curve for ethanol.

TABLE I

DETERMINATION OF ETHANOL WITH ALCOHOL OXIDASE

Ethanol (mg/100 ml)			*Relative error (%)*
Added	*Found*[a]		
0.50	0.48		4.2
1.00	0.94		6.2
2.50	2.56		2.5
5.00	5.09		1.8
7.50	7.43		1.0
		Av. rel. error	3.2

[a] Average of 3 determinations.

centrations as high as 5–10 mg/100 ml in this study agrees with the value given by the former.

Blood from normal individuals who have not ingested ethanol may contain from 10–50 mg/100 ml ethanol. According to the American Medical Association[15], very few persons are drunk at blood ethanol levels of 50 mg/100 ml, more than 50% are grossly intoxicated at levels of 150 mg/100 ml, and practically all persons are drunk at levels above 350 mg/100 ml. Levels as low as 100 mg/100 ml have been recommended as presumptive evidence that a driver is under the influence of alcohol[16]. The method described here has adequate sensitivity for the measurement of blood alcohol. A 25-fold dilution of blood samples with buffer would put probable final sample concentrations within the linear range of measurement. Successful application of glucose enzyme electrodes to blood glucose measurements[1, 2] indicates that no serious problems should be encountered in the application of this method to blood alcohol measurements.

Effect of pH

The effect of pH on the initial reaction rate of 10 mg/100 ml ethanol solutions is shown in Fig. 2. The optimal pH was 7.8. This value allows amperometric hydrogen peroxide monitoring at potentials as low as 0.35 V (*vs.* S.C.E.).

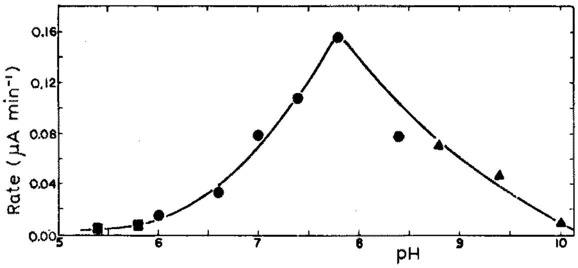

Fig. 2. Effect of pH on the activity of alcohol oxidase. (■) Acetate buffer; (●) phosphate buffer; (⬢) tris–HCl buffer; (▲) glycine buffer.

Selectivity

Calibration curves for the measurement of methanol, ethanol, and allyl alcohol are shown in Fig. 3. The selectivity found in this study is in accordance with that given by others using alcohol oxidase from *Basidiomycete*[13,14,17]. Table II contains the relative reactivities of several substrates with alcohol oxidase from *Basidiomycete* and *Botrytis cinerea.* The enzymes from both sources do not appreciably oxidize secondary and branched-chain primary alcohols. With the enzyme from *Basidiomycete,* reactivity decreases as the chain length of the normal primary aliphatic alcohol increases. The trend is opposite for the enzyme from *Botrytis cinerea.* Unsaturation in the chain enhances the activity of the enzyme from both sources.

The fact that alcohol oxidase from *Basidiomycete* is three times more active for methanol than ethanol presents no problem to blood ethanol measurement because the concentration of methanol will normally be negligible compared to

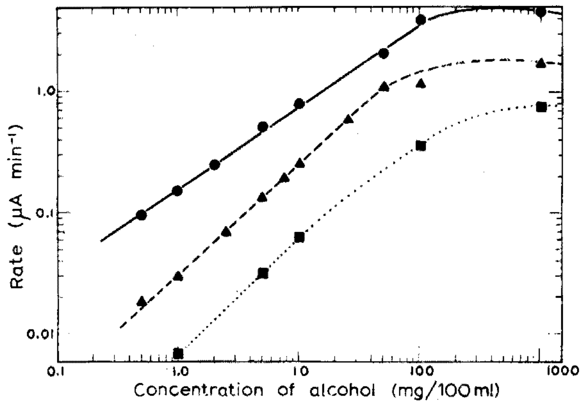

Fig. 3. Calibration curves for alcohol oxidase systems. (●) Methanol; (▲) ethanol; (■) allyl alcohol.

TABLE II

SUBSTRATE SELECTIVITY OF ALCOHOL OXIDASE

Substrate	*Relative reactivity*	
	Basidiomycete[14,17]	*Botrytis*[18] *cinerea*
Methanol	100	0.0
Ethanol	28	1.0
Allyl alcohol	17	122
n-Propanol	5.3	0.25
n-Butanol	2.1	19.0
Isobutanol	0.1	0.0
sec-Butanol	0.01	
n-Pentanol		38.0
n-Hexanol		137
n-Heptanol		126

that of ethanol. In any case, it is potentially useful for the detection and determination of ethanol in the absence of interfering alcohols and is more selective than alcohol dehydrogenase[19].

The authors gratefully acknowledge the financial assistance of the National Science Foundation (Grant No. GP-31518) in carrying out this research.

SUMMARY

A rapid, simple method for the measurement of ethanol or methanol has been developed by coupling amperometric hydrogen peroxide monitoring to the alcohol oxidase enzyme system. Alcohols can be measured within several seconds at concentrations as low as 0.5 mg/100 ml. The only reagent required is alcohol oxidase in phosphate buffer.

RÉSUMÉ

Une méthode ampérométrique, rapide et simple est proposée pour la mesure de l'éthanol ou du méthanol, à l'aide d'une électrode alcool-oxydase. Les alcools peuvent être dosés en quelques secondes, jusqu'à la concentration minimum de 0.5 mg/100 ml. Le seul réactif nécessaire est l'alcool-oxydase en tampon phosphate.

ZUSAMMENFASSUNG

Für die Bestimmung von Äthanol oder Methanol wurde eine schnelle, einfache Methode entwickelt, bei der die amperometrische Messung von Wasserstoffperoxid mit dem Alkoholoxidase-Enzym-System verbunden wird. Alkohole können innerhalb weniger Sekunden bei so niedrigen Konzentrationen wie 0.5 mg/100 ml bestimmt werden. Als einziges Reagenz ist Alkoholoxidase in Phosphatpuffer erforderlich.

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SHORT COMMUNICATION

Fluorescences of *m*- and *p*-hydroxybenzoic acids and their methylated derivatives

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A great deal has been written about the electronic spectra and the acid–base properties of salicylic acid. The anomalous dissociation constants and absorption spectral shifts accompanying dissociation are recognized to result from intramolecular hydrogen-bonding in the ground electronic state[1,2]. The anomalous Stokes shift of the fluorescence spectrum has been attributed to intramolecular proton transfer from the hydroxyl group to the carboxyl group during the lifetime of the lowest excited singlet state[3]. This has been verified for the uncharged salicylic acid and also shown to occur in the singly charged anion, by fluorimetric titrimetry[4].

Although salicylic acid is the best known of the hydroxybenzoic acids, *m*- and *p*-hydroxybenzoic acids are also of analytical interest because of their use as intermediates in the preparation of dyes and fungicides (alkyl esters of *p*-hydroxybenzoic acid are widely employed as stabilizers of commercial, packaged pharmaceutical preparations). From the standpoint of physical chemistry, the comparison of the pH dependence of the electronic spectra, especially the fluorescence spectra, of *m*- and *p*-hydroxybenzoic acid, with those of salicylic acid are of importance in leading to an understanding of proton-transfer phenomena in electronically excited states. In this communication, the acidity dependences of the *m*- and *p*-hydroxybenzoic acids, their esters and methyl ethers, are discussed.

Experimental

Absorption spectra were taken on a Beckman DB-GT spectrophotometer. Fluorescence spectra were recorded on a Perkin-Elmer MPF2A fluorescence spectrophotometer whose monochromators were calibrated against the xenon line emission spectrum and whose output was corrected by means of a rhodamine-B quantum counter. pH measurements were made on an Orion Model 801 pH meter with a Beckman silver–silver chloride–glass combination electrode.

m-Hydroxybenzoic acid, *p*-hydroxybenzoic acid, their methyl esters and methyl ethers (Aldrich Chemical Co., Inc., Milwaukee, Wis.) were recrystallized from chloroform. Reagent-grade sulfuric acid (Mallinckrodt Chemical Works, St. Louis, Mo) diluted with water was used to prepare solutions below pH 3. The corrected Hammett acidity scale[5] was used to calibrate the concentrated sulfuric acid solutions. Phosphate and acetate buffers, and carbonate-free sodium hydroxide solutions were used in pH studies. Distilled deionized water was used throughout.

Results and Discussion

The long-wavelength absorption and fluorescence maxima of *m*-hydroxybenzoic acid, *p*-hydroxybenzoic acid and their methylated derivatives in Hammett acidity and pH regions are presented in Table I.

TABLE I

LONG-WAVELENGTH ABSORPTION ($\bar{\nu}_a$) AND FLUORESCENCE ($\bar{\nu}_f$) MAXIMA OF THE VARIOUS PROTOTROPIC SPECIES DERIVED FROM *m*-HYDROXYBENZOIC ACID, *p*-HYDROXYBENZOIC ACID, THEIR METHYL ESTERS AND THEIR METHYL ETHERS

(Spectral maxima are given in $cm^{-1} \cdot 10^{-4}$)

	m-Hydroxybenzoic acid		*p-Hydroxybenzoic acid*	
	$\bar{\nu}_a$	$\bar{\nu}_f$	$\bar{\nu}_a$	$\bar{\nu}_f$
Cation (H_0–10)	3.22	[a]	3.43	2.81
Neutral Molecule (pH 2.0)	3.36	2.78	3.90	3.11
Singly Charged Anion (pH 7.0)	3.49	2.93	4.10 (3.60)[b]	3.26
Doubly Charged Anion (pH 12.0)	3.20	2.46	3.57	2.97
	m-Anisic acid		*p-Anisic acid*	
	$\bar{\nu}_a$	$\bar{\nu}_f$	$\bar{\nu}_a$	$\bar{\nu}_f$
Cation (H_0–10)	3.17	[a]	3.33	[a]
Neutral Molecule (pH 2.0)	3.36	2.80	3.92	3.16
Anion (pH 7.0)	3.54	3.01	4.01 (3.60)[b]	3.31
	Methyl m-hydroxybenzoate		*Methyl p-hydroxybenzoate*	
	$\bar{\nu}_a$	$\bar{\nu}_f$	$\bar{\nu}_a$	$\bar{\nu}_f$
Cation (H_0–10)	3.26	[a]	3.42	[a]
Neutral Molecule (pH 2.0)	3.36	[a]	3.92	3.10
Anion (pH 11.0)	3.05	[a]	3.39	2.89

[a] No fluorescence was observed from these species.

[b] Long wavelength shoulder (in parentheses), presumably the 0–0 feature of the ^1L_b band.

The dissociation constants of the hydroxybenzoic acids (pK_a) and their methylated derivatives in the ground electronic state were estimated from the inflection points in the absorptiometric pH titrations (Table II). These constants are in good agreement with those previously reported for the same compounds[6–10] except that the pK_a values for the equilibria between the cations and the neutral molecules are more acidic in the present measurements, owing to the employment of the revised acidity scale of Jorgenson and Hartter[5]. Additionally, the value for the pK_a of the equilibrium between the singly and doubly charged anions derived from *m*-hydroxybenzoic acid is about 0.5 units lower than that previously reported[8]; on the basis of Hammett substituent parameters, the present value seems more reasonable.

TABLE II

GROUND-STATE pK_a VALUES (FROM ABSORPTIOMETRY) AND FLUORIMETRIC TITRATION INFLECTION POINTS ($\mathrm{pH}_{\frac{1}{2}}$) FOR THE VARIOUS PROTOTROPIC INTERCONVERSIONS OF *m*- AND *p*-HYDROXYBENZOIC ACID AND THEIR METHYLATED DERIVATIVES

(C = cation, N = neutral molecule, A = singly charged anion, D = doubly charged anion)

		$C \rightleftharpoons N+H^+$	$N \rightleftharpoons A+H^+$	$A \rightleftharpoons D+H^+$
m-Hydroxybenzoic acid,	pK_a	−8.4	4.0	9.8
	$\mathrm{pH}_{\frac{1}{2}}$	−1.0	4.0[a]	9.8 (4.0)[a]
m-Anisic acid,	pK_a	−8.8	3.9	
	$\mathrm{pH}_{\frac{1}{2}}$	1.9	3.9	
Methyl-*m*-hydroxybenzoate,	pK_a	−8.8	9.1	
	$\mathrm{pH}_{\frac{1}{2}}$	—	—	
p-Hydroxybenzoic acid,	pK_a	−7.6	4.5	9.3
	$\mathrm{pH}_{\frac{1}{2}}$	−1.1	4.5	9.3
p-Anisic acid,	pK_a	−7.6	4.3	
	$\mathrm{pH}_{\frac{1}{2}}$	0.2	6.6	
Methyl-*p*-hydroxybenzoate,	pK_a	−8.2	8.2	
	$\mathrm{pH}_{\frac{1}{2}}$	0.6	4.8	

[a] The fluorescence of the dianion of *m*-hydroxybenzoic acid is diminished at pH 9.8 with concurrent appearance of the monoanion fluorescence. However, complete elimination of both monoanion and dianion fluorescences occurs below pH 4.

The pK_a values for the equilibria between the cations and neutral molecules derived from the methyl esters are reported here for the first time. No evidence was found for acid-catalyzed solvolyses of the esters rapid enough to be interferences in determining the latter pK_a values.

The fluorescences of all species studied, except those of the dianions and monoanions derived from *m*- and *p*-hydroxybenzoic acid are extremely weak. Previous investigators had observed fluorescences from only the dianions of the *m*- and *p*-hydroxybenzoic acids[11,12]. The present study suggests that only the fluorescences of the dianions are sufficiently intense for analytical work. Moreover, all species derived from the methyl ester of *m*-hydroxybenzoic acid, were non-fluorescent.

In all cases where fluorescent conjugate pairs were observed (both acid and base fluorescent), the shift of the fluorescence spectrum with successive dissociations paralleled that of the absorption spectrum. This situation is opposite to that observed in salicylic acid[4] and indicates the absence of intermolecular phototautomerism in the neutral molecules and monoanions of *m*- and *p*-hydroxybenzoic acid and their methylated derivatives.

Proton exchanges in the lowest excited singlet state, as indicated by pH-dependent shifting or quenching of the fluorescence spectrum in regions in which the ground-state equilibria do not occur, were evident in several instances (Table II). Except in the case of methyl *m*-hydroxybenzoate, in which all species were non-fluorescent, all cations and their conjugate bases, and the neutral molecules, demonstrated at least partial attainment of equilibrium in the fluorescent state. In contrast, approach to excited-state equilibrium was observed between the neutral molecule and monoanion in only two (*p*-anisic acid and methyl *p*-hydroxybenzoate) of six possible compounds, illustrating the effect of the low concentration of H^+ and OH^- on the rates of proton exchange reactions and thus the low probability of attainment of equilibrium during the lifetime of the excited state, in the mid pH region.

While the dianion and monoanion derived from *p*-hydroxybenzoic acid do not approach equilibrium in the excited state, the dianion and monoanion of *m*-hydroxybenzoic acid are partially equilibrated in the excited state. This is shown by the presence of some dianion fluorescence in the pH region 8 to 4 where the monoanion is essentially the only species excited. The fluorescences of the dianion and monoanion of *m*-hydroxybenzoic acid are quenched by hydrogen ion below pH 4 as the monoanion is converted to the neutral molecule.

TABLE III

FLUORESCENCE MAXIMA ($\bar{\nu}_f$) OF *m*- AND *p*-HYDROXYBENZOIC ACID AND THEIR METHYL ESTERS IN VARIOUS MEDIA

	$\bar{\nu}_f$ *(cm*$^{-1}\cdot$*10*$^{-4}$*)*		
	in $CHCl_3$ + *2% TFA*[a]	*in* $CHCl_3$	*in* $CHCl_3$ + *2% morpholine*
m-Hydroxybenzoic acid	2.62	2.90	2.95
Methyl *m*-hydroxybenzoate	2.86	2.92	2.41
p-Hydroxybenzoic acid	3.20	3.22	—
Methyl *p*-hydroxybenzoate	3.24	3.29	3.00

[a] Trifluoroacetic acid.

That the emissions of most species derived from *m*- and *p*-hydroxybenzoic acid are quenched partly or completely by interaction with the aqueous solvent is illustrated by the fact that in chloroform (Table III) most of these species fluoresce more intensely (albeit still very weakly) than in water. The large red shift of the fluorescence of *m*-hydroxybenzoic acid in chloroform, on the addition of a small amount of trifluoroacetic acid, indicates that the neutral molecule is protonated in the excited state and that the excited cation fluoresces in chloroform even though no fluorescence is detectable from this species in water. The small shifts of the fluorescences in chloroform of methyl *m*-hydroxybenzoate, methyl *p*-hydroxybenzoate and *p*-hydroxybenzoic acid on addition of trifluoroacetic acid suggest that here protonation does not occur in the excited state and that the small changes in the fluorescence maxima are due to hydrogen bonding with trifluoracetic acid. Addition of a small amount of morpholine to the chloroform solutions of the compounds produces the fluorescences of the singly charged anions except in the case of *p*-hydroxybenzoic acid where the singly charged anion is apparently non-fluorescent in chloroform.

The failure of the *m*- and *p*-hydroxybenzoic acids, their singly charged anions and their methyl esters to undergo phototautomerization in aqueous solutions can be explained in terms of the short life-times of their lowest excited singlet states. The quantum yield of fluorescence (ϕ_f) and the lifetime of the lowest excited singlet state (τ_f) are proportional to one another according to:

$\tau_f = \phi_f \tau_N$, where τ_N is the natural lifetime of the excited state. τ_N is essentially the reciprocal of the intrinsic molecular probability of fluorescence and is approximately inversely proportional to the integrated intensity of the absorption band corresponding to the lowest excited singlet state. For benzenoid molecules τ_N ranges from about 10^{-8} to 10^{-7} s. Now the integrated intensity of an absorption band does not generally vary much from solvent to solvent, so that for the same molecular species τ_N is approximately constant. Thus a very small quantum yield of fluorescence translates into a very short lifetime of the lowest excited singlet state (i.e. $\tau_f \ll 10^{-8}$–10^{-7} s). In the compounds studied here, the species which are theoretically capable of phototautomerism have very weak fluorescences and therefore very short-lived excited singlet states. Phototautomerism in these compounds requires successive protonation and deprotonation; reactions each of which typically have first-order or pseudo-first-order rate constants of about 10^8 s^{-1}. Since the ground-state neutral and anionic species exist from pH 4 to pH 10 in these compounds, there does not appear to be sufficient time for bimolecular phototautomerism to occur after excitation. In methyl salicylate where biprotonic phototautomerism has been observed[4] from pH 0 to 9, the fluorescence intensities and therefore the lifetimes of the excited states of the excited neutral molecule and zwitterion are much longer than in the compounds studied here. The reasons for the low quantum yields of fluorescence and short lifetimes of the excited states of the *m*- and *p*-hydroxybenzoic acids and their monoanions and esters relative to those of the corresponding species derived from salicylic acid are not well understood. However, the presence of the internal hydrogen bond in the *o*-isomers is likely of great importance in this regard and possibly acts as a partial deterrent to quenching interactions with the solvent.

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SHORT COMMUNICATION

Determination of cadmium in air by non-flame atomic absorption spectrometry

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(Received 29th May 1973)

It has been stated recently[1] that environmental cadmium presents the most insidious and most widespread health hazard of any of the trace elements. When inhaled, cadmium is completely absorbed from the lungs, and it is known that cadmium induces arterial hypertension and affects the kidneys. Cadmium in air originates from operations such as smelting, pigment manufacture and silver brazing[2, 3].

Most methods currently used for the determination of cadmium in air require sampling of large volumes and involve some sample preparation. Of the recently developed methods[4, 5] the more sensitive employed a heated long-path absorption tube[5]. We have recently reported the determination of lead in air by non-flame atomic absorption spectrometry[6] and the present study uses a similar approach for the measurement of cadmium.

Experimental

Apparatus. The instrumentation, which consisted of a Varian Techtron AA5 spectrophotometer, a Model 63 carbon rod atomizer and the air sampling equipment, has already been described[6].

Reagents. All chemicals used were analytical-reagent grade. A 1000-p.p.m. stock standard solution of cadmium chloride (Hartman-Leddon Co., Philadelphia) was used for the preparation of standards by suitable dilution with twice-distilled water.

Procedure. The GSWP Millipore filter (Millipore Filter Corp., Bedford, Mass.) with a mean pore size of 0.22 μm was treated with (1+2) hydrochloric acid, washed with distilled water and dried before use. This operation reduced the blank cadmium level by about 30%. A disc cut from the filter was inserted into the graphite sampling cup. The particulate matter from samples of up to 400 ml was collected on the filter by drawing the air through the cup with a portable pump. The collected sample was treated directly in the cup with 2 μl of 1000 p.p.m. phosphoric acid. Aqueous cadmium standards with the same addition of phosphoric acid were deposited on the filter disc in the sampling cup. The cups were clamped between the support rods, and the preselected sequence of drying, ashing and atomization was performed; the settings used were established as described below. The peak height of the absorption signal at 228.8 nm recorded during the atomization step was measured from the recorder tracing and corrected for the blank reading.

Results and discussion

A recorder trace for an air sample containing cadmium is shown in Fig. 1. The first two peaks are non-atomic signals caused by decomposition of the filter material. It was confirmed with a hydrogen continuum lamp that no non-atomic absorption occurred during the atomization step.

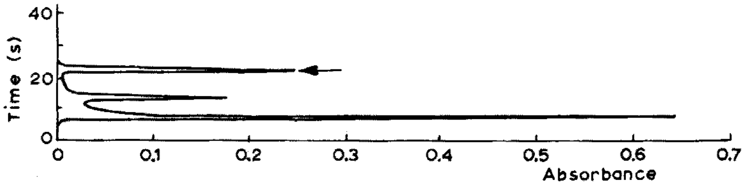

Fig. 1. Recorder trace for a 400-ml air sample containing 0.18 μg Cd m^{-3}, measured at the 228.8-nm cadmium line. →denotes the cadmium atomic absorption peak which includes 0.04 absorbance from the blank.

The optimal heating program was established as follows. It was found that a drying time of 15 s was sufficient; the drying voltage was then adjusted so that the first non-atomic peak appeared just before the completion of the drying stage (indicating decomposition of the filter). An ashing time of 10 s was suitable, provided that the ashing voltage was such that the recorder pen returned to the baseline after the second non-atomic peak. It is essential to ensure that there is no non-atomic contribution to the atomic peak generated during the atomization stage. An atomization time of 3 s was used and the voltage was selected so that no peak resulted when the heating sequence was repeated without further sample addition.

Cadmium (b.p. 767°) is relatively volatile and it is thus not possible to ash the organic filter material without losing some metal during the ashing stage. It was necessary, therefore, to convert the cadmium in the sample to a less volatile form to prevent any loss. Cadmium phosphate, which has a relatively high boiling point, appeared to have the desired property. Consequently an excess of phosphoric acid was added to form the phosphate. A further advantage of adding phosphoric acid to both the aqueous standards and the collected particulates was that the analyte was essentially in the same form in standards and samples. If the analyte in the sample is different in form to that in the standard, the difference in volatility will cause the respective atomic peaks to occur at different times (hence at different temperatures) during atomization. Interferences will then be encountered.

Unlike chemical interferences found in flames, the formation of volatile compounds of the analyte element during atomization can result in loss of the analyte by chemical interferences in the vapour phase. For example, cadmium would be lost as the halide (except for fluoride) in the presence of an excess of these anions. The loss of cadmium can be prevented by forming a less volatile compound such as cadmium phosphate. The great variability in sample matrix composition makes it impossible to match samples and standards, and the nature of the technique precludes the use of the standard addition method. It appears, therefore, that the addition of phosphoric acid is the best available means of preventing chemical interferences.

The calibration curve constructed from the peak readings of the aqueous standards was linear over the concentration range studied (up to 0.6 absorbance). The absolute sensitivity was $1.5 \cdot 10^{-12}$ g which corresponds to 0.008 μg Cd m^{-3} with a 200-ml air sample. The relative standard deviation calculated from the series of measurements of aqueous cadmium standards containing up to $2 \cdot 10^{-10}$ g cadmium was 4%. This is the only estimate of the precision of the procedure, because, unlike the experiment designed to establish the precision of the analysis of airborne lead[6], we were unable to produce an atmosphere containing a constant concentration of cadmium particulates. For the same reason, the accuracy of the method could not be established. However, the precision and accuracy of the analysis for cadmium should be of the same order as that found for lead[6].

Cadmium could not be detected in an open environment in the vicinity of smelting operations despite the extremely good sensitivity attainable. However, cadmium was found in a closed environment where silver brazing operations were being carried out. Up to 0.75 μg Cd m^{-3} was found close to silver brazing under a fume hood, the concentration falling rapidly to 0.05 μg Cd m^{-3} at a distance of 5–10 m. Since it has been reported that cigarettes contain cadmium[2], cigarette smoke was analyzed and found to contain a cadmium concentration of *ca.* 0.1 μg m^{-3}.

The proposed technique combines freedom from contamination (essential in view of the very low level of airborne cadmium) with speed of analysis.

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SHORT COMMUNICATION

Difficulties in the determination of arsenic by atomic absorption spectrometry

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Previous communications from this laboratory have described a system for the continuous determination of lead[1], cadmium[2,3] and mercury[2,4,5] in the atmosphere. The present study was undertaken in an effort to extend this procedure to include arsenic. Although the resonance lines of arsenic lie in the vacuum ultraviolet at 189.0, 193.7 and 197.2 nm, this presented no problem as the system employed permitted the use of resonance lines as low as 184.9 nm[4]. The various techniques used in the calibration of the instrument for different metals have been compared[6].

An initial investigation showed that on occasion ambient air gave an arsenic atomic absorption signal even after correction for the molecular absorption in this spectral region. The results indicated that arsenic in the atmosphere could be directly detected by atomic absorption. It was confirmed that the 189.0-nm line is the most sensitive for arsenic, followed by the 193.7-nm line[7,8].

Equipment

The equipment used has been previously described[4]. The r.f. carbon atomization cell was of the design described for cadmium determinations[5]. A Glomax demountable hollow-cathode lamp (Barnes Engineering Company) operated in a helium atmosphere was used as the radiation source.

Calibration methods

The use of arsine. The initial approach adopted to calibrate the instrument for arsenic was to inject arsine gas diluted with clean air into the system. Arsine is readily decomposed at temperatures above 230° into arsenic and hydrogen. However, it was found that arsine decomposed even at room temperature, so that quantitative data based on the concentration of arsine introduced were unreliable. Moreover, some arsenic metal plated out in cool zones of the equipment between the carbon bed at 1600° and the water-cooled brass adaptor of the inlet head; and arsenic was held up in the carbon bed for varying unpredictable periods of time.

Calibration by direct droplet injection–H_2 absorption. Any organic solvent introduced into the system was decomposed to carbon monoxide and hydrogen. The latter absorbed at wavelengths below 200 nm. In an effort to decrease this molecular background, formic acid was used as a solvent because of its low

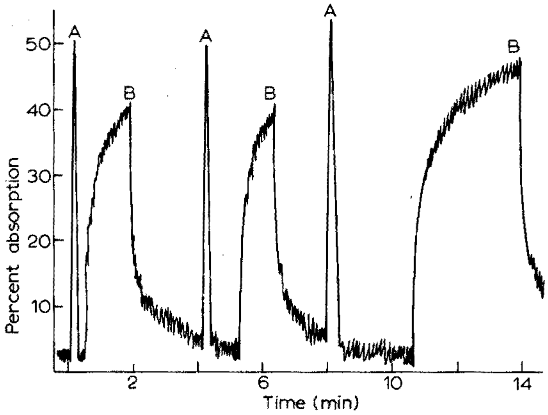

Fig. 1. Absorption trace for arsenic injection in formic acid. A, points of injection; B, delayed arsenic signals.

hydrogen content. Solutions of arsenic in formic acid were prepared by dilution of μl-quantities of an aqueous 1000 μg ml^{-1} arsenic stock solution with formic acid. A diagram of a typical absorption trace is shown in Fig. 1. These delayed absorption peaks were established to be due to arsenic atomic absorption rather than molecular absorption (measured by the deuterium lamp).

To investigate this further, arsenic was introduced into the carbon bed by the direct drop technique, and by injecting into a carbon disc and dropping the disc onto the bed. Results indicated loss of arsenic on the carbon disc and again suggested interaction between arsenic and carbon. In further studies, the carbon bed depth was varied as was the air flow rate. The absorption signal increased with decreased contact with the carbon bed and with a decreased time between atomization and absorption measurement.

Each of these observations indicated a carbon–arsenic interaction, although no C–As compounds have been reported in the literature.

Atomic form of arsenic

Vapor pressure data indicate that vaporized arsenic exists mostly in the form of As_4 molecules up to temperatures of 800°. The following equilibria exist at all temperatures.

$$As_4 \rightleftarrows 2\,As_2 \rightleftarrows 4\,As \tag{1}$$

At a total pressure of 750 mm Hg, the partial pressures of the three species at temperatures of 800, 1000 and 1200° are given in Table I. These data indicate that the formation of As_4 molecules is favored at the temperatures present in the atomizer cell under equilibrium conditions and in the absence of other competing equilibria.

If arsenic compounds are reduced to arsenic atoms in the atomizer, the latter will readily form As_2 and As_4 molecules. The rate of formation depends on the kinetics of equilibrium (1). It would be expected that at low arsenic concentrations, the rate of formation of As_2 and As_4 would be significantly different from the rate for high concentrations of arsenic.

TABLE I

PARTIAL PRESSURES OF As_4, As_2 AND As MOLECULES

Species	*Partial pressure (mm of Hg)*		
	800°	*1000°*	*1200°*
As_4	696.2	529.4	452.6
As_2	47.6	198.4	216.4
As	5.21	22.3	81.0

Molecular absorption by arsenic vapor

Since arsenic vapor is distributed in an equilibria involving arsenic molecules, it was decided to measure the molecular absorption of an arsenic system. A cell was evacuated and doped with·a small quantity of elemental arsenic; the cell was heated to various temperatures and the arsenic allowed to equilibrate. The molecular absorption was measured with a deuterium lamp as source; the spectral slit width was 0.125 nm. The results are shown in Fig. 2. Wide-band molecular absorption was observed for the arsenic vapor in the wavelength region 185–280 nm. The degree of absorption was temperature-dependent, again indicating the importance of equilibria conditions. The results showed that the As_4 species absorbed weakly but that As_2 absorbed more strongly across the spectral range examined. Moreover, there were variable degrees of molecular absorption across the three resonance lines of arsenic .It seemed clear that equilibria conditions would greatly affect the absorption measured for a given concentration of arsenic, which would be particularly important for flame atomizers; any small change in the part of the flame monitored would introduce a large change in the absorption signal.

Many workers have found that the determination of arsenic by atomic absorption has been difficult and unreliable. In flame atomizers atomic arsenic can be quickly changed to molecular arsenic with a severe loss in sensitivity. In non-flame atomizers the problem is further complicated by a possible interaction with carbon. Only under rigidly controlled conditions will reproducible data be obtained. However, with variation in sample composition such control may not always be possible.

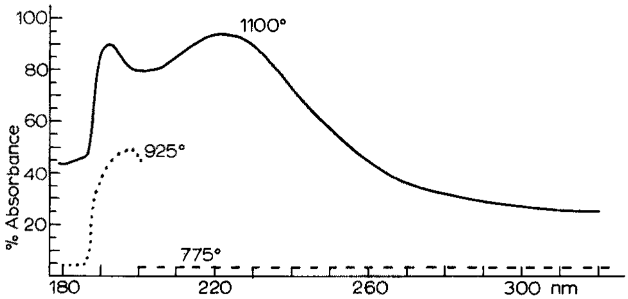

Fig. 2. Molecular absorption spectra for arsenic at 1100° (——), 925° (····), and 775° (– – –).

This investigation was supported by research grant R 800771, Air Pollution Control Office, Environmental Protection Agency.

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SHORT COMMUNICATION

A carbon tube for the analysis of water by flameless atomic absorption spectrometry

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The analysis for trace elements of water samples from environmental surveys has created an urgent need for a technique which is both simple and rapid, to cope with the large number of samples generated and to provide meaningful results at very low levels of concentration. Techniques such as neutron activation analysis and spark-source mass spectrometry are capable of providing the high sensitivity required, but these techniques require expensive equipment and are time-consuming. Flameless atomic-absorption spectrometry provides both high sensitivity and rapid sample throughput for a number of elements and has the great advantage of being adaptable to commercial atomic-absorption spectrometers. This technique was therefore investigated for the analysis of natural water samples derived from environmental surveys.

Although commercial flameless atomizers are available, it was decided to construct a simple apparatus to enable investigation of electrode dimensions and designs with a view to optimizing the various parameters involved. This has resulted in the development of a carbon tube which gives high sensitivity and good reproducibility and is therefore very suitable for water analysis. This note describes the unit and gives details of its performance.

Experimental

A Varian-Techtron AA-3 atomic-absorption spectrometer was used for all measurements. The power supply was a 3-kW transformer with drying, ashing and atomizing cycles controlled by a 3-stage sequential timer. Argon was used as shield gas.

With the flameless atomization device, various types of carbon filaments were examined; most success was achieved with a tube design. Kirkbright[1] described several graphite tubes and tube furnaces in his review, and Amos *et al.*[2] and Norval and Butler[3] also described graphite tubes for flameless atomic absorption. These tubes have been either parallel-sided or reduced in diameter in the middle section. The final design used here (Fig. 1) was chosen because of the high sensitivity, good reproducibility and ease of operation.

Several types of spectrographic graphite were tested; Ringsdorff RWO grade gave the best durability and electrical characteristics, as well as high sensitivity and suitable precision.

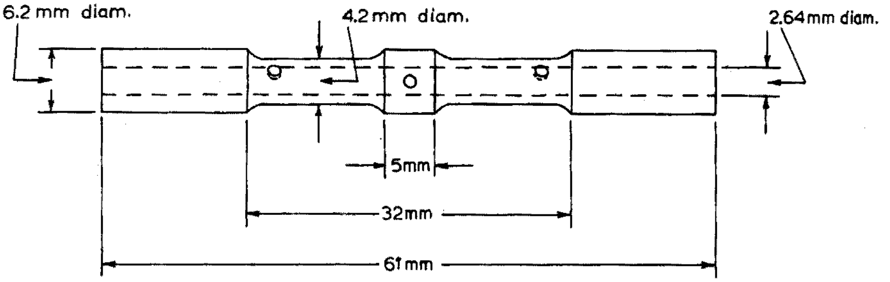

Fig. 1. Carbon tube atomizer.

Commercial electrodes have been manufactured from 5-mm diameter graphite rod and viewed in a transverse direction. The carbon tube described here was machined from 6.2-mm (0.25-in.) diameter graphite rod and viewed longitudinally through the 2.64-mm diameter hole (number 37 drill). With the longitudinal hole positioned on the optical axis, the three small holes (1.32-mm diameter, number 55 drill) were positioned so that the two extreme holes were vertical. The centre hole was located at an angle of *ca.* 45° to the vertical.

With this arrangement, the cold ends of the carbon tube and the electrode holders acted as a shield to prevent much of the light from the glowing carbon entering the slit of the monochromator.

Eppendorf Autopipettes with disposable tips were used for introducing samples of up to 25 μl into the carbon tube through the centre hole. Extra care during the drying cycle must be taken with such large samples.

Calibration and reproducibility. The calibration recordings shown in Fig. 2 are for absorption readings on aqueous solutions of lead in 2% nitric acid made up in demineralized water. Observations were made at 283.3 nm because of the high noise level in the hollow-cathode lamp at the more sensitive 217.0-nm line. The absorbance *versus* concentration plot is curved; this is largely attributable to the response characteristics of the measuring electronics.

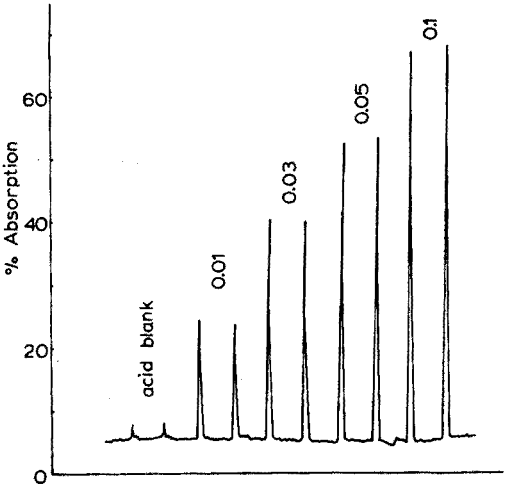

Fig. 2. Calibration for lead (5-μl samples; 283.3 nm). Concentrations in μg Pb ml^{-1}.

The absolute sensitivity calculated from the calibration data is $3 \cdot 10^{-12}$ g for 1% absorption. The high sensitivity may be attributed to increased residence time of the atomic vapours in the light path. At the commencement of the atomization cycle the reduced diameter sections reach a higher temperature than the central compartment containing the solution residue. This prevents diffusion of the vapours towards the cooler ends of the tube. Poorer sensitivity was observed with an electrode which was manufactured without any reduced diameter regions.

The long-term reproducibility of the carbon tube is exceptionally good. Samples of 5 μl of a lead solution containing 0.1 μg ml^{-1} ($5 \cdot 10^{-10}$ g) gave a mean absorbance value of 0.43 with a coefficient of variation of 4% over a period of four weeks, for several different tubes.

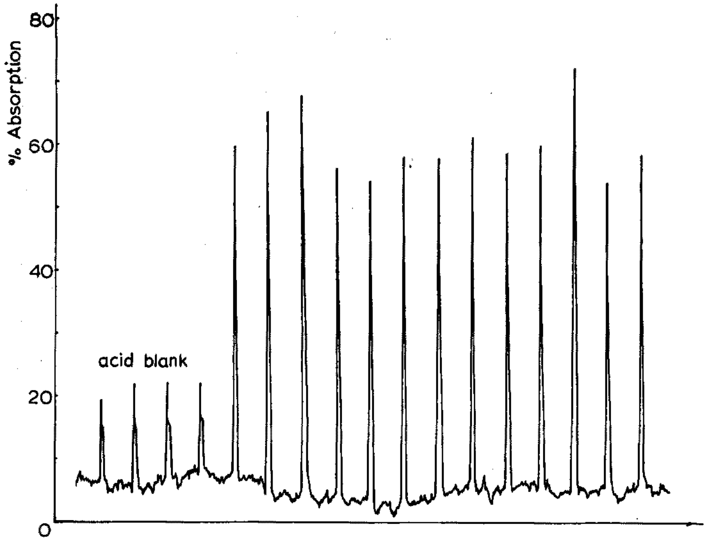

Fig. 3. Reproducibility for $5 \cdot 10^{-11}$ g of lead (283.3 nm; 5 × scale expansion).

Figure 3 demonstrates the reproducibility that can be obtained for 5 μl of a 0.01-μg ml^{-1} lead solution ($5 \cdot 10^{-11}$ g). The coefficient of variation of the peak absorbance is 15%.

In our experience, the reproducibility is most affected by the manner in which the sample is introduced into the electrode. Reproducibility was found to deteriorate gradually as the centre hole becomes enlarged through repeated sample loading. This becomes apparent after about 200 burns, even though very little thermal erosion of the graphite is evident after this time.

Applications

The carbon tube described has been used extensively for the determination of lead in natural waters. Cobalt has been determined in waters, but the sensitivity is not greatly different from that of conventional atomic absorption, hence water concentrates are preferred even though any concentration step greatly increases the chance of contamination.

Beryllium has been determined in liquid effluent in the range 0.01–0.05 μg ml^{-1} with 5-μl samples.

Discussion

Memory effects and sensitivity were found to depend on the position of the two extreme holes. If these are close together then reduced sensitivity is observed; if they are far apart or absent then increased memory effects occur. The magnitude of these effects is small for volatile elements such as lead, but for less volatile elements such as cobalt, sensitivity can vary greatly. It was also found that a hole in the underside of the electrode which allowed the argon gas to purge through the tube, greatly reduced the sensitivity.

Results with the carbon tube described have shown that volatile elements can be determined very simply and with high sensitivity. For less volatile elements, the concentration in solution that may be determined is not greatly different from conventional atomic absorption, and the conditions necessary for atomization give a reduced lifespan to the carbon tubes. In view of the ease of sample introduction and the greater sample volume which can be used, this is a convenient method for determining volatile elements, and has been found very suitable for rapidly processing large batches of samples.

Further investigations on the applications of the device to other elements are presently being undertaken.

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SHORT COMMUNICATION

Spectrophotometric determination of uranium in ores after decomposition in a Teflon pressure bomb

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The usual chemical procedure for determining uranium in an ore is to decompose the −200-mesh sample in a Teflon beaker with a mixture of nitric and hydrofluoric acids. Often a residue remains and the last traces of uranium may have to be extracted by fusion with sodium carbonate[1, 2] or boric oxide[3].

Bernas[4] has reported a method for the rapid decomposition of silicates by hydrofluoric acid at 110° in a sealed Teflon vessel. The additional cost of the Teflon-lined pressure bomb compared to a Teflon beaker, is justified by the avoidance of a subsequent fusion of the residue. Moreover, samples larger than −200 mesh can be decomposed readily in a bomb, which is advantageous when the distribution of uranium between various sized particles of ore is studied in leaching experiments.

This communication describes the spectrophotometric determination of uranium in ores with 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (bromo-PADAP)[5] after the decomposition of ores in a Teflon-lined pressure bomb.

Experimental

Apparatus and reagents. Teflon-lined decomposition vessel from Uni-seal Decomposition Vessels Ltd., Haifa, Israel.

Standard uranium solution, 1 mg ml^{-1}. Dissolve 0.1792 g of pure U_3O_8, ignited at 900°, in 20 ml of 2.5 *M* nitric acid, and dilute to 100 ml with water. Prepare 10 and 20 μg U ml^{-1} standard solutions from this stock solution by dilution.

The complexing solution, dilute complexing solution, buffer solutions of pH 7.85 and 8.35, and TOPO solution were the same as described previously[5].

Silica–fluoroboric acid solution. Dissolve 0.107 g of pure silicon dioxide in 40 ml of water containing 3.6 ml of 40% hydrofluoric acid. Add 1 ml of aqua regia and 1.3 g of boric acid, and warm to dissolve. Cool. Dilute to 100 ml with water.

Sample preparation

Weigh 0.2 g of rock sample (−200 mesh) into the Teflon-lined decomposition vessel. Add 1 ml of aqua regia, making certain that the sample becomes thoroughly wetted; then add 3.6 ml of 40% hydrofluoric acid and close the vessel by hand-tightening the screw cap containing the Teflon sealing disc. Place the vessel in an air oven for 50 min at 150°, and cool to ambient temperature in a stream of compressed air.

Unscrew the lid, insert the spout and transfer the decomposed sample with the aid of a stream of water into a 250-ml polythene beaker containing 1.3 g of boric acid. Dilute to 50 ml and heat on the hot-plate to dissolve the fluoride precipitate. Cool. Transfer into a 100-ml volumetric flask and dilute to volume with water. Store in a polythene bottle.

Recommended procedure

Direct method. Pipette a sample aliquot (not more than 5 ml) containing 0–200 μg of uranium into a 50-ml volumetric flask. Add 4.0 ml of complexing solution and set aside for 15 min. Then proceed as described previously[5] for *Uranium in waters*, doubling the amounts of all the reagent solutions added. (The volume of 0.9 ml of 2.5 *M* sodium hydroxide is required to neutralise a 5-ml sample aliquot.)

Prepare a blank and a standard by pipetting a volume of the silica–fluoroboric acid solution equal to the sample aliquot into two 50-ml volumetric flasks. Add to the standard solution between 20 and 100 μg of uranium and continue as for the sample solutions.

Extraction method. Pipette an aliquot (not more than 50 ml) of the sample solution (1–150 μg of uranium) into a 100-ml separating funnel. Add 7 ml of 15 *M* nitric acid and dilute to *ca.* 60 ml with water. Add 10 ml of 2% sodium fluoride solution and 4 ml of freshly-prepared 5% ascorbic acid solution and mix. Pipette in 5.00 ml of 0.1 *M* TOPO and extract for 1 min. Allow the phases to separate and discard the aqueous phase.

Pipette 2.00 ml of organic phase into a dry 25-ml volumetric flask and continue as described previously[5].

Blanks and standards should be prepared with a volume of silica–fluoroboric acid solution equal to the sample aliquot and carried through the complete extraction procedure.

TABLE I

DISSOLUTION TEMPERATURE AND TIMES FOR TEFLON-LINED PRESSURE VESSEL

Mineral	*Mesh size*	*Temp.* (°)	*Time*[a] (*min*)
Acidic silicate rock	−200	130	65
	−200	150	50
	−72, +200	150	75
	−44, +72	150	100
	−44, +72	170	80
Uranite[b], uranophane and silica	−200	150	100

[a] Minimal time required for complete dissolution.

[b] Uranite in allanite or stillwellite. The leached uranite nucleus is encased in concentric shells of uranophane (Ca–U–Si–H_2O) and silica.

Discussion

Although Bernas[4] stated that quantitative decomposition of silicates is achieved within 30–40 min at temperatures as low as 110°, it was found that longer decomposition times and higher temperatures were required to dissolve completely uranium ore samples. Table I shows that for complete dissolution of a −200-mesh ore sample containing acidic silicates, a minimal time of 50 min at 150° must be used. Samples of larger particle size required longer reaction times and higher temperatures.

Effect of boric acid. Bernas[4] recommended the addition of 2.8 g of boric acid per 100 ml of solution containing 3.6 ml of 40% hydrofluoric acid. In this work the effect of boric acid on the uranium–bromo-PADAP complex was studied. The boric acid added after sample dissolution was varied between 0.9 and 2.8 g. Results for the direct method showed that maximum absorbance was obtained when the amount of boric acid was between 1.1 and 1.3 g (Fig. 1).

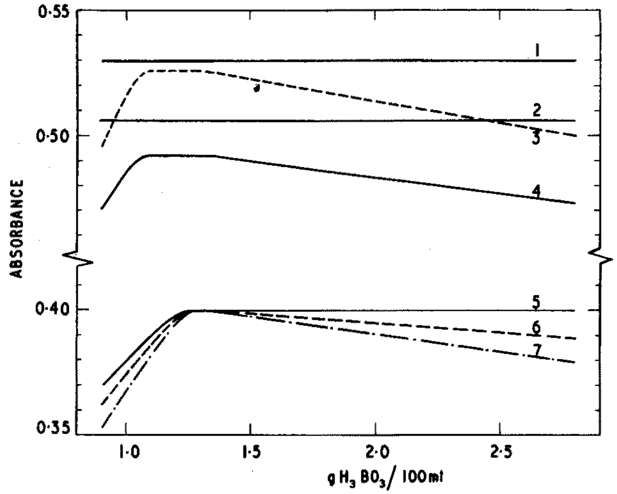

Fig. 1. The effect of boric acid on the absorbance. Direct method (91.4 μg U/50 ml): (1) 5 ml of sample with and without 2.5 mg Si; (2) 5 ml of sample with 5 mg Si; (3) 10 ml of sample without Si; (4) 10 ml of sample with 5 mg Si. Extraction method (36.6 μg U/25 ml): (5) no Si added; (6) 25 mg Si; (7) 50 mg Si.

It was decided to add 1.3 g of boric acid because attack on the glassware decreased with increasing boric acid concentration; this addition converted all the hydrofluoric acid present to fluoroboric acid. The largest sample aliquot allowable was 5 ml, and should contain no more than 2.5 mg of silicon. Larger sample aliquots or more silicon produced lower absorbances (Fig. 1).

For the extraction method, maximal absorbance occurred when 1.25–1.3 g of boric acid was added after sample dissolution. Amounts of silicon up to 50 mg per extraction did not affect the results.

Effect of fluoroboric acid on colour formation. It was found that a standing time of 15 min after the addition of complexing agent was required, otherwise high results were obtained. The reaction time is required for the complexing reagents to form the necessary metal complexes. No standing time was required before the extraction with TOPO solution.

Study of interferences. The effects of interfering elements in the direct method were determined in the presence of 5 ml of silica–fluoroboric acid solution, in solutions containing 0 and 90 μg of uranium. The following elements caused an error equivalent to less than 2 μg of uranium (0.010 absorbance in 1-cm cell): 5 mg of Cd, Co, Fe and Ni; 2.5 mg of Al, Ba, Bi, Cu, Ga, Ge, Hg, Mg, Mn, Mo, Pb, SO_4^{2-}, Th, W and Zn; 1 mg of Ce, Lu, Nb, PO_4^{3-}, Sb, Sn, Ti, Zr; 0.5 mg of Be and La; 0.2 mg of Cr; 0.01 mg of V(V).

In the extraction method the extractions were carried out from 50 ml of silica–fluoroboric acid solution. The interfering ions were added to blanks and to solutions containing 90 μg of uranium. The following elements caused an error equivalent to less than 2 μg of uranium in the original 50 ml: 500 mg of Cl^- and PO_4^{3-}; 250 mg of SO_4^{2-}; 30 mg of Al; 20 mg of As, Ce, Cr, Cu, Fe, Mo and Sn; 10 mg of Nb,Sb,Th (after centrifuging), Ti and V. Zirconium between 5 and 25 mg gave an error of −5% at any uranium concentration.

Applications

The results obtained for a variety of uranium ores are shown in Table II. The results by the direct method are in good agreement with results obtained by extraction. This is because the aliquot size is limited to 5 ml and therefore the error from silicon and the various other interfering ions is minimized.

TABLE II

SPECTROPHOTOMETRIC DETERMINATION OF URANIUM IN ORES (−200 mesh)

Sample	*HF–HNO_3 dissolution*[a] *U (p.p.m.)*	*Teflon pressure bomb*	
		Direct method U (p.p.m.)	*Extraction method U (p.p.m.)*
A21[b]	337	347	331
A24[b]	227	240	217
A26[b]	345	362	334
A30[b]	351	358	357
B21	736	735	725
B24	618	620	600
B26	745	760	751
B30	937	950	939
2-116	503	507	510
G40	531	541	545
G34	1.07%	1.08%	1.08%
Std. No. 3[c]	0.634%	0.633%	0.631%

[a] Ref. 2.

[b] −44, +200 mesh.

[c] AAEC uranium standard ore No. 3, containing 0.748±0.008% U_3O_8 (0.634% U).

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SHORT COMMUNICATION

The automatic spectrophotometric determination of manganese(II) after cation-exchange chromatography

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A simple method for the automatic determination of trace amounts of zinc(II) after cation-exchange chromatography, was recently reported[1]. It is now shown that trace amounts of manganese(II) can be determined similarly after separation from other metal ions. A great deal of information is available on the ion-exchange separation of the transition metals (see, for example, refs. 2 and 3); in the proposed method, a single eluant containing ammonium chloride and thiocyanate is used. Manganese(II) in the range 3.0–15.0 μg can be determined without interference and with a maximum error of 3%. The method can be applied to determinations of manganese in river waters.

Experimental

Apparatus. A Shimadzu MPS-50 L recording spectrophotometer was used for the measurement of absorbances with a 5-mm flow cell.

Resin and reagents. Cation-exchange resin, Amberlite CG-120 (type 3, 400–600 mesh, hydrogen form) was loaded into a jacketed glass column, to give a resin bed 8 × 200 mm. The standard solution of manganese was prepared from manganese sulfate in distilled water containing a little sulfuric acid. Zincon (0.1 mg), 2-carboxy-2′-hydroxy-5′-sulfoformazylbenzene, was dissolved in 20 ml of 1 *M* sodium hydroxide and diluted to 1 l with distilled water; this solution was prepared weekly. A Clark and Lubs buffer solution of pH 9.2 was prepared. All other reagents were of the highest purity available.

Procedure. Figure 1 shows the analysis system. Glacial acetic acid is added to the sample solution to give a 4% acetic acid solution. The ion-exchange column is heated at 82° to obtain sharp elution peaks. Stopcock 2 is closed and stopcock 1 opened, while the sample solution is passed through the column and discarded through cock 1, manganese being adsorbed quantitatively on the resin. The pump speed is increased during this process. Elution with a mixture (1+1) of 1.0 *M* ammonium chloride and 1.0 *M* ammonium thiocyanate is begun and the first 30 ml of eluate are discarded. Stopcock 1 is then closed and stopcock 2 opened. Eluted manganese is recorded at 690 nm as a peak on the recorder and the amount of manganese is calculated from a calibration curve based on peak area measurements.

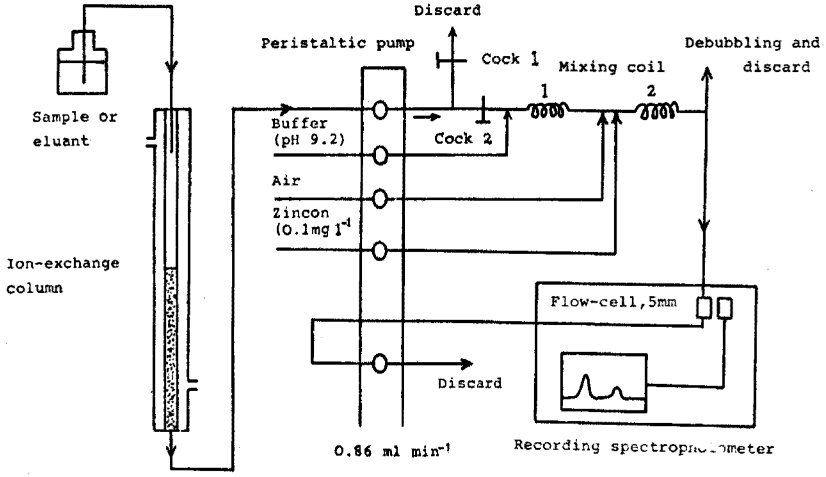


Fig. 1. Automatic analysis for manganese(II) in solutions. All systems are joined with teflon tubing (i.d. 0.8 mm). Mixing coils were made of teflon tube (i.d. 0.8 mm, length 1.5 m).

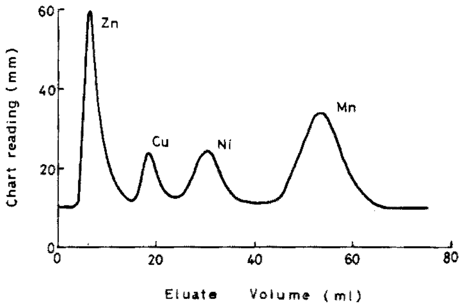
Separation of manganese from other metal ions

Manganese(II) has been separated from Co(II), Cu(II), Fe(III), Ni(II), and Zn(II) from hydrochloric acid solutions by anion-exchange chromatography[2], and from Cd(II), Co(II), Cu(II), Fe(III), Pb(II), and Zn(II) from hydrochloric acid–acetone mixtures by cation-exchange chromatography[3]; however, these stepwise elution procedures require many different eluants. It is convenient in automatic systems to have a single eluant which separates many metal ions. When Mn(II) is adsorbed quantitatively on the cation-exchange resin (hydrogen form) from a solution of 4% acetic acid, Al(III), Be(II), Bi(III), Cd(II), Co(II), Cr(III), Cu(II), Fe(III), Ni(II), Ti(IV), and Zn(II) are also adsorbed. In elution studies with 1.0 *M* ammonium chloride, only Co(II), Cu(II), Mn(II), Ni(II), and Zn(II) were eluted among these adsorbed ions; indeed, a very good separation of Zn(II), Cu(II) and Mn(II) was achieved, elution occurring in the order given. However, Mn(II) was not separated from Co(II) and Ni(II) with this eluant.

Further elution studies showed that a (1+1) mixture of 1.0 *M* ammonium chloride and 1.0 *M* ammonium thiocyanate gave a good separation of Mn(II) from Co(II) and Ni(II); Fig. 2 shows a separation of four metal ions with this eluant. Manganese(II) can be separated from all other metal ions. The results obtained are listed in Table I.

Determination of manganese with zincon

Zincon is generally used as a color reagent for zinc(II) and copper(II). However, Co(II), Ni(II) and Mn(II) can also be determined with zincon if these ions are suitably separated; Co(II) and Ni(II) react with zincon in weakly alkaline solution to yield green complexes, maximum absorbance occurring at 670 nm for the cobalt complex and 680 nm for the nickel complex. The manganese complex with zincon is yellow so that there is no absorbance maximum above 600 nm. However peaks corresponding to manganese could be recorded at such wavelengths owing to the decreased absorbance of the zincon solution when the manganese



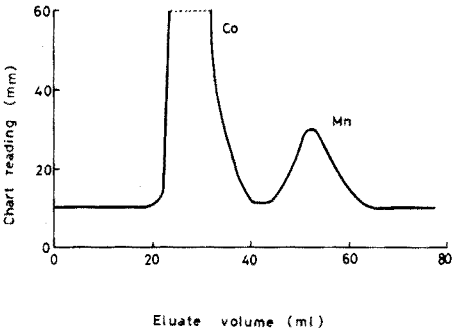


Fig. 2. Separation of Mn(II) from Cu(II), Ni(II) and Zn(II). Mn(II) 12.0 μg, Cu(II) 30.0 μg, Ni(II) 5.0 μg, Zn(II) 25.0 μg. Eluant, (1+1) mixture of 1.0 *M* NH_4Cl + 1.0 *M* NH_4SCN. Measured at 690 nm.

Fig. 3. Separation of 10 μg Mn(II) from 100 μg Co(II). Eluant and wavelength as in Fig. 2.

TABLE I

VOLUME DISTRIBUTION COEFFICIENTS OF METAL IONS AT A COLUMN TEMPERATURE OF 82°

Metal ion	D_v[a] *(1 M NH_4Cl)*	D_v[a] *(1 M NH_4Cl+1 M NH_4CNS)*
Mn(II)	12.5	4.6
Fe(III)	not eluted	2.6
Co(II)	12.0	2.2
Ni(II)	12.6	2.2
Cu(II)	7.8	1.0
Zn(II)	3.0	0.1

[a] $D_v = V/X - \varepsilon$ (*V*, peak elution volume; *X*, bed volume (10.0); ε, relative interstitial volume (0.4)).

complex was formed. A study of the optimal wavelength in the region 600–720 nm with 10.0 μg of manganese showed that maximal peak height was recorded at 670–690 nm. A wavelength of 690 nm was selected, because of the possible interferences by the copper and nickel complexes when a simple thiocyanate eluant was used.

There was a straight-line relationship between the peak areas and manganese concentrations in the range 3.0–15.0 μg; above 15.0 μg, the calibration plot curved towards the peak area axis.

Determination of manganese in the presence of other metal ions

The proposed method was applied to the determination of 10.0 μg of manganese in the presence of 100 μg of Co(II), Fe(III), or Ni(II), and 500 μg of Al(III), Be(II), Bi(III), Cd(II), Cr(III), Cu(II), Ti(IV), or Zn(II). There was no significant interference, the maximal error being 3%. The separation of 10.0 μg of manganese from 100 μg of cobalt is shown in Fig. 3.

Determination of trace amounts of manganese in river water

The method can be applied to the determination of traces of manganese in almost neutral solutions such as natural water. The manganese contents of Kiso-gawa river were determined; Table II shows the results.

TABLE II

DETERMINATION OF MANGANESE(II) IN RIVER WATER

	Sampling at point A[a], June 20th (p.p.b.)	*Sampling at point B[a], June 15th (p.p.b.)*	*Sampling at point C[a], June 17th (p.p.b.)*
Kiso-gawa river	34	12	<3

[a] Points: A, Inuyama district, 60 km upstream; B, Kisogawa-cho district, 32 km upstream; C, Kuwana district, 8 km upstream from bay.

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SHORT COMMUNICATION

The extraction of nickel from various salt solutions with oxine in chloroform

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(Received 4th May 1973)

Nickel has been shown to be extracted with a chloroform solution of oxine from acidic aqueous perchlorate solutions as $Ni_2(Ox)_3(HOx)_3ClO_4$, and from alkaline solutions as $Ni_2(Ox)_4(HOx)_2$, where HOx represents 8-hydroxyquinoline[1]. In the present work the extraction of nickel from aqueous solutions containing various common inorganic alkali metal salts has been studied by means of absorbance measurements of the organic extracts, and the results are compared with extraction from perchlorate solutions.

Experimental

Materials and apparatus. All these were essentially the same as in the previous work[1].

Extraction of nickel. A volume (10 ml) of an oxine solution in chloroform and an equal volume of an aqueous alkali metal salt solution containing nickel ion ($1.00 \cdot 10^{-4}$ *M*) at a constant ionic strength of 0.1 were equilibrated in a glass-stoppered cylindrical tube for 1 h. The pH of the aqueous phase was adjusted by addition of a suitable acid or sodium hydroxide, and the ionic strength was kept constant by addition of sodium sulfate and a suitable salt, while the concentration of the anion in question was maintained at the desired concentration. The mixture was then centrifuged and the absorbance of the organic phase and the pH of the aqueous phase were measured. All the experiments were carried out at 20°.

Results

The absorbance spectra of the nickel–oxine complexes extracted into oxine solutions in chloroform from the various salt solutions were practically the same as those from the perchlorate solutions[1]. Figures 1 and 2 show the absorbance at 400 nm of the extracted nickel–oxine complexes from the various salt solutions as a function of pH, where C and C' are the initial aqueous and organic concentrations of the species shown by the subscripts, respectively. The absorbance by excess of oxine was negligible at 400 nm. The absorbance–pH curves are essentially analogous to those of the perchlorate system[1]; they have a low constant absorbance in the low pH region, but a high constant absorbance in the high pH region where nickel is comletely extracted. The rising parts of the absorbance–pH curves differ from each other, depending on the kind and concentration of the salt used.

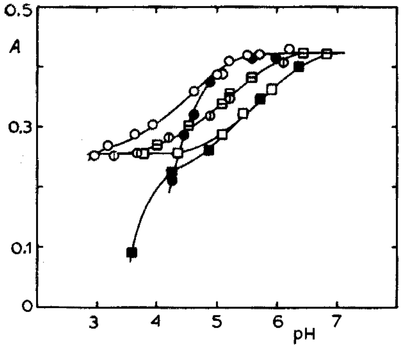

Fig. 1. Absorbance of organic extract as a function of pH (chloride and nitrate systems). C^0_{HOx}, C_{Cl}; (●) 0.01 *M*, 0.1 *M*; (⦶) 0.1 *M*, 1.0 *M*; (○) 0.1 *M*, 0.1 *M*. C^0_{HOx}, C_{NO_3}: (■) 0.01 *M*, 0.1 *M*; (⊟) 0.1 *M*, 0.02 *M*; (□) 0.1 *M*, 0.1 *M*.

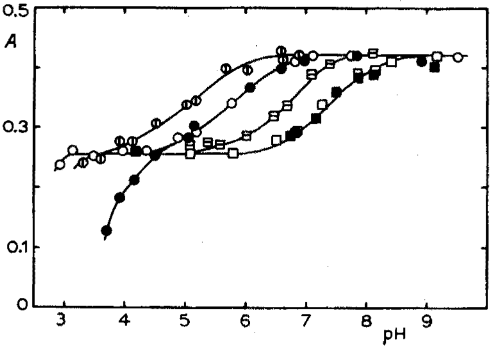

Fig. 2. Absorbance of organic extract as a function of pH (bromide and iodide systems). C^0_{HOx}, C_{Br}: (●) 0.01 *M*, 0.1 *M*; (⦶) 0.1 *M*, 0.02 *M*; (○) 0.1 *M*, 0.1 *M*. C^0_{HOx}, C_I: (■) 0.01 *M*, 0.1 *M*; (⊟) 0.1 *M*, 0.02 *M*; (□) 0.1 *M*, 0.1 *M*.

By analogy with the perchlorate system[1], the change of the absorbance of the organic extract with change of pH can be assumed to correspond to the following reaction.

$$Ni_2(Ox)_3(HOx)_3X_{org} \rightleftharpoons Ni_2(Ox)_4(HOx)_{2org} + H^+_{aq} + X^-_{aq} \tag{1}$$

$$K_{org,x} = \frac{[Ni_2(Ox)_4(HOx)_2]_{org}[H][X]}{[Ni_2(Ox)_3(HOx)_3X]_{org}} \tag{2}$$

where org and aq refer to the organic and aqueous phases, respectively, and X is the inorganic anion in question. In eqn. (1) the dissociation of the binuclear complexes was assumed to be negligible. Equation (2) can be rewritten as previously described[1] as:

$$K_{org,x} = \frac{d - d_{min.}}{d_{max.} - d}[H][X] \tag{3}$$

where $d_{min.}$ and $d_{max.}$ are the minimal and maximal absorbances of the organic extracts, respectively, at the pH of optimal extraction and d is the absorbance at an arbitrary pH. Sulfate ion was regarded as an inert ion in reaction (1).

Plots of log $\{(d - d_{min.})/(d_{max.} - d)\}$ against pH$-$log[X] are shown in Fig. 3, straight lines with unit slopes being obtained as expected. The values of $K_{org,x}$ were determined graphically; the results are summarized in Table I.

The extraction constant of nickel from an aqueous solution containing an anion X^-, K_x, can be calculated from:

$$K_x = \frac{[Ni_2(Ox)_3(HOx)_3X]_{org}}{[Ni]^2[HOx]^6_{org}[X]} \tag{4}$$

$$K_x = \frac{K_s}{K_{org,x}} \tag{5}$$

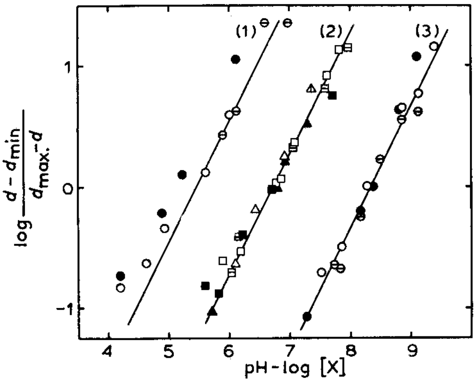

Fig. 3. Determination of $K_{\mathrm{org,x}}$ values. Chloride systems (1) C^0_{HOx}, C_{Cl}: (○) 0.1 *M*, 0.1 *M*; (⊖) 0.01 *M*, 0.1 *M*; (●) 0.1 *M*, 1.0 *M*. Bromide (triangles), nitrate (squares) (2) and iodide (circles) (3) systems C^0_{HOx}, C_{X}: (△ □ ○) 0.1 *M*, 0.1 *M*; (△ ⊟ ⊖) 0.01 *M*, 0.1 *M*; (▲ ■ ●) 0.1 *M*, 0.02 *M*. The chloride system of $C^0_{\mathrm{HOx}} = 0.1$ *M* and $C_{\mathrm{Cl}} = 1.0$ *M* is shown for reference.

TABLE I

EQUILIBRIUM CONSTANTS FOR EXTRACTION OF NICKEL FROM VARIOUS SALT SOLUTIONS

X	*log* $K_{org\,x}$	*log* K_x	r_c^a *(Å)*	r_h^b *(Å)*	$pH_{\frac{1}{2}}$	
					Calcd.	*Found*
Cl^-	−5.42	4.60	1.81	1.81	2.63	2.65
Br^-	−6.72	5.90	1.95	1.92	2.40	
I^-	−8.33	7.51	2.16	2.16	2.16	
NO_3^-	−6.72	5.90		2.03	2.40	2.46
ClO_4^-	−8.75[1]	7.93		2.31	2.10	2.12

[a] Ionic radius[2]. [b] Hydrated radius[3].

where K_s is the extraction constant of nickel from the sulfate solution as $Ni_2(Ox)_4(HOx)_2$; log K_s had already been determined to be −0.82[1]. Thus, K_x was calculated from $K_{\mathrm{org.x}}$ (Table I). The $pH_{\frac{1}{2}}$ value at which nickel is 50% extracted with an equal phase volume of chloroform solution of 0.1 *M* oxine and 0.1 *M* aqueous salt solution was calculated from K_x by means of eqn. (6).

$$K_x = \frac{[H]_{\frac{1}{2}}\left(1+\frac{[H]}{K_1 D_R^0}\right)^6}{C_{Ni}(C^0_{HOx})^6[X]} \tag{6}$$

where K_1 and D_R^0 are the acid dissociation constant of the oxinium ion and the distribution coefficient of oxine, respectively. The values p$K_1 = 5.14$ and log $D_R^0 = 2.64$ were used[1]. The experimental $pH_{\frac{1}{2}}$ values are in good agreement with the calculated ones as in Table I.

A plot of log K_x against the ionic radius[2] or the hydrated ionic radius[3] gave an excellent straight line correlation. The extraction constant is favored by the increasing radius of the anion, which is the general trend observed in the extraction of ion-association complexes[4].

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SHORT COMMUNICATION

Determination of silica in silicates containing phosphorus, titanium and zirconium by a modified procedure

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In a recent paper[1], a specially developed procedure was described for the gravimetric determination of silica in silicates containing phosphorus, titanium and zirconium, by means of oxine-12-molybdosilicate. After fusion of the sample with boron trioxide and lithium carbonate, the cake was dissolved in 0.01 *M* hydrochloric acid containing EDTA, in order to avoid hydrolysis of titanium and zirconium, and in contact with a strongly acidic cation-exchange resin, *e.g.*, Amberlite IR-120 (H^+), in order to remove cations. The resultant solution was then treated with an anion-exchange resin, Amberlite IRA-400 (HCO_3^-), which takes up phosphate and leaves silicic acid in solution, for the final determination. For a 0.2 % relative error, the tolerance limits for phosphorus and titanium were fixed as 2% P_2O_5 and 5% TiO_2, respectively; the tolerance limit fixed for titanium can be taken as approximately valid for titania plus zirconia.

In the present communication, a simplified procedure with somewhat better tolerance limits for phosphorus, titanium and zirconium is described.

Experimental

Reagents. Boron trioxide, sodium hydroxide pellets and solutions of thymol blue, ammonium molybdate and 8-hydroxyquinoline, as well as the saturated wash solution, were prepared as described previously[1]. Osmium tetroxide solution (0.01 *M*) was prepared by dissolving 0.25 g of OsO_4 in 100 ml of 0.05 *M* sulfuric acid.

Ultrasonic generator. Operating frequency, 20 kHz; cavitation bath, 16 cm diameter, 10 cm height; power density in the cavitation bath, 2 W cm^{-2}

Ion-exchange column. The standard type[2] with a height of 370 mm and a diameter of 14 mm was used. The column was prepared with two beds, the upper bed (100 mm) consisting of Amberlite IR-120 (H^+; 30–50 mesh), and the lower bed (250 mm) of Amberlite IR-45 (Cl^-; 30–50 mesh). The resins in the column were kept under 0.04 *M* hydrochloric acid.

Procedure. Fuse 0.1000 g of the finely powdered sample with boron trioxide and lithium carbonate as described previously[1]; after fusion for 30–45 min, remove the burner and allow the crucible to cool to room temperature, so that the fused mass solidifies to a transparent glass.

Prepare 1 l of 0.04 *M* hydrochloric acid containing 2 ml of 30% hydrogen peroxide (perhydrol). Transfer about 800 ml of this solution to a 1000-ml beaker and reserve the rest in a wash bottle for later use.

Introduce the platinum crucible into the solution contained in the beaker. Rinse the cover of the crucible into the beaker with some of the reserved solution. Put the beaker in the cavitation bath of the ultrasonic generator; usually 1–2 min of ultrasonic action suffices to detach completely the cake from the crucible. Then, add 10 g of Amberlite IR-120 (H^+) resin, suspend the crucible in the solution, cover the beaker, and stir magnetically until the cake has completely dissolved, (*ca.* 35–45 min). Remove and rinse the crucible with a stream of the reserved solution. Filter the solution through paper (S & S 589, black ribbon) into a 1-l volumetric flask, wash with the reserved solution, and dilute to the mark.

Pass a 50-ml aliquot through the ion-exchange column at a flow-rate of 2 ml min^{-1}, collecting the effluent in a 150-ml platinum dish (or paraffin-coated beaker) containing 1 g of sodium hydroxide. Stir this solution carefully to dissolve the solid reagent, adding 2 drops of 0.01 *M* osmium tetroxide. Wash the ion-exchange column three times with 10-ml portions, and once with a 50-ml portion, of 0.04 *M* hydrochloric acid.

Add 8 drops of thymol blue solution and then neutralize with (1+1) hydrochloric acid, dropwise, until the color changes from yellow to red. Transfer the solution to a 600-ml beaker and rinse the dish into the beaker with a stream of water. Then add (1+9) hydrochloric acid, acetic acid and molybdate solutions, and complete the determination of silica as described previously.

Results and discussion

The optimal sample size and the methods of attack and dissolution of the cake to ensure the conversion of all silica to the monomeric form have already been discussed[1]. Ultrasonics were used to detach the cake from the crucible, instead of the thermal shock used previously; the glass could be rapidly removed as a whole without damage to the platinum crucible.

The cake was dissolved in 0.04 *M* hydrochloric acid containing hydrogen peroxide, in contact with Amberlite IR-120 (H^+) resin. Titanium(IV) hydrolyzes readily to form hydrous oxides $TiO_2 \cdot x\ H_2O$. In the absence of hydrogen peroxide, titanium(IV) would be transformed to non-sorbable complexes[2]; furthermore, titanium(IV) phosphates may precipitate from aqueous solutions. The addition of hydrogen peroxide converts any titanium in the sample to perhydroxy complexes, thus avoiding both hydrolysis and precipitation of titanium(IV). According to Mori *et al.*[8], below pH 2 the orange coloured species $[Ti(OH)_2(H_2O)(H_2O_2)]^{2+}$ or $(TiO_2 \cdot aq)^{2+}$ is formed. This perhydroxy complex is held strongly by cation-exchange resins.

The aqueous chemistry of zirconium(IV) is very complex. In the older literature, the "zirconyl" ion, ZrO_2^{2+}, was generally considered the prevailing species in aqueous solutions of zirconium(IV), but many studies have now shown that zirconium(IV) hydrolyzes and polymerizes, and also forms complexes readily with many anionic species. These processes depend upon pH and concentration, and may also be time-dependent. Matijevic *et al.*[9] determined the charge of the cationic species in zirconium(IV) nitrate and sulfate solutions by the coagulation method. Below pH 2.5 the prevailing species in dilute solutions of zirconium(IV) nitrate is

Zr^{4+}; in the range 5–5.7, a triply charged hydrolysis product is formed, which is either dimeric $Zr_2(OH)_5^{3+}$, or trimeric $Zr_3(OH)_9^{3+}$. In solutions of zirconium(IV) sulfate, a slow hydrolysis from Zr^{4+} to triply charged species, and eventually precipitation, occurs. Moreover, zirconium(IV) can be precipitated from acidic solution by phosphate ion. The addition of hydrogen peroxide does not prevent this precipitation, but the dissolution of the cake in the presence of the cation-exchange resin ensures the uptake of zirconium(IV) at least up to a certain limit.

The stirring of the solution in contact with Amberlite IR-120 resin favors the dissolution of the cake by the removal of cations from the solution. However, as the solution is 0.04 *M* in hydrochloric acid, the uptake of cations attains only an equilibrium condition. The use of 0.04 *M* hydrochloric acid assures a relatively rapid dissolution of the cake (35–45 min), and prevents any precipitation of cations. It is therefore preferable to 0.01 *M* or 0.025 *M* hydrochloric acid: concentrations above 0.04 *M* are not recommended because the uptake of phosphate in the subsequent column stage would be hindered.

Complete removal of the interfering substances is achieved by passing an aliquot of the solution through a double-bed ion-exchange column. Any residual cations are removed on the upper layer; the resin must be kept in contact with 0.04 *M* hydrochloric acid, to avoid any hydrolysis on initial contact with the sample solution. The lower part of the column comprises a weakly basic chloride anion-exchanger which retains phosphate; the uptake of phosphate becomes more difficult as the hydrochloric acid concentration is increased, but 0.04 *M* hydrochloric acid is the minimum concentration necessary to prevent precipitation of cations. Accordingly, the column dimensions and the flow rate are critical parameters. The dimensions specified are suitable for samples containing up to 5% TiO_2 and 5% P_2O_5, if an aliquot of 50 ml and a flow rate up to 2 ml min^{-1} are used. The content of P_2O_5 in silicate minerals and rocks rarely exceeds 2%; thus the specified column should allow the use of 100-ml aliquots, which would be convenient for samples with relatively low silica content ($<$ 50% SiO_2). If necessary, the second resin bed could be suitably enlarged. The use of this two-bed column has the advantage that both resins can be simultaneously regenerated by passing hydrochloric acid.

The effluent, free from interfering substances, is received in a sodium hydroxide solution. In 0.1 *M* sodium hydroxide solution, silicic acid is completely depolymerized; thus, on suitable acidification, all silicic acid is present in the necessary monomeric form. The addition of osmium tetroxide to the alkaline solution, while the percolation is in progress, ensures the complete decomposition of the hydrogen peroxide before the column stage is concluded; this is necessary to avoid any reducing effects in the subsequent stages of the analysis.

The proposed method was tested by analyzing samples of a soda feldspar, practically free from those substances, to which phosphorus, titanium and zirconium were added in known amounts. The silica content of the sample, by the earlier procedure[4], was shown to be 65.49%. Table I presents the results obtained. High precision and accuracy were achieved when levels of 5% P_2O_5 and 5% TiO_2 were present simultaneously. The modified procedure allows a P_2O_5 content higher than the previous one (5% instead of 2%). Contents higher than 5% P_2O_5 and 5% TiO_2 probably can be tolerated by using proper column dimensions and flow

TABLE I

RESULTS OBTAINED FOR SODA FELDSPAR[a]

Sample	*SiO_2 found (%)*	*Relative error (%)*
Soda feldspar with addition of:		
5% P_2O_5 and 5% TiO_2	65.46	−0.05
	65.50	+0.02
	65.47	−0.03
	65.48	−0.02
	mean = 65.48	
2.5% P_2O_5 + 3% TiO_2 + 1% ZrO_2	65.47	−0.03
2.5% P_2O_5 + 5% TiO_2 + 1% ZrO_2	65.43	−0.09
5% P_2O_5 + 3% TiO_2 + 1% ZrO_2	65.36	−0.20
5% P_2O_5 + 5% TiO_2 + 1% ZrO_2	65.36	−0.20
5% P_2O_5 + 5% TiO_2 + 1.3% ZrO_2	65.28	−0.30
5% P_2O_5 + 5% TiO_2 + 2% ZrO_2	65.01	−0.72

[a] Containing 65.49% SiO_2; this value was used in estimating relative errors.

rate. When phosphorus, titanium and zirconium were present simultaneously at different levels, zirconium was found to have a lowering effect on the results. If a 0.2% relative error is regarded as permissible, the results show that the modified procedure is applicable to silicate samples containing up to 1% ZrO_2 together with 5% P_2O_5 and 5% TiO_2.

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SHORT COMMUNICATION

The decomposition of hydrogen peroxide in alkaline solution

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Hydrogen peroxide is often used as an oxidizing agent in alkaline medium. The excess can be decomposed by boiling the alkaline solution, particularly in the presence of platinized platinum, but prolonged boiling is necessary to destroy the last traces of hydrogen peroxide. In a micromethod for the determination of chromium[1], to overcome this prolonged boiling period, chlorine water was added, followed by potassium cyanide to remove the excess of chlorine. More frequently, the decomposition of hydrogen peroxide is hastened by the addition of appropriate catalysts. Feigl *et al.*[2], for instance, oxidized chromium(III) in alkaline solution with hydrogen peroxide, and removed the excess of peroxide by boiling with some nickel salt (5 ml of 5% nickel nitrate solution). The catalytic action of osmium tetroxide is much better, mere traces being needed to destroy the hydrogen peroxide at room temperature in a few minutes[3].

In the work described here, the decomposition of hydrogen peroxide in alkaline solution was examined in relation to a procedure for the determination of silica in silicates, in which the sample melt from a boron trioxide–lithium carbonate fusion is dissolved in dilute hydrochloric acid containing hydrogen peroxide[4]; the peroxide serves to convert any titanium(IV) to the perhydroxy complex, thus preventing its interference. After the removal of interferences by ion exchange, the excess of hydrogen peroxide must be destroyed before the final stages of the analysis[4]. Some methods of solving this practical problem are compared below. The observations may be useful in other cases.

Experimental

A sample solution was prepared by mixing 1 ml of perhydrol with 1 l of distilled water. A 50 ml aliquot was acidified with 20 ml of 1.5 *M* sulfuric acid and then titrated with *ca.* 0.1 *N* potassium permanganate solution. This titration value was used for comparison to establish the relative amounts of hydrogen peroxide destroyed by the methods studied.

For each test, a 50-ml aliquot of the hydrogen peroxide solution was treated with 1 g of sodium hydroxide, and the particular decomposition method was applied. Then the solution was acidified with 25 ml of 1.5 *M* sulfuric acid and titrated with 0.1 *N* potassium permanganate solution.

The solution of osmium(VIII) employed as catalyst was the usual 0.25% osmium tetroxide in 0.05 *M* sulfuric acid; 2 drops were used for the test.

The ultrasonic generator had the following characteristics: operation frequency, 20 kHz; cavitation bath, 16 cm diameter, 10 cm height; power density in the cavitation bath, 2 W cm^{-2}.

Results and discussion

The results obtained with the different decomposition treatments for hydrogen peroxide are shown in Table I. It can be seen that decomposition of hydrogen peroxide by simple heating on a water bath is slow; a considerable portion remains undecomposed after 1 h. The relative amount destroyed depends on the time of heating, but also varies with the particular glassware used. The effect of ultrasonics at a power density of 2 W cm^{-2} is very small.

TABLE I

DECOMPOSITION OF HYDROGEN PEROXIDE IN ALKALINE SOLUTION

Treatment	*Time of treatment (min)*	*Fraction destroyed (%)*
Heating on a water bath	30	69.0
	60	93.4
	90	86.8
Action of ultrasonics	15	1.8
Leaving in platinum dish at room temperature	15	72.6
Leaving in platinum dish with application of ultrasonics	10	79.2
Heating in platinum dish	10	96.8
on a water bath	15	97.9
Addition of OsO_4 and	2	99.0
waiting at room temperature	5	100
Addition of OsO_4 and	1	99.6
application of ultrasonics	1.5	100
Addition of OsO_4 and heating on water bath	2	100

A large part of the hydrogen peroxide can be decomposed simply by allowing the solution to stand in a platinum dish at room temperature for a reasonable period of time. The decomposition is catalysed by contact with the walls of the platinum dish. The rate of decomposition increases appreciably with simultaneous application of ultrasonics and, more notably, with heating of the solution.

The decomposition of hydrogen peroxide is extraordinarily accelerated by the catalytic effect of osmium tetroxide, only traces of which are needed. At room temperature, 5 min suffices for complete decomposition. Further experiments showed that solutions 5 times richer in hydrogen peroxide are still completely decomposed at room temperature in the course of 5 min. Occasionally, no evolution of gaseous bubbles is observed; since the solution does not consume permanganate, the oxygen presumably forms a supersaturated solution and in fact, the application of ultrasonics to the solution after 5 min causes a prompt effervescence. The rate of decomposition in the presence of osmium tetroxide is significantly increased by the simultaneous application of ultrasonics or heat. The addition of more than 2 drops of osmium tetroxide solution can also be used to accelerate the decomposition.

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Analytica Chimica Acta, 69 (1974) 231–234

SHORT COMMUNICATION

Flotation of traces of tin(IV) ions with iron(III) hydroxide and paraffin

Application to analysis of high-purity zinc metal

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Flotation of various particles, molecules and ions in solution has been studied by many workers[1,2], but the literature which deals with the separation by this technique of trace elements from matrix elements in trace analysis is scanty[3,4]. The present paper describes a new technique for the separation of microgram quantities of tin from large amounts of zinc. Tin(IV) ions are co-precipitated with iron(III) hydroxide, and then floated with the aid of a hot ethanolic paraffin solution and small nitrogen bubbles (0.5–1 mm diam.). For a large volume of solution, flotation is more rapid and convenient than filtration or centrifugation of the precipitates. The separation of tin from paraffin is very easy because the latter is insoluble in hot hydrochloric acid. This separation technique has been successfully applied to the spectrophotometric determination of tin at the p.p.b. level in high-purity zinc metal.

Apparatus

A Fujitsu well-type NaI(Tl) scintillation counter, a Hitachi model 139 spectrophotometer with matched 2-cm cells, and a Hitachi-Horiba model M-5 pH meter were used. The flotation cell consisted of a 40 mm i.d. × 250 mm long glass tube tapered at the lower end with a sintered-glass disk (No. 4, 40 mm diam.) near the lower end.

Reagents

Iron solution (5 mg Fe ml^{-1}). Dissolve ammonium iron(III) sulfate in water, and standardize by the gravimetric oxine method.

Paraffin solution. Dissolve 100 mg of paraffin wax (m.p. 56–58°) in 25 ml of 99.5% ethanol at about 65°. Prepare immediately before use.

Standard tin solution. Dissolve tin metal in 6 *M* hydrochloric acid to prepare a stock solution (100 μg Sn ml^{-1}). Dilute this stock solution to 1 or 2 μg Sn ml^{-1} with water immediately before use.

Tartaric–oxalic acid mixture. Dissolve 20 g of tartaric acid and 0.4 g of oxalic acid dihydrate in 100 ml of water.

Phenylfluorone solution. Dissolve 50 mg of phenylfluorone in 480 ml of 99.5% ethanol and 20 ml of 6 *M* hydrochloric acid.

All reagents used were of reagent grade and employed without further purification. Water was purified by distillation and ion exchange. ^{113}Sn–^{113m}In, ^{64}Cu, ^{65}Zn, and ^{124}Sb were used as tracers.

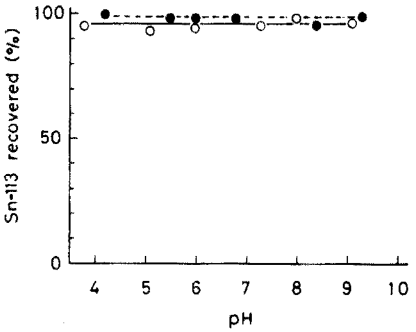
Standard procedure

All experiments were carried out by the following standard procedure unless otherwise stated.

Place about 190 ml of acidic sample solution containing μg quantities of tin(IV) in a 300-ml beaker, and add 6 ml of iron solution. Adjust the pH to 6 with aqueous ammonia (15 *M* and 0.1 *M*) to precipitate iron(III) hydroxide. Add 25 ml of hot (65°) paraffin solution while stirring the sample solution with a glass rod, and immediately transfer the contents of the beaker quantitatively to a flotation cell with continuous stirring. Pass nitrogen from the lower end of the cell for 1–2 s to effect complete agitation followed by flotation of the iron(III) hydroxide. Transfer the scum to an Allihn filtering tube with a sintered-glass disk (No. 4, 20 mm diam.) using a dropping pipet with a 2-mm mouth and a glass spoon. Wash the scum with a few ml of hot 0.1 *M* aqueous ammonia. Add 4 ml of hot 6 *M* hydrochloric acid to dissolve the iron(III) hydroxide, and collect the filtrate in a suitable vessel.

Results and discussion

The optimal pH. Figure 1 shows the percentage of tin(IV) coprecipitated with iron(III) hydroxide as a function of the pH of solution. In this experiment, the iron(III) hydroxide was separated by centrifugation (not by flotation), dissolved in 6 *M* hydrochloric acid, and then used for the γ-activity measurement after 1 day (to attain the transient equilibrium of ^{113}Sn–^{113m}In). In the presence of zinc, ammonium chloride was added to prevent the precipitation of zinc hydroxide, but this was insufficient between pH 7 and 9. The flotation of iron(III) hydroxide with paraffin was easiest in the pH range 6–8. Therefore, the optimal pH for the separation of tin(IV) from zinc was about 6.



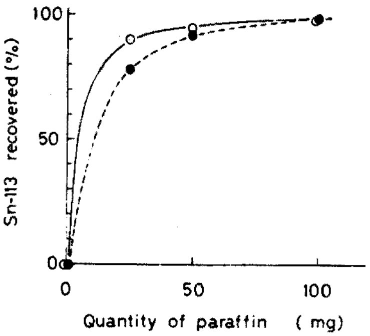


Fig. 1. Coprecipitation of tin(IV) with iron(III) hydroxide. (○) 50 ml of solution containing 1 μg of Sn(IV) and 10 mg of Fe(III). (●) 50 ml of solution containing $ZnCl_2$ (1 g as Zn), 5 g of NH_4Cl, 1 μg of Sn(IV), and 10 mg of Fe(III).

Fig. 2. Effect of quantity of paraffin on tin recovery at pH 6. (○) 200 ml of solution containing 2 μg of Sn(IV) and 30 mg of Fe(III). (●) 200 ml of solution containing $ZnCl_2$ (10 g as Zn), 30 g of NH_4Cl, 2μg of Sn(IV), and 30 mg of Fe(III).

TABLE I

TIN RECOVERY AS A FUNCTION OF QUANTITY OF IRON(III)

(Solution volume 200 ml, pH 6, 2 μg Sn(IV), 100 mg paraffin)

Fe(III) (mg)	10	20	30
^{113}Sn recovered (%)	92	96	98

Required quantities of iron(III) and paraffin. Thirty mg of iron(III) and 100 mg of paraffin were required for satisfactory recovery of tin(IV) from 200 ml of solution, as shown in Table I and Fig. 2.

Concentration factors. The zinc accompanying tin through the standard procedure was determined by EDTA titration after removal of iron by precipitation as hydroxide, in order to obtain the concentration factors of tin with respect to zinc. The concentration factors were 100–150 for 10 g of zinc (in the presence of 30 g of ammonium chloride) and 200–350 for 5 g of zinc (in the presence of 10 g of ammonium chloride).

Applicability to flotation of other elements. The proposed method was also applicable to the flotation of 1–3 μg of antimony(V), copper(II), and zinc in 200 ml of solution, with greater than 95% yields at pH 7.0, 7.6, and 8.0, respectively.

Determination of traces of tin in zinc

Because large amounts of zinc interfere with the photometric determination of traces of tin with phenylfluorone[5], the proposed method was employed as a preconcentration technique for the analysis of high-purity zinc metal.

Recommended procedure. Place a weighed 10- or 20-g sample and 25 or 50 ml of water in a covered 500-ml beaker, and add cautiously 50 or 100 ml of (1+6) mixture of nitric and hydrochloric acids in small portions. After the vigorous reaction has ceased, heat gently to dissolve. Transfer half or quarter of the solution to a 300-ml beaker, and dilute to about 170 ml with water. Add 6 ml of iron solution and 10 g (for 5 g of zinc) or 30 g (for 10 g of zinc) of ammonium chloride, neutralize the solution to pH 6 with aqueous ammonia, and proceed as described under Standard procedure.

Adjust the pH of the filtrate (obtained by treating the scum with 6 *M* hydrochloric acid) to 1.5 with aqueous ammonia (15 *M* and 6 *M*), and transfer the solution to a 10-ml volumetric flask. Add 0.5 ml of tartaric–oxalic acid mixture, 1.0 ml of 0.5% polyvinyl alcohol, enough ascorbic acid to reduce iron(III), and 1.0 ml of phenylfluorone solution, dilute to the mark with water, and shake vigorously for 30 s. After 30 min, measure the absorbance at 510 nm against a reagent blank including all the reagents used for the photometric determination and 30 mg of iron(III).

Construct a calibration curve by taking 0–5 μg of tin(IV) and 30 mg of iron(III) and proceeding as in the last paragraph.

The equation of the calibration curve was (absorbance)$=0.135\cdot(\mu$g Sn), with a maximum deviation of about 0.1 μg of tin. In the photometric determination of 0.5 or 1.0 μg of tin, no interference resulted from the presence of 100 mg of zinc(II), 50 μg of bismuth(III), and 0.5 μg of germanium(IV).

TABLE II

DETERMINATION OF TIN IN ZINC METAL

Sample taken (g)	*Aliquot taken*	*Sn found (μg)*	*Sn in sample (p.p.m.)*
20.0	1/4	0.7	0.14
	1/4[a]	1.9	0.18
	1/2	1.3	0.13
10.0	1/2	0.8	0.16
	1/2	1.0	0.20
			Av. 0.16

[a] 1.0 μg of tin(IV) was added.

Table II shows the analytical results for a sample of commercial high-purity zinc (99.999%) by the recommended procedure. No contamination with tin during the whole procedure was detected. A tracer experiment showed that no loss of tin occurred during the separation step. The time required for a determination was about 2 h.

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Analytica Chimica Acta, 69 (1974) 235–237

SHORT COMMUNICATION

Turbidimetric assay of the acidic polysaccharides in agar

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Quaternary ammonium compounds such as cetylpyridinium chloride, cetyltrimethylammonium bromide, cetyldimethylbenzylammonium chloride, lauryltrimethylammonium chloride, and myristylpyridinium chloride, react with anionic functions of the water-soluble polysaccharides to form insoluble precipitates[1]. This property has been employed for the separation of acidic from neutral polysaccharides. Scott[2] has extended this observation to develop a sensitive method for the determination of sulphated polysaccharides. The method is based on the spectrophotometric determination of cetylpyridinium chloride (CPC) in the supernate at 260 nm after the removal of the acidic polysaccharide–CPC complex. The method permits a rapid determination of monoester sulphate and carboxyl functions in polysaccharides, since only the sulphate group is ionized in dilute mineral acid. Stoloff and Blethen[3] observed that this procedure gave higher values for the sulphate content than those obtained by the barium sulphate precipitation method; they preferred to introduce a non-ionic surfactant with a low absorption at 260 nm, to displace the CPC bound to the colloidal precipitate. Since the acidic polysaccharides are quantitatively precipitated with CPC, it was of interest to know if the suspension of this compound could be assayed turbidimetrically. Optimal conditions for the turbidimetric assay of the acidic polysaccharide in agar are described below.

Experimental

Water distilled in an all-glass unit was used. All reagents were of AnalaR grade.

Urea solution. Urea (BDH) was recrystallized from ethanol (95%). Acidification of urea just before use ensured the removal of cyanate by decomposition. Freshly prepared urea (10 *M*) solution from the recrystallized urea gave a negative test for cyanate. Routinely the urea solutions were acidified to pH 4.2 with citric acid (1 *M*) before use and the desired pH was obtained with sodium hydroxide (1 *M*).

Agar solution. Disperse the agar (Agar-Agar Trading Co., Osaka, Japan) in buffered urea (10 *M*, pH 6.2) and decolorize by treatment with activated charcoal followed by precipitation with ethanol[4]. Dissolve the purified agar (0.5–1 g) in 100

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ml of buffered urea (10 *M*, pH 6.2) by heating on a water bath (98°). Filter through a fritted glass funnel (G3). Use the purified agar as a working standard for the calibration curve for the determination of acidic polysaccharides. Purified agaropectin, if available, would be a suitable choice as standard.

CPC reagent. Dissolve cetylpyridinium chloride (2 g; Merck) in 100 ml of 0.02 *M* citrate buffer (pH 6.2).

Recommended procedure. To 2.5 ml of the solution containing 2–20 mg of agar in buffered urea (10 *M*, pH 6.2), add 2.5 ml of the CPC reagent. Mix well and heat on a boiling water bath (98°) for 20 min with occasional stirring. Cool to room temperature (26–28°), and measure the turbidity with a Spectronic-20 (Bausch and Lomb) at 400 nm in test-tubes (12-mm diameter) against a reagent blank.

The calibration curve obtained under these conditions was linear over the range 0–20 mg of agar.

Results and discussion

According to Araki[5], agar, the gel-forming polysaccharide, consists of two

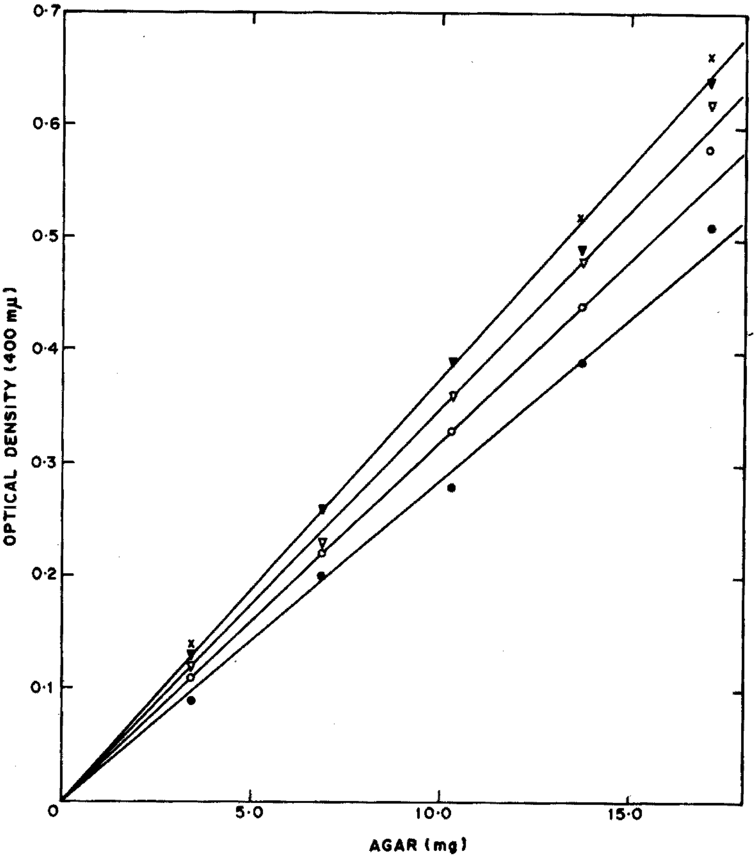

Fig. 1. Influence of heating time on the formation of turbidity of CPC–agaropectin complex at pH 6.2. (●) 0 min; (○) 5 min; (▽) 10 min; (▼) 15 min; (×) 20 min.

main polysaccharides, the neutral polysaccharide agarose and the acidic sulphated polysaccharide agaropectin. The determination of these acidic sulphate and carboxyl groups in agar is difficult, because the agar solution on cooling forms thick gels. The hydrophilic gel matrix of agar is held together by hydrogen bonds; there are no covalent cross-linkages. The gel matrix is stable over a pH range 4.0–9.0 and melts at 40°. However, agar solution (2% w/v) maintains sufficient fluidity at 40° when dispersed in buffered urea solution (10 *M*, pH 6.2) and the turbidity produced can be determined from the absorbance at 400 nm. The method is suitable for judging the absence of agaropectin in the purified agarose preparations.

The optimal period of heating and the pH for the production of reproducible turbidity, were studied for the conditions given in the recommended procedure. The effect of heating time is shown in Fig. 1. The effect of pH was studied over the range 3–8 for a heating time of 20 min; the turbidity increased with pH in the range 3–5.5, but then remained constant up to pH 8. These results indicated that heating for a period of 20 min at 98° with a pH of about 6.0 gave the most reproducible turbidity for the agaropectin in agar.

Purified agarose[4] on treatment with CPC reagent under these conditions gave no absorption at 400 nm, indicating that it was practically free from agaropectin. This was in agreement with other data (sulphur $<0.07\%$).

The method can be extended for the assay of the anionic groups in any water-soluble polymer that will form a precipitate with CPC, if a suitable standard is used.

We wish to thank Prof. H. J. Arnikar for his interest and help.

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SHORT COMMUNICATION

A silicone-rubber surfactant electrode

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Liquid ion-exchange electrodes responsive to ionic surfactants have been developed by Gavach *et al.*[1,2], and by Birch and Clarke[3]. These electrodes give Nernstian response over moderate ranges, and should prove useful for direct measurements of surfactant concentrations for surface chemistry studies and in potentiometric titrations[2]. Cottrell[4] has used a coated wire, polyvinyl chloride electrode for determining very small concentrations of surfactants. The present note describes the development and use of silicone-rubber electrodes responsive to cationic detergents.

Preparation of electrodes

Samples of hexadecyltrimethylammonium bromide and sodium dodecylsulphate were used as obtained (BDH Ltd.).

Hexadecyltrimethylammonium dodecylsulphate, precipitated by mixing solutions of hexadecyltrimethylammonium bromide and sodium dodecylsulphate, was dialysed with a suitable membrane (Visking tubing, Gallenkamp Ltd.) for 48 h until free of sodium bromide. The material was air-dried at 100°, and was then ground to pass a B.S. 120-mesh sieve. Further grinding gave particles of about 20 μm, as indicated by electron microscopy, and this material was used to prepare the membranes.

Silastomer 70 (1 g) and 0.25 g of Catalyst BC (Hopkin and Williams Ltd.) were mixed on a watch glass and a known amount of hexadecyltrimethylammonium dodecylsulphate was stirred in thoroughly. The resultant mixture was then pressed between two flat Perspex plates, separated by means of thin card, 0.035 cm apart; a heavy weight, 4–5 kg, was placed on top of the plates. After the silicone rubber had been allowed to cure for from 12 to 24 h, a circular membrane (diameter 1.3 cm) was cut from the material. This was attached by means of a self-curing silicone sealant (RTV-108 Translucent Adhesive Sealant, General Electric Corp., New York) to the base of a high-resistance lead glass tube, 24 h being allowed for curing. The glass tube was washed carefully before use, dried at 105°, and then treated whilst still hot with a silanizing agent (Repelcote, Hopkin and Williams Ltd.). This treatment improved the stability of the signal obtained from the prepared electrodes.

A silver–silver chloride wire was used as the internal reference electrode. The electrode responses were constant when 10^{-1} M potassium chloride solution was used as the inner reference solution. The addition of hexadecyltrimethylammonium bromide to this solution caused the silver chloride to be stripped by detergent action from the silver wire causing an erratic response. The silver wire was soldered to the inner wire of the coaxial cable connected to the pH meter; it was not found necessary to screen the electrode further. A Suba-seal rubber cap was attached to the top of the glass tube and was used to position the internal reference electrode.

Electrode response

Potentiometric measurements were made against an Orion double-junction reference electrode with a Pye 290 pH meter in a cell having a flow-through water jacket maintained at 25°.

The electrodes were found to respond to cationic detergents but not to anionic detergents; the slight response obtained with sodium dodecylsulphate, and also with sodium tetraphenylboron, ammonium reineckate, potassium hexacyanoferrate(III), and potassium dichromate were of opposite slope to that expected for anionic response and were shown to be due to the cations added.

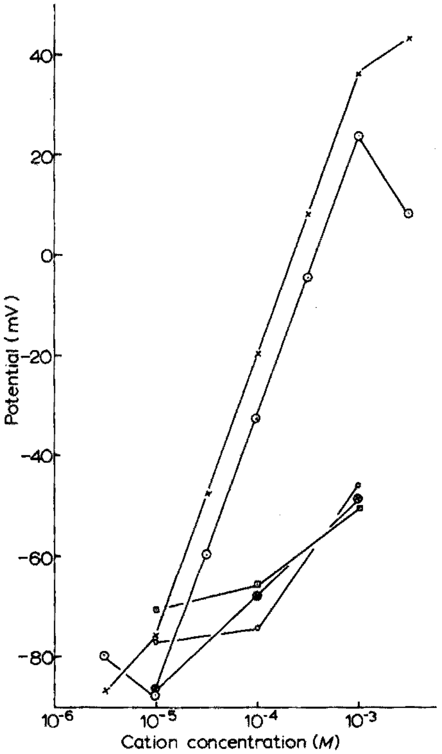
Electrodes containing between 40 and 60% (w/w) of surfactant material were examined. Electrodes containing 40% (w/w) were most suitable, giving a steady potential response within 1 min with a potential–concentration slope of 56 mV per decade change in concentration in the range 10^{-5}–10^{-3} M of hexadecyltrimethylammonium bromide or hexadecylpyridinium bromide (see Fig. 1). Electrodes containing 50% (w/w) of surfactant material gave a response of 57 mV per decade but the time required to obtain a steady potential response was 10–15 min at 10^{-4}–10^{-3} M concentrations and 20 min at 10^{-5} M concentration. Electrodes containing 60% (w/w) surfactant material responded even more slowly. Subsequent work was carried out using electrodes containing 40% of surfactant material. A similar electrode prepared from undialysed material gave a fast response but of only 32 mV per decade change in concentration.

Electrodes showed a strong memory effect on going from high to low concentrations of cationic surfactant. After use the electrode was immersed in sodium dodecylsulphate or in sodium tetraphenylboron solution (10^{-4} M) for 30

TABLE I

STABILITY OF ELECTRODE RESPONSE

Time after preparation and soaking (days)	*Potential in 10^{-4} M hexadecyltrimethylammonium chloride (mV)*	*Potential–concentration slope (mV per decade)*
0	−33	56
21	+ 3	52
54	−16	46
68	−20	44



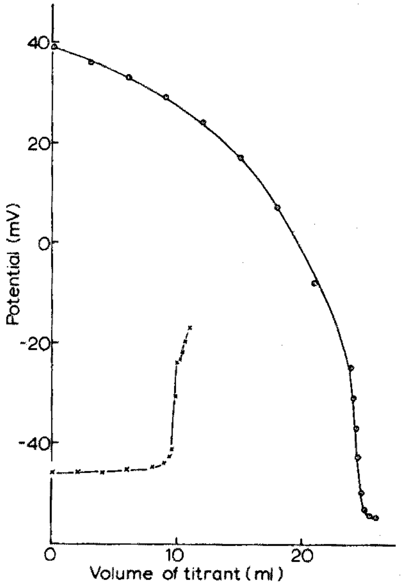


Fig. 1. Response of electrode to surfactant and other ions. (×) Hexadecylpyridinium bromide; (⊙) hexadecyltrimethylammonium bromide; (⊗) sodium chloride; (○) sodium dodecylsulphate; (□) sodium tetraphenylboron.

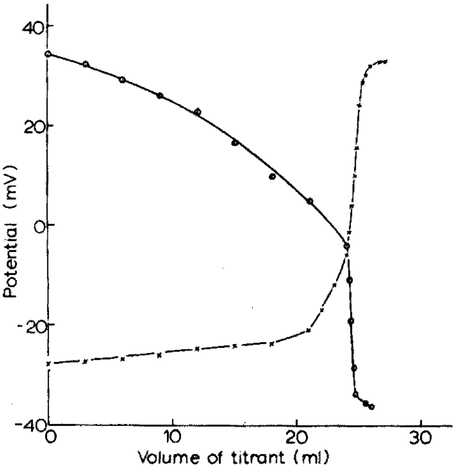
Fig. 2. Potentiometric titrations (10^{-3} *M* solutions). (⊙) Titration of 25 ml of hexadecyltrimethylammonium bromide with sodium dodecylsulphate solution (e.p. 24.5 ml); (×) titration of 10 ml of sodium dodecylsulphate solution with hexadecyltrimethylammonium bromide solution (e.p. 9.8 ml).

min, the solution being stirred with a magnetic stirrer, and then for a few minutes each in three amounts of deionized water. The electrode was then stored in deionized water until required, when it could be used without further pretreatment.

The effect of age on electrode response is shown in Table I. Between the time intervals recorded in Table I the electrode was used extensively to detect end-points in various potentiometric titrations. Although the absolute potential given in a particular solution was fairly constant over one or two days, it clearly varied considerably over a longer period of time.

Potentiometric titrations

Solutions (10^{-3} *M*) of sodium dodecylsulphate, sodium tetraphenylboron, ammonium reineckate, potassium hexacyanoferrate(III) (A.R.) and potassium dichromate (A.R.) were titrated with a 10^{-3} *M* solution of hexadecyltrimethylammonium bromide using the surfactant electrode to detect the end-point. A limited number of titrations were also made with hexadecylpyridinium bromide.



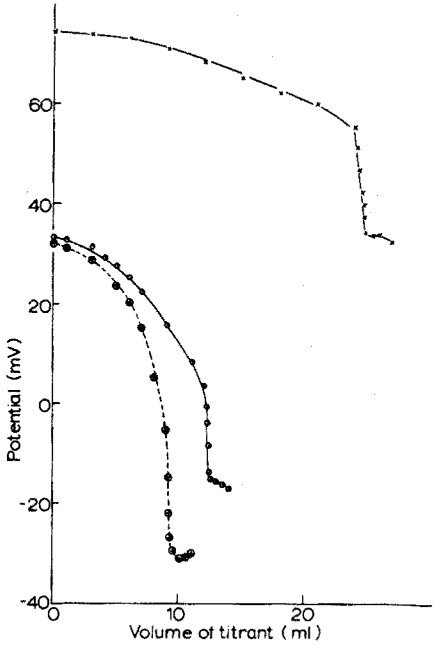


Fig. 3. Potentiometric titrations (10^{-3} *M* solutions). (⊙) Titration of 25 ml of hexadecyltrimethylammonium bromide solution with sodium tetraphenylboron solution (e.p. 24.8 ml); (×) titration of 25 ml of sodium tetraphenylboron solution with hexadecyltrimethylammonium bromide solution (24.7 ml).

Fig. 4. Potentiometric titrations (10^{-3} *M* solutions). Titrations of 25 ml of hexadecyltrimethylammonium bromide solution with (×) ammonium reineckate solution (e.p. 24.9 ml); (⊙) potassium dichromate solution (e.p. 12.35 ml); (⊗) potassium hexacyanoferrate(III) solution (e.p. 8.1 ml).

Titration curves are shown in Figs. 2–4. The end-points were determined by means of first derivative curves, and were satisfactorily reproducible.

Results and discussion

The heterogeneous solid-state electrode described responds only to detergent cations and not to detergent anions. A steady potential response is reached within 1 min and the electrode can be used conveniently to detect the end-points in the titration of cationic detergents with a range of precipitants. The absolute potential of the electrode, however, drifted badly over a period of days (see Table I), and the difference in potential response per decade change in concentration also decreased quite rapidly, falling to 44 mV per decade after two months of use.

The relative advantages and disadvantages of solid-state and liquid-junction electrodes are well established. With a liquid-junction electrode there is a greater danger of contaminating the test solution and this is important in on-line use and for biochemical use in direct contact with a bloodstream, for example. This is not an obvious disadvantage in the present case, however, as samples would normally be disposable. The heterogeneous solid-state electrodes in general take longer to prepare. If the present system were to be put into routine use, then a large sheet of the impregnated silicone rubber should be prepared so that the membrane can be replaced fairly readily when this becomes necessary. In the present work excellent results were obtained with a membrane which was soaked and used five weeks after preparation, and there seems to be no reason why longer storage times should be detrimental to the membranes.

The authors wish to thank Dr. A. M. G. Macdonald for helpful discussion on the preparation of silicone-rubber electrodes.

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SHORT COMMUNICATION

Determination of ammonia in tobacco and tobacco smoke with an ammonia electrode

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(Received 17th September 1973)

Many classical analytical methods have been described for the determination of ammonia in various matrices[1–4], but few of these are suitable for the determination of ammonia in tobacco or tobacco smoke. Such determinations usually involve distillation of the ammonia and subsequent titrimetric or colorimetric measurements[5–8]. The distillation and titration methods are generally tedious, time-consuming, and non-specific. A gas-chromatographic method[9] overcomes some of the problems, but still requires a lengthy separation step to isolate the ammonia from the bulk of the sample. In view of the complex compositions of both tobacco and smoke, the need for a simple technique for determining ammonia in these materials was indicated. This paper describes a method in which an ammonia electrode is used for determining ammonia in tobacco and tobacco smoke.

Experimental

Apparatus. An Orion Model 95-10 ammonia electrode was used in conjunction with the Orion Model 407 specific-ion meter.

The cigarettes were smoked by a one-port syringe-type smoking machine which was made in these laboratories. The machine was adjusted to operate at standard conditions (one 35-ml, 2-s puff per min).

An all-glass steam distillation unit of 125-ml capacity was used.

Reagents. Ammonia calibration standard solutions of 0.1 p.p.m. ammonia, 1.0 p.p.m. ammonia, and 10 p.p.m. ammonia were prepared from reagent-grade ammonium chloride.

Buffer solution was prepared by dissolving 10.2 g of dipotassium hydrogenphosphate and 4.8 g of borax in 1 l of ammonia-free water. The alkaline solution used in conjunction with the buffer solution was prepared by dissolving 5 g of borax in 100 ml of 0.5 *M* sodium hydroxide.

Cigarettes and filters. The cigarettes used were two domestic brands (tobacco blends), one European brand (dark tobacco), and two non-commercial types (burley tobacco and flue-cured tobacco). One of the domestic brands was an 85-mm filter cigarette and the other an 85-mm non-filter cigarette. All cigarettes were conditioned at 60% relative humidity at 75°F for 48 h before use.

Filters examined for their capacity to remove ammonia from cigarette smoke were:

1. Cellulose acetate: 1.6 den. per fil., 48,000 total den. tow; length 20 mm; 2.7 in. pressure drop.
2. Paper: length 20 mm with 2.5 in. pressure drop.
3. Cellulose-acetate-activated carbon (100 mg)—length 20 mm, 3.0 in. pressure drop. (Carbon was held in place with two 7.5 mm, 3.3 den. per fil., 44,000 total den. cellulose acetate rods.)
4. Cellulose acetate: 3.3 den. per fil., 44,000 total den. tow; treated with an acidic additive, 20 mm length, 3.0 in. pressure drop.
5. Cellulose acetate: high efficiency, 1.6-den. per fil. tow, 25 mm length, 2.4 in. pressure drop.

Determination of ammonia in tobacco. Place a weighed sample of tobacco (100–200 mg) in a steam-distillation flask. To the flask add 20 ml of buffer solution and 5 ml of alkaline solution, steam-distil, collecting the distillate (80 ml) in 10 ml of 0.1 M hydrochloric acid and then dilute to 100 ml with distilled water. Transfer the solution to a 250-ml beaker, and stir at the rate established during calibration of the instrument with the known samples. Dip the ammonia electrode in the solution, and add 2 ml of aqueous 10 M sodium hydroxide. Read the previously calibrated meter directly in p.p.m. ammonia. Calculate the percentage of ammonia in the tobacco from the concentration in solution, volume of solution, and weight of the tobacco sample.

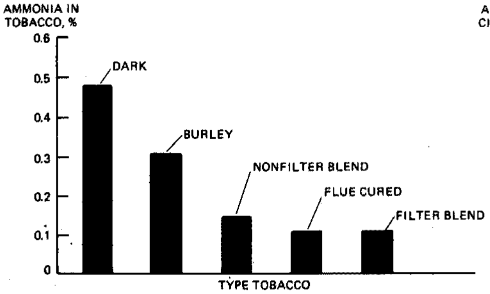
Determination of ammonia in cigarette smoke. Two cigarettes were smoked to 27-mm butt lengths under standard conditions, the smoke then being passed through a scrubber flask containing 50 ml of 0.1 M hydrochloric acid to collect the ammonia. Transfer the contents of the flask to a steam-distillation unit and add 2 ml of aqueous 10 M sodium hydroxide. Steam-distil until about 80 ml of distillate has collected in a 100-ml volumetric flask containing 10 ml of 0.1 M hydrochloric acid. Fill the flask to the mark with distilled water, and transfer the solution to a 250-ml beaker. Stir at the predetermined rate, add 2 ml of 10 M sodium hydroxide, and read the amount of ammonia in p.p.m. directly from the previously calibrated meter. Calculate the amount of ammonia per cigarette from the ml of distillate, p.p.m. of ammonia, and number of cigarettes smoked.

The precision was determined from 7 repeat analyses. The percentage of ammonia removed from the tobacco smoke by various filters was based on the amount of ammonia delivered by a control cigarette equipped with a length of tobacco column equal to that of the test filter.

Comparison of the electrode method with a gas-chromatographic method. To evaluate the accuracy of the electrode method, the ammonia in the tobacco and tobacco smoke from a non-filter cigarette was determined by a gas-chromatographic method similar to that described by Ayers[9]. An 8-ft $\times \frac{1}{8}$ in stainless-steel column packed with Chromosorb 103 was used; the column temperature was controlled at 100°, and a thermal conductivity detector was used. The method for collection of the ammonia was identical to that described above, but it was necessary to concentrate the acidic steam-distillate until the ammonia concentration was about 20 p.p.m.

Results and discussion

Ammonia content of various cigarette tobaccos. The ammonia contents of several tobaccos used in cigarettes are shown in Fig. 1. The cigarettes made from dark tobacco contained 0.48% ammonia which was the highest of the commercial cigarettes tested. Cigarettes made from burley tobacco contained 0.31%, and those prepared from flue-cured tobacco contained 0.11% ammonia. The blended tobacco of the non-filter domestic cigarette contained 0.14% ammonia, and the blended tobacco of the filtered domestic brand contained 0.11% ammonia.



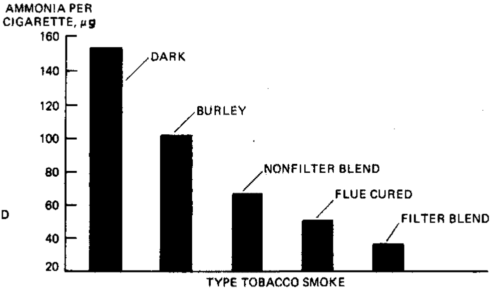


Fig. 1. Ammonia content of various cigarette tobaccos.

Fig. 2. Ammonia content of smoke from various cigarettes.

Ammonia in the smoke of various cigarettes. The smoke from the dark tobacco cigarettes contained 153 µg of ammonia per cigarette (Fig. 2). Smoke from the burley and the flue-cured tobacco cigarettes contained 102 µg and 51 µg of ammonia per cigarette, respectively. Smoke from the non-filter, blended-tobacco cigarettes contained 67 µg of ammonia per cigarette while smoke from the filter in blended-tobacco cigarettes contained 36 µg of ammonia per cigarette. The lower amount of ammonia in the smoke of the filter cigarettes was primarily due to ammonia removal by the filter.

Removal of ammonia from cigarette smoke by filters. The amounts of ammonia removed from cigarette smoke by certain filters are shown in Table I. The most efficient filter for removing ammonia from smoke was the high-efficiency cellulose acetate filter.

The ammonia in cigarette smoke is present as the ammonium ion. This is indicated[10] by the pH of cigarette smoke, which ranges from 5.4–6.4. In Fig. 3

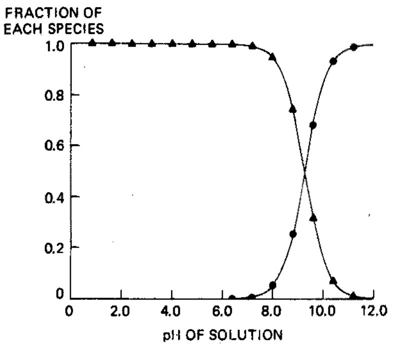


Fig. 3. Fraction of NH_4^+ and NH_3 *vs.* pH; (▲) NH_4^+; (●) NH_3.

TABLE I

EFFICIENCY OF FILTERS FOR REMOVING AMMONIA FROM CIGARETTE SMOKE

(The tobacco used contained 0.14% ammonia)

Filter	*Pressure drop (in)*	*Ammonia delivery (μg)*	*Ammonia removal by filter (%)*
Tobacco	0.6	67	—
Cellulose acetate[a]	2.4	47	30
Cellulose acetate[b]	3.1	27	60
Paper	2.5	39	42
Acetate–carbon[c]	3.0	38	44
Acetate–additive[d]	3.0	36	46

[a] Regular acetate filter; [b] high-efficiency acetate filter; [c] 100 mg activated carbon between two 7.5-mm acetate plugs; [d] acetate filter containing a polyol and an acidic additive.

The ammonia in cigarette smoke is present as the ammonium ion. This is indicated[10] by the pH of cigarette smoke, which ranges from 5.4–6.4. In Fig. 3 the fraction of ammonium and ammonia are plotted *vs.* pH. In the pH range of cigarette smoke, the amount of unprotonated ammonia is negligible. Therefore, ammonia in cigarette smoke is primarily ammonium ion entrained in the particulate phase, and the amount of ammonia removed by a filter roughly parallels the amount of total particulate matter removed by a filter. This observation is further supported by the fact that the filter containing activated carbon did not selectively remove the ammonia from cigarette smoke.

Accuracy and precision. The precision of the method was determined from the repeated analyses of the smoke from the domestic brand 85-mm non-filter cigarette. The mean delivery of ammonia was 67 μg with a standard deviation of 2.2 and a coefficient variation of 3.3%.

An attempt was made to compare the results with those obtained by the classical Nessler method[4]. However, several compounds in tobacco smoke interfered with the Nessler method, even after steam distillation of the sample. A gas-chromatographic method, similar to that of Ayers[9], was used to obtain a measure of the accuracy of the method. This method was less sensitive than the electrode method, and it was necessary to concentrate the acidified steam-distillate about 20-fold; the average amount of ammonia found in the smoke of the non-filter cigarette was 67 μg which is identical to the 67 μg obtained with the electrode method.

The gas-chromatographic method was also used to determine the ammonia content of tobacco; the amount found was 0.13%, which agreed very closely with the 0.14% obtained by the electrode method.

Effect of amines on electrode response. Amines were the compounds which were most likely to interfere with the electrode response during the ammonia determinations. No interference was observed when methylamine was added in amounts equal to the ammonia concentration. It was therefore concluded that the relatively low amounts of amines in cigarette smoke (10–15 μg of methylamine)[11] would not significantly affect the ammonia determination.

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BOOK REVIEWS

Organic Reaction Mechanisms 1971, Edited by B. Capon and C. W. Rees, Interscience Publishers–John Wiley and Sons, London, 1972, price £16.00.

Once again it is a pleasure to have at hand the latest volume in this series of annual surveys of the literature. It is now so well-established and its readers will be so familiar with the lay-out and general presentation that it is difficult to find anything fresh to say about the series.

New readers will be impressed with the timeliness and thoroughness of the book, features which have characterised the whole series. There has been an inevitable increase (about 10%) in the number of references culled (5000) as compared with last year. The most important are described and discussed; the rest are listed. The team of reviewers, almost the same as last year, has done a good job. The format is up to the usual high standard, but the printers have altered the type setting to something smaller and more "modern". In the opinion of the reviewer this does not make for easier reading.

E. J. Forbes (Birmingham)

Absorption Spectra in the Ultraviolet and Visible Region, Vol. XVII, Edited by L. Lang, Akademiai Kiado, Budapest, 1972, 400 pp. + Index.

This is a continuation of the valuable collection of absorption spectra of new and recent compounds; the present volume contains information about many compounds of pharmaceutical interest.

L. Sachs, *Statistische Methoden*, Springer-Verlag, Berlin, 2. Aufl., 1972, xiii + 105 S., Preis geheftet DM 8,80; US $2.80.

This small paperback is intended as an aid to practical scientists, medical workers, psychologists and sociologists. Various corrections to the earlier edition have been made, and improved and extended methods have been introduced.

Vocabulary of Surface-active Agents, C.I.D., 64 Avenue Marceau, 75-Paris-8e, 2nd Ed., 1972, 136 pp., price 20.00 F + postage.

This is a vocabulary containing 213 terms which has been prepared by the Comité International des Dérivés Tensio-actifs (C.I.D.). The terms and definitions are described in the official languages of the committee—French, German and English. The terms are those recommended by the committee in consultation with other international bodies.

W. W. Schulz and G. E. Benedict, *Neptunium-237: Production and Recovery*, U.S. Atomic Energy Commission, Office of Information Services, 1972, viii + 85 pp., price $3.00 (obtainable from N.T.I.S., Department of Commerce, Springfields, Va. 22151).

This is one of the A.E.C. Critical Review Series, now being released by the U.S.A.E.C. The topics covered are supply and demand, recovery of neptunium from aqueous solutions, and non-aqueous methods for the recovery and separation of neptunium. Most of the information is relevant to production-scale work.

H. B. Gray, *Elektronen und Chemische Bindung*, Walter de Gruyter, Berlin, 1973, xi+194 S., Preis DM 24,00.

A translation of the English edition of 1964, this paperback is intended for undergraduate students, and is largely concerned with molecular orbital theory.

G. Charlot, *Cours de Chimie Analytique Générale. Tome III. Exercices*, Masson, Paris, 1972, 212 pp., prix 48 F.

This paperback is Part III of a series of texts based on the courses of analytical chemistry at the University of Paris. It is devoted to exercises in the theory of equilibria in homogeneous media, heterogeneous media and separations. Worked examples are provided, and the text should be very useful to all teachers of analytical chemistry.

R. A. Durst, *Electrochemical Analysis Section, N.B.S., Summary of Activities, June 1970–June 1971*, N.B.S. Tech. Note 583, U.S. Gov. Printing Office, Washington, D.C. 20402, Issued February 1973, 113 pp., price $0.75.

Developments in coulometric and polarographic analysis are considered. Work on pH and ionic activity standards, potentiometry of complexing agents and calcium, and determination of dissolved oxygen is summarized.

M. R. Litzow and T. R. Spalding, *Mass Spectrometry of Inorganic and Organometallic Compounds*, Elsevier Scientific Publishing Company, London, 1973, xv+620 pp, price Dfl. 150.00 (about $52.60 or £21).

This is the first comprehensive treatise devoted exclusively to the study of inorganic compounds by mass spectrometry. The text is divided into twelve chapters, with the first three forming an introduction to the subject of mass spectrometry. There is a comparatively brief and uniformative section devoted to instrumentation; no complete instruments are described, there is no mention of modern chemical ionisation sources or of alternative mass analysers of the radiofrequency type such as the monopole. Succeeding sections deal with the interpretation of mass spectra according to the origin of the ion and with the types of information which are accessible with the aid of a mass spectrometer. This last section incorporates an excellent comprehensive description of the many alternative methods of determining appearance potentials from ionisation efficiency curves. However, some more extended account of electron monochromators would be appropriate here and the suggestion that photoelectron spectrometry has recently made mass spectrometric determinations of ionisation potentials obsolete is misleading. In fact, such measurements have always been inferior to those made with an ionisation chamber and a vacuum ultraviolet monochromator.

The following nine chapters are each devoted to a separate group of elements covering the whole periodic table from the alkali metals to the rare gases.

The spectra of the elements and isotopic abundances are reported, followed by more detailed information about the organometallic derivatives. Probably the most interesting section is that devoted to the oxides, fluorides and oxyfluorides of xenon, the bonding being sufficiently strong for many of the structures to survive electron impact.

It is difficult to assess the total coverage of this book over so wide a subject but random checks are not encouraging. There is, for example, no mention of the very extensive work carried out with uranium and the transuranic elements during the developments of atomic energy. Similarly, there appears to be no mention of mass spectral studies on the oxides and oxyacids of chlorine, while the section dealing with co-ordination complexes is very cursory indeed.

However, the authors have undertaken a formidable task in assembling so much complex information in a single book and the results of their labours will be invaluable to those involved in teaching or carrying out research work in the ever widening field of inorganic chemistry. It is suggested that, in any future edition, the meagre index be replaced by a compound index which will permit the reader to discover as rapidly as possible whether the mass spectrum of any particular organometallic compound has been recorded.

J. R. Majer (Birmingham)

ERRATUM

Masakichi Nishimura and Shinichiro Noriki, A precise spectrophotometric determination of a major constituent in pure materials, *Anal. Chim. Acta*, 66 (1973) 351–357.

Page 352, equation (2) should read:

$$A = \varepsilon_{\mathrm{MI}}[\mathrm{MI}] + \varepsilon_{\mathrm{I}}[\mathrm{I}] = (\varepsilon_{\mathrm{MI}}\mathrm{Y} + \varepsilon_{\mathrm{I}})C_{\mathrm{I}}/(1+\mathrm{Y})$$

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