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Vol. **70**, No. **1**	May 1974	
Vol. **70**, No. **2**	June 1974	**(completing Vol. 70)**
Vol. **71**, No. **1**	July 1974	
Vol. **71**, No. **2**	August 1974	**(completing Vol. 71)**
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Vol. **72**, No. **2**	October 1974	**(completing Vol. 72)**
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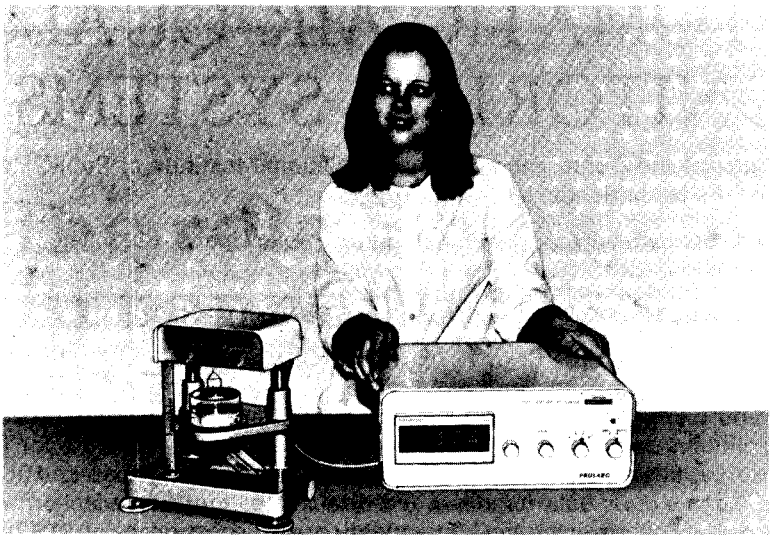

Tensiomètre PROLABO à équilibrage automatique et lecture numérique directe en dyne/cm

Ce nouveau tensiomètre automatique est destiné comme le tensiomètre Dognon-Abribat à la mesure précise des tensions superficielles et interfaciales. C'est un instrument élaboré aux possibilités très étendues:

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Les résultats sont remarquablement **reproductibles;** la sensibilité de l'instrument est de **0,1 dyne/cm.**

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Les indications du tensiomètre étant **instantanées,** on peut suivre l'évolution de la tension au cours du temps, et **enregistrer** graphiquement ses variations.

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On comprend donc l'intérêt du tensiomètre pour l'étude des solutions détergentes, des liquides de traitement de minerais, des bains d'électrolyse, des colles, des peintures et vernis, des catalyseurs, des suspensions, des solutions colloïdales, etc. . . Sant compter l'étude des liquides biologiques.

La tension superficielle des liquides est une constante physique dont la mesure systématique se révèle fructueuse. Elle est à la fois un indice de pureté et de propreté superficielle.

Si le tensiomètre manuel de Dognon-Abribat est un excellent instrument lorsqu'il s'agit de faire épisodiquement quelques mesures ou d'organiser des travaux pratiques d'élèves, on préfèrera le nouveau tensiomètre automatique dans les laboratoires de recherches, les laboratoires de mesures physiques et les laboratoires de contrôle industriel devant exécuter de nombreuses mesures.

Le même tensiomètre convient pour faire rapidement et avec précision des mesures isolées ou en série, ou pour suivre et enregistrer en continu des tensions variables. Dans beaucoup de problèmes pratiques, l'étude des variations de la tension sous l'effet de divers paramètres est du plus haut intérêt. Ses qualités offrent des possibilités nouvelles pour l'étude scientifique des tensions superficielles et interfaciales et pour son exploitation pratique en milieu industriel.

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AUTOMATIC AIR QUALITY MONITORING SYSTEMS

Proceedings of the Conference held at the National Institute of Public Health, Bilthoven, The Netherlands, 5-8 June, 1973.

edited by **T. SCHNEIDER,** National Institute of Public Health, Bilthoven, The Netherlands.

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This symposium was organized by the National Institute of Public Health in Bilthoven, The Netherlands. Its purpose was to set up an exchange of knowledge on existing and planned automated air quality monitoring systems and the analysis of the air pollution data. It comprised the following subjects: monitors for measurement of air pollution and their use in the systems approach; design and application of automatic systems; data handling and data evaluation in connection with large systems; use of models for the determination of dispersion of air pollution; and application of monitoring systems within the existing international cooperation. As a result of the symposium a plan has been developed for future international cooperation between national research institutes and governmental agencies in the field of monitoring of pollution with a systematic and harmonized approach.

CONTENTS:

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Optimal Substance Separation with Pre-packed Columns

Analytica Chimica Acta, 69 (1974) 253–258

N.M.R. DETERMINATION OF WAX ESTERS IN MARINE LIPIDS

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(Received 10th September 1973)

High-resolution nuclear magnetic resonance (n.m.r.) spectroscopy has been extensively applied in the study of lipids[1–4], fatty acids[5–9], wax esters[10] and lipoproteins[11]. The application of n.m.r. in most investigations[4–10] has been to the structural elucidation of fatty components isolated from natural lipids. However, it has also become a useful tool for determining the degree of unsaturation and average molecular weight of natural fats[2], and for the determination of various fatty acids in triglyceride lipids[3,4].

Wax esters are known to be important constituents of some marine oils and lipids[12]. Chromatographic methods have shown the presence of up to 80% wax ester in some cases[12–14]. There is a need for an accurate rapid estimation of wax esters in natural samples. Integration of the n.m.r. spectra of the regions corresponding to terminal methyl groups and to methylene protons of both wax esters and triglycerides, followed by a simple calculation, provides such a method.

EXPERIMENTAL

Samples

Commercial samples of Spermaceti (BDH, Ltd) and a commercial quality lard (Bio-Research Laboratories, Montreal) were used as model samples for wax esters (WE) and triglycerides (TG), respectively. The lard sample was from a lot which had been characterized for use in nutritional studies and shown to be essentially pure triglyceride. Two different but typical samples of marine lipids containing wax esters such as commercial sperm whale (*Physeter catodon*) oil[13] from a local reduction plant and total lipids[12] extracted from barracudina (*Paralepis rissoi krøyeri*) by the method of Bligh and Dyer[15] were used. Analytical data for these lipid samples are listed in Table 1.

Apparatus

A Varian Associates T-60 spectrometer was used to determine the 60-MHz room-temperature spectra used in this work. In determining the integral trace, particular attention was paid to the proper adjustment of phase so that the baseline at the peaks of interest was as flat as possible.

TABLE I

ANALYTICAL DATA OF FOUR LIPID SAMPLES

	Model TG	*Model WE*	*Barracudina oil*	*Sperm whale oil*
Iodine value (Wijs)	27	4–5	124	87
Free fatty acid (as oleic acid) (%)	—	—	1.0	0.6
Phospholipids (%)	—	—	0.4	0.6
Av. mol.wt.	869	442	502	531
Av. mol.wt. (TG only)			882	895
Av. mol.wt. (WE only)			432	419

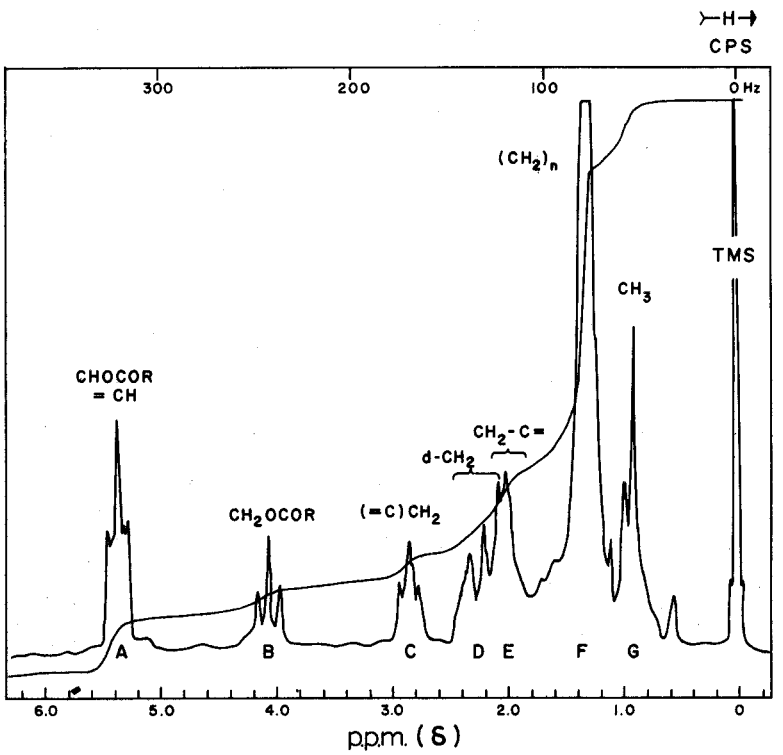

Fig. 1. N.m.r. spectrum (60-MHz) of barracudina lipids.

Procedure

Spectra were recorded for 10% (w/w) solutions of the lipid samples in $CDCl_3$ containing 1% (v/v) tetramethylsilane (Stohler Isotope Chemicals). Figure 1 shows one such spectrum, that of the lipids extracted from barracudina oil. The spectrum may be arbitrarily divided into seven groups of signals, which have been labelled A to G in the Figure. Assignment of these groups of signals is

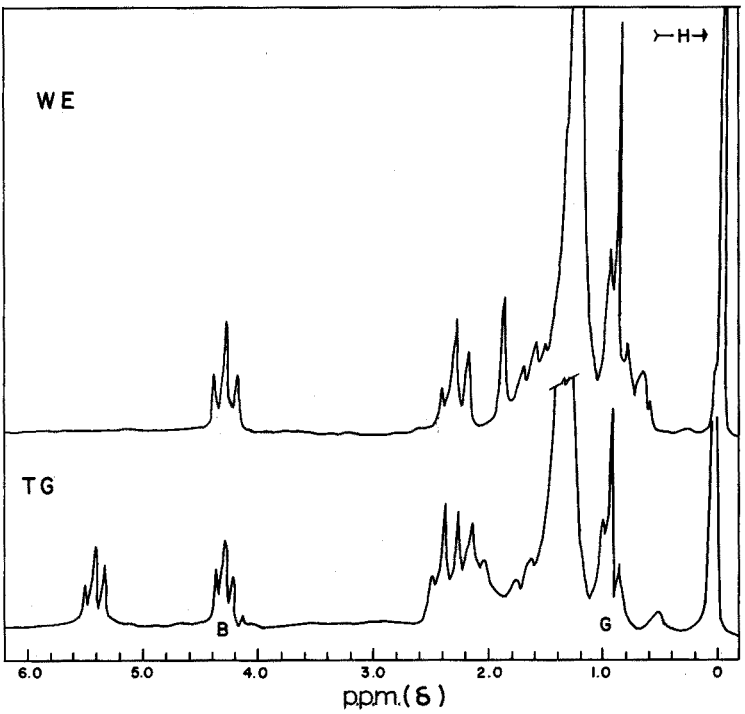

Fig. 2. N.m.r. spectrum (60-MHz) of model wax esters and triglycerides.

straightforward, especially after inspection of the spectra of the model wax ester and triglyceride samples shown in Fig. 2. Group A signals are those of olefinic protons superimposed on the signal of the methine proton of the glyceryl moiety of the triglycerides. Group B contains the signals of methylene protons, respectively, in the glyceryl moiety of triglycerides and in the $-COOCH_2-$ group of wax esters. Groups C, E and F are from their chemical shifts the signals of CH_2 groups bonded respectively to two sp^2 carbons, one sp^2 and one sp^3 carbon, and to two sp^3 carbons. Group D is the signal of CH_2 groups in the α-position to the carboxyl group in the fatty acid moieties of wax esters and triglycerides. Group G contains the signals for the protons of terminal methyl groups in both triglycerides and wax esters.

For each spectrum six integral traces were recorded. From each integral trace the values of the integrals (in arbitrary units) for groups B and G were determined. Where a sloping baseline was observed, the integral was measured from a point halfway between the signal of interest and the new low field group to a similar midpoint between the signal of interest and the next high field group. The average value of the six integrals measured for groups B and G were used in the calculation described in the next section.

For comparison of the n.m.r. method with established methods, wax esters and triglycerides were separated by a thin-layer chromatographic method and quantitatively recovered[12,14]. The average molecular weights of the two fractions were determined by depression of the freezing point in benzene. From the weight

percent wax esters and triglyceride given by the t.l.c. method and these average molecular weights, the mole percent of each in the total oil or lipid sample may be calculated.

Calculation

Let m_1 and $1-m_1$ be the mole fractions of triglyceride and wax esters respectively, in a sample containing mostly those two types of lipid. If each proton contributes an amount P to the observed integral trace, the general molecular structures of triglycerides and wax esters lead to these values of the integrals I_b and I_g for the group B and G signals:

$$[4m_1+2(1-m_1)]P=I_b \quad (1)$$

$$[9m_1+6(1-m_1)]P=I_g \quad (2)$$

When I_b and I_g are measured for an experimental mixture, eqns. (1) and (2) can be solved simultaneously for m_1 and P. The solution for m_1 is:

$$m_1 = \frac{2I_g}{2I_g-3I_b} - 2 \quad (3)$$

from the value of the mole fraction m_1 the mole percent wax esters in the sample is seen to be:

$$\mathrm{WE} = \left(3 - \frac{2I_g}{2I_g-3I_b}\right) \times 100 \quad (4)$$

RESULTS AND DISCUSSION

It might seem unusual to determine the mole percent wax ester by integration of two signals both of which have a contribution from both wax and triglyceride molecules, but the alternative would be to use the integral of group A signals. The model wax ester spectrum (Fig. 2) shows that there are no observable signals in this region, while the model triglyceride spectrum has a strong absorption. It is entirely possible, however, that in a natural lipid sample the A group signals would have a contribution from olefinic protons in wax ester molecules[10], which would invalidate any method of analysis assuming group A signals to arise solely from triglyceride.

The error inherent in the measurement of n.m.r. integrals is much greater than any correction required to account for the distribution of peak intensity resulting from the natural distribution of carbon isotopes[2]. The 1.108% ^{13}C content of natural samples means that part of the absorption intensity of *e.g.* a CH_2 proton signal is contained in the so-called ^{13}C satellite signals equally disposed about the $^{12}CH_2$ signal. Both B and G group signals are slightly affected by this, but to different extents, each less than 1% of the observed intensity.

Table II compares the result of the application of the n.m.r. method based on B and G group integrals with the result of quantitative t.l.c. followed by colligative property determination of average molecular weight. The agreement between the two methods was very acceptable, but even more remarkable is

TABLE II

MOLAR PERCENT OF WAX ESTERS IN MODEL MIXTURES AND NATURAL LIPIDS AS DETERMINED BY N.M.R. AND T.L.C. METHODS

Lipids	*Mole % (±s)*	
	T.l.c.	*N.m.r.*
Model wax ester	98.5	98.0±1.8
Mixture A	2.6	2.9±0.2
Mixture B	17.4	17.0±0.8
Sperm whale oil	78.5±5.4	76.2±3.0
Barracudina oil	80.9±5.0	84.6±4.1

the saving of time and effort effected by the n.m.r. method. After extraction of the lipid from a natural fat, analysis of the wax ester content by the n.m.r. method can easily be carried out in 10 min, with spectrometers which are relatively unsophisticated and easy to operate. With this method and the T-60 spectrometer, wax ester contents as low as 3 mg in a 50 mg sample of natural lipid can be determined with an overall error of less than 7%.

SUMMARY

The proton n.m.r. spectra of lipids containing triglycerides and wax esters dissolved in $CDCl_3$ are characterized by seven sets of signals. The areas of the signals of terminal methyl group and of methylene protons of both wax ester and triglyceride were integrated. These were used to calculate the content of wax esters in lipids or oils. The rapid n.m.r. procedure is directly usable for natural lipids containing as low as 3 mg of wax esters in 50-mg samples with an error of about 7%. The method described compares favorably with t.l.c. determination.

RÉSUMÉ

Les spectres r.m.n. de lipides contenant triglycérides et cires (esters) dissous dans $CDCl_3$ sont caractérisés par sept séries de signaux. Les surfaces des signaux du groupe méthyl terminal et des protons méthylène, de la cire et du triglycéride, sont intégrées. Ceci permet de calculer la teneur des cires dans lipides ou huiles. Le procédé r.m.n. rapide est directement utilisable pour des lipides naturels, contenant au minimum 3 mg de cire dans 50 mg d'échantillon, avec une erreur d'environ 7%.

ZUSAMMENFASSUNG

Die Protonen-n.m.r.-Spektren von Lipiden, die Triglyceride und Wachsester enthalten, die in $CDCl_3$ gelöst werden, werden durch sieben Folgen von Signalen charakterisiert. Die Flächen der Signale der endständigen Methylgruppe und der Methylenprotonen sowohl von Wachsester als auch von Triglycerid wurden

integriert. Diese wurden für die Berechnung des Gehalts an Wachsestern in Lipiden oder Ölen verwendet. Das schnelle n.m.r.-Verfahren ist mit einem Fehler von etwa 7% unmittelbar auf natürliche Lipide anwendbar, die nur 3 mg an Wachsestern in Proben von 50 mg enthalten. Die beschriebene Methode ist günstig im Vergleich zu der dünnschichtchromatographischen Bestimmung.

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Analytica Chimica Acta, 69 (1974) 259–265

DETERMINATION OF STRONTIUM IN HUMAN TOOTH ENAMEL BY ATOMIC ABSORPTION SPECTROMETRY

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The strontium level of tooth enamel varies considerably depending on the geographical area[1], and appears to be correlated to the levels present in food and drinking water. The present investigation was concerned with the levels of strontium in teeth from different areas in Great Britain in relation to strontium levels of the drinking water. There is some evidence that strontium may reduce dental caries; for example, the prevalence of dental caries is reduced in Ohio[2,3,4] where there are high levels of strontium in drinking water and in Gloucestershire[5] where high levels are found in the soil.

Strontium determinations in biological materials by both emission and atomic absorption spectrometry are subject to serious interferences. Enamel is apatitic in nature and can be represented by the simple formula $Ca_{10}(PO_4)_6X_2$, where X is usually hydroxyl or fluoride, hence the sample will contain 36.75% calcium and 17.4% phosphorus. Webb and Wordingham[6] determined the ratio of strontium to calcium in bone and teeth by flame emission photometry; the sensitivity of the method was reduced by the interference of calcium and phosphate, but this was overcome by surrounding the oxygen–propane flame with a mantle of oxygen.

When strontium is determined by atomic absorption, the absorption is reduced by anions such as phosphate and sulphate, and by cations such as calcium and aluminium, owing to the formation of stable compounds in air–acetylene flames. Such interferences have been studied by David[7] and in more detail by Trent and Slavin[8]. David[7] demonstrated that strontium absorption was seriously depressed by the combined presence of calcium and phosphate in plant materials, probably because strontium enters the calcium phosphate lattice which is scarcely dissociated in the flame; phosphate was therefore removed with an anion-exchange resin, and a standard addition method was used to compensate for any calcium interference. In some cases[9,10], both calcium and phosphate have been removed by ion exchange. Coprecipitation of strontium and calcium oxalates has also been used to overcome some interferences in urine[11], bone[12] and other biological materials[13]. The addition of an excess of lanthanum has been recommended by Trent and Slavin[8] to control chemical interferences in the analysis of fish, hay, bone and milk, and by Belcher and Brooks[14] for coal ash. For urine analysis, Montford and Cribbs[15] coprecipitated strontium with lanthanum, and dissolved the precipitate in hydrochloric acid for the determination. However, both ion-exchange and coprecipitation techniques are time-consuming; and if lanthanum addition is used, the phosphate concentration must be less than 300 p.p.m., which would necessitate intolerable dilutions for a biological sample.

The higher temperature of the nitrous oxide–acetylene flame reduces chemical interferences to some extent, but the sensitivity is low, owing to ionization effects. Trent and Slavin[8] showed that ionization was prevented by the presence of a more easily ionized metal such as sodium or potassium.

The investigation described here was concerned with the chemical interferences produced by calcium and phosphate at the levels found in human tooth enamel, and the methods by which these interferences may be reduced.

EXPERIMENTAL

Apparatus

A Perkin-Elmer model 303 atomic absorption spectrophotometer fitted with a recorder–readout accessory and a Hitachi 165 recorder was used. The standard air–acetylene burner head (0.0115 × 4 in) was used for air–acetylene (air 21.2 l min^{-1}, acetylene 4.5 l min^{-1}) whilst the flat-topped nitrous oxide–acetylene burner head (0.019 × 2 in) was used for the nitrous oxide–acetylene flame (nitrous oxide 12.5 l min^{-1}, acetylene 7.2 l min^{-1}). The spectral source was a strontium "Intensitron" hollow-cathode lamp operated at 460.7 nm.

Procedure

Separate the enamel and dentine from the tooth by mechanical means, and dry the enamel at 105° for 48 h in an air oven.

Digest 0.1 g of enamel in 1 ml of concentrated (62%) perchloric acid in a Kjeldahl flask. When dissolution is complete, transfer the solution quantitatively to a 10-ml graduated flask. To overcome ionization effects, add 1 ml of sodium chloride solution (0.02 g ml^{-1}, A.R. sodium chloride) and dilute the solution to volume.

Prepare standards containing 2000 p.p.m. sodium chloride with 0, 20, 40 and 60 p.p.m. strontium for the standard addition technique. Add 0.1 ml of each addition standard to different 2-ml aliquots of the sample solution to give "added" strontium concentrations of 0, 0.0952, 1.904 and 2.857 p.p.m. strontium. Aspirate the solutions containing sample plus addition against a blank of 2000 p.p.m. sodium chloride solution, in the air–acetylene flame.

By extrapolation of the graph of absorbance against "added" strontium concentration, obtain the level of strontium in the sample solution after correcting for the dilution produced by the standard addition technique.

RESULTS AND DISCUSSION

Interference studies

Aqueous strontium standards (2.0 p.p.m.) were prepared containing increasing concentrations of sodium or potassium as their chlorides. The strontium absorption was observed in both air–acetylene and nitrous oxide–acetylene flames. Figure 1 shows the enhancement caused in the nitrous oxide–acetylene by an alkali metal. This enhancement rises to a maximum, after which further additions of the alkali metal produce no effect; the enhancement was greater for potassium than sodium. In the air–acetylene flame little change was caused by either potassium or sodium (Fig. 1). In the subsequent interference studies, the standards contained either 2000 p.p.m. potassium chloride or 2000 p.p.m. sodium chloride.

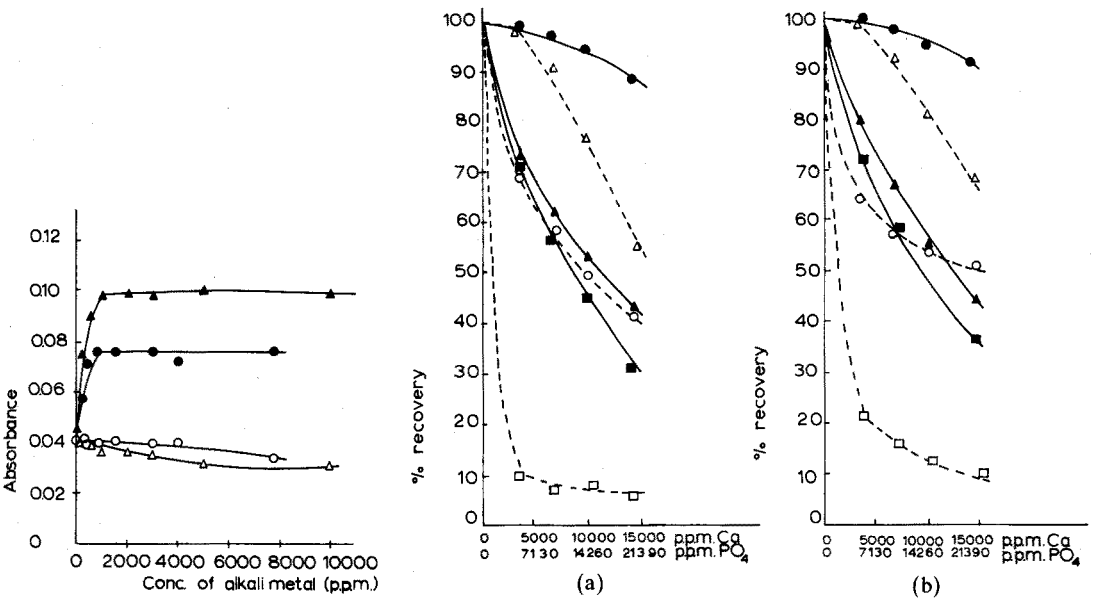

Fig. 1. Effect on strontium (2.0 p.p.m.) absorption due to increasing concentrations of potassium or sodium. Nitrous oxide–acetylene: (▲) potassium, (●) sodium. Air–acetylene: (△) potassium, (○) sodium.

Fig. 2. Depression of strontium (2.0 p.p.m.) absorption in nitrous oxide–acetylene or air–acetylene flames by increasing concentrations of calcium, phosphate or combined calcium and phosphate. (a) Standards containing 2000 p.p.m. sodium chloride. (b) Standards containing 2000 p.p.m. potassium chloride. Nitrous oxide–acetylene: (▲) Ca, (●) PO_4, (■) Ca–PO_4. Air–acetylene: (△) Ca, (○) PO_4, (□) Ca–PO_4.

Strontium standards (2.0 p.p.m.) were prepared containing either calcium or phosphate in a concentration range corresponding to the minimal to maximal levels possible in an aqueous solution of enamel. Similar standards containing both calcium and phosphate in the same ratio as in enamel were also prepared. The results of these interference studies are shown in Fig. 2. The interferences were generally less in the higher temperature flame, but nevertheless were still significant. Phosphate interference was considerably less in the nitrous oxide–acetylene than air–acetylene flame, but calcium interference was unexpectedly greater in the nitrous oxide–acetylene flame. With the nitrous oxide–acetylene flame, these high calcium concentrations produce an intense flame emission which may contribute to the depression; this emission produced a high degree of noise on the photomultiplier, so that high noise suppression was essential, which was undesirable especially when scale expansion was used. The greatest interference was produced by calcium and phosphate present together, this was greater in air–acetylene than nitrous oxide–acetylene flames, and was due to the refractory nature of the calcium phosphate formed in the cooler flame. In the nitrous oxide–acetylene flame, the depression produced by calcium and phosphate together was approximately equal to the sum of their separate depressions, which indicates that the depression was due to the separate effects of calcium and phosphate, rather

than formation of calcium phosphate. In the air–acetylene flame, the combined interference showed no simple relationship to the separate depressions; the depression was significant even at the lowest calcium–phosphate concentration where the separate effects of both elements were considerably less.

Interference produced by up to 300 p.p.m. phosphate has been overcome by the addition of lanthanum[8]. The use of lanthanum was investigated at the phosphate levels found in enamel. Standards of 2.0 p.p.m. strontium containing 3680 p.p.m. calcium, 5200 p.p.m. phosphate and 2000 p.p.m. potassium chloride were buffered with increasing concentrations of lanthanum. The precipitate of lanthanum phosphate was filtered and the filtrate aspirated. In the nitrous oxide–acetylene flame, the absorption did not change with increasing lanthanum concentration. However, in the air–acetylene flame, the absorption increased with lanthanum concentration up to 1.5% (w/v) lanthanum, after which there was no further increase. The plateau region corresponded to the absorption of a standard of 2.0 p.p.m. strontium, 3680 p.p.m. calcium and 2000 p.p.m. potassium chloride, *i.e.* the initial standard without phosphate. This agrees with the findings of Fig. 2, for in the air–acetylene flame, the removal of phosphate by precipitation from the calcium–phosphate standard would lead to increased absorption, the interference of calcium being less than that of combined calcium and phosphate. In the nitrous oxide–acetylene flame, the depression produced by calcium is not greatly affected by phosphate, so that removal of phosphate has little effect.

Strasheim *et al.*[10] reported an enhancing effect of methanol. In this study, no enhancement was found in air–acetylene flames, whilst in nitrous oxide–acetylene flames, the enhancement was only 10% at methanol concentrations of greater than 60% (v/v), but at this concentration, "salting out" of sodium chloride or potassium chloride occurred.

Suitable acids for the digestion of tooth enamel are sulphuric acid–nitric acid mixtures or perchloric acid. Both sulphuric acid[8,16] and nitric acid[8] have been reported to interfere with the atomic absorption of strontium, and in addition precipitation of calcium sulphate can occur, which may lead to coprecipitation of strontium sulphate. Perchloric acid limits the use of potassium chloride as an ionization suppressant, owing to the low solubility of potassium perchlorate.

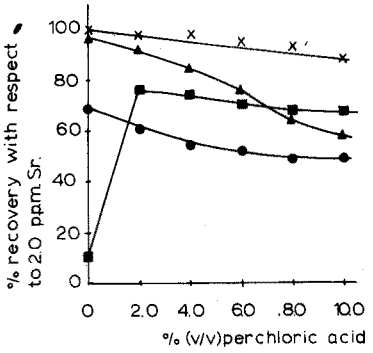

Fig. 3. Effect on strontium (2.0 p.p.m.) absorption of increasing concentrations of perchloric acid in the air–acetylene flame. (×) 2.0 p.p.m. Sr, (▲) +5200 p.p.m. PO_4, (●) +3680 p.p.m. Ca, (■) +(5200 p.p.m. PO_4–3680 p.p.m. Ca).

Therefore, in the analysis of enamel, sodium chloride was used to overcome ionization after digestion with perchloric acid. The influence of perchloric acid on the absorption was investigated in the presence of calcium and phosphate separately and together.

Figure 3 shows that in the air–acetylene flame, the strontium (2.0 p.p.m.) absorption decreased slightly with increased perchloric acid concentration, probably because of viscosity effects. Similar effects were observed with standards containing either calcium or phosphate. However, when calcium and phosphate were present together, the absorption was significantly increased in the presence of perchloric acid. In all cases, with the nitrous oxide–acetylene flame, absorption was slightly reduced with increasing acid concentration. These results indicate that in the air–acetylene flame the presence of perchloric acid overcomes, to a significant extent, the combined calcium and phosphate interference, without time-consuming chemical separation techniques or the addition of lanthanum.

Recovery studies

The recoveries of strontium added to samples of dried enamel before digestion with perchloric acid are summarized in Table I; the analysis was carried out by the procedure described. Analysis of enamel without any addition gave a mean strontium content of 119.4 p.p.m. (4 determinations; coefficient of variation, 0.75%).

TABLE 1

RECOVERY STUDIES

(Strontium present in enamel, 119.4 p.p.m.)

Sr added (p.p.m.)	19.60	33.27	109.14
Sr recovered (p.p.m.)	136.7	150.8	225.4
% Recovery	98.3	98.8	98.6

An estimate of the precision was obtained from the results of five aqueous enamel samples containing 1.12 p.p.m. strontium. These samples gave a mean absorbance of 0.0113; the standard deviation was 0.00016 and the coefficient of variation, 1.5%.

Conclusions

Calcium and phosphate at the levels found in tooth enamel have serious effects on the strontium absorption in both air–acetylene and nitrous oxide–acetylene flames, but particularly in the former where the absorption is reduced to 10% of its original value. However, in the air–acetylene flame, perchloric acid significantly decreases the calcium–phosphate interference, so that a standard addition method can be applied. The sensitivities for enamel were 0.36 p.p.m. for 1% absorption and 0.25 p.p.m. for 1% absorption, for the air–acetylene and nitrous oxide–acetylene flames, respectively. Although the nitrous oxide–acetylene flame offers the higher sensitivity, air–acetylene is preferable because of the lower noise level found. The nitrous oxide–acetylene flame should be used only if a suitable absorption peak cannot be obtained for 3× scale expansion with the air–

acetylene flame. Sample solutions should be prepared containing sodium chloride to allow for use of the higher temperature flame, if this is considered necessary.

Chemical separation of calcium or phosphate, or the addition of lanthanum, can be avoided if perchloric acid is used for sample dissolution. The remaining interference can be compensated for by the standard addition method, which ensures that the sample and standard are matched with regard to interference and total solids. Under the conditions used, the strontium calibration graph was linear up to 6.0 p.p.m. The recovery studies showed that digestion of tooth enamel with perchloric acid coupled with the method of standard addition is highly reliable.

The interest and encouragement of Professor G. S. Nixon in this work is gratefully acknowledged.

SUMMARY

Interferences in the atomic absorption of strontium by calcium and phosphate at the levels found in human tooth enamel were investigated for both air–acetylene and nitrous oxide–acetylene flames. In air–acetylene flames, the interferences can be reduced by the addition of lanthanum(III); but perchloric acid, used for sample dissolution, causes a significant reduction in the calcium–phosphate interference, so that a standard addition method can be applied, without the need for any chemical separations. An average recovery of 98.6% for added amounts of strontium to enamel showed the proposed method to be reliable.

RÉSUMÉ

On examine l'influence du calcium et du phosphate sur l'absorption atomique du strontium en quantité de l'ordre de celle présente dans l'émail dentaire humain. Ces essais ont été effectués dans des flammes air–acétylène et protoxyde d'azote–acétylène. En flammes air–acétylène, les interférences peuvent être réduites par addition de lanthane(III); cependant l'acide perchlorique utilisé pour la dissolution de l'échantillon réduit également l'interférence calcium–phosphate. On peut par conséquent utiliser une méthode avec addition d'étalon, sans qu'il soit nécessaire d'effectuer une séparation chimique. Cette méthode est satisfaisante; on retrouve en moyenne 98.5% du strontium ajouté à l'émail.

ZUSAMMENFASSUNG

Störungen bei der Atomabsorption von Strontium durch Calcium und Phosphat bei den Gehalten, die in menschlichem Zahnschmelz gefunden werden, wurden für die Luft–Acetylen- und für die Lachgas–Acetylen-Flamme untersucht. In Luft–Acetylen-Flammen können die Störungen durch Zugabe von Lanthan(III) vermindert werden; jedoch setzt Perchlorsäure, die für die Auflösung der Proben verwendet wird, die Calcium–Phosphat-Störung erheblich herab, so dass eine Standard-Zumischmethode ohne irgendwelche chemische Trennungen angewendet werden kann. Die Zuverlässigkeit der vorgeschlagenen Methode wurde durch zum Zahnschmelz hinzugegebene Strontiummengen belegt, die im Mittel zu 98.6% wiedergefunden wurden.

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ATOMIC-ABSORPTION SPECTROMETRIC DETERMINATION OF SILVER, BISMUTH AND CADMIUM IN SULFIDE ORES BY DIRECT ATOMIZATION FROM THE SOLID STATE

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In analysis for trace components in sulfide minerals and ores, the analyst is frequently compelled to decompose large amounts of sample and to perform tedious and time-consuming separation and concentration steps. The introduction of the furnace techniques of atomic absorption spectrometry has increased considerably the sensitivity of the method, and many trace elements of minerals and ores can now be determined rapidly and conveniently. However, practically all of the furnace methods for solid materials are still based on an atomization from the liquid state.

The main advantages of atomizations directly from the solid state are that time-consuming decompositions can be omitted, and that analyses can be made without the addition of reagents and without separation and/or concentration steps, so that the risk of losing the element to be determined or introducing contaminations is substantially reduced.

There appears to be nothing in the literature on the direct use of the furnace technique in the analysis of sulfide ores.

Reactions in the furnace

In the types of sulfide ores discussed here, trace amounts of silver, bismuth and cadmium may occur as separate minerals; however, they are more likely to be found in the lattices of the main sulfide minerals. The concentrations of the three elements are normally in the p.p.m. range. A detailed elucidation of the behaviour of sulfides during heating in the graphite furnace was found difficult; however, some general conclusions can be drawn.

In the temperature range 600–700°, the di- and polysulfides give off sulfur and a distinct smell of sulfur dioxide can be observed. At temperatures around 1500°, the monosulfides either decompose thermally or react with carbon. Sulfides high in iron, *e.g.* pyrite, give a residue (probably iron carbide) in the furnace.

EXPERIMENTAL

Apparatus

Measurements were made with a Perkin-Elmer 303 atomic absorption spectrophotometer equipped with a deuterium arc source background corrector. The construction of the graphite furnace (heated by a high-frequency induction

generator) has been described elsewhere[1]. The signals were registered with a 2-channel recorder; one of the channels plotted the peak (in percent absorption), and the other recorded the integrated peak area after a transformation of the percent absorption signals into absorbance by means of a logarithmic amplifier, and integration with a home-made electronic integrator.

Weighings were made with semi-micro or micro balances.

Reagents and standards

Standard solutions were prepared from high-purity metals and the acids were of "Suprapur" quality (E. Merck). The furnace was purged with argon (purity 99.99% by volume). Graphite of the quality employed for constructing the furnace was pulverized in an agate mortar and used as a dispersing agent.

Separate primary standard solutions (1000 p.p.m. of the metal) were prepared by dissolving the proper amounts of the metals in a small excess of nitric acid. Secondary standard solutions were prepared by dilution with water; sulfuric acid was added to the standard solutions prepared for analysis by the boat-technique in order to obtain a sulfate concentration corresponding to that of the sample solutions. The analysis by the latter method was based on pipetting a constant volume (0.4 ml) of sample or standard solutions into the tantalum boat.

For the determination of cadmium, a solid synthetic standard was prepared; the matrix contained 40.1% FeS, 10.5% ZnS, 2.4% PbS, 0.2% CuS and 18.3% S, and corresponded to the composition of the ASK sulfide ore. The components were mixed intimately by grinding in an agate mortar, cadmium sulfide was added, and, through two dilution steps, standards covering the range 150–453 p.p.m. cadmium were prepared. This solid synthetic standard was used in the analysis of samples high in cadmium, such as the ASK sulfide ore.

Samples low in cadmium, *e.g.* the SU-1 sulfide ore, were analyzed with the ASK reference schist as standard, the content of cadmium in the latter sample being known from earlier determinations[2].

Preparation and decomposition of the samples

The samples were normally ground manually in an agate mortar to pass a 270-mesh sieve (sieve opening 53 μm). As will be seen from Table III (on page 272), some determinations of cadmium in the SU-1 and ASK sulfide ores were made without grinding. The former sample had the following screen test[4]: -100 mesh, 93.34%; 100 mesh–65 mesh, 5.36%; $+65$ mesh, 1.30%. A screen test of the latter sample gave the following result: -250 mesh, 94.81%; 250 mesh–100 mesh, 5.00%; $+100$ mesh, 0.19%.

In connection with the determinations of cadmium, the following two series of decompositions were made.

Ten 0.2000-g portions of the ASK sulfide ore were transferred to polycarbonate 125-ml Erlenmeyer flasks equipped with plastic stoppers; 2.5 ml of concentrated hydrofluoric, 2.0 ml of hydrochloric and 2.0 ml of nitric acids were added, the flasks were stoppered, and the samples were decomposed by heating for 3 h on a boiling water bath. The solutions were finally diluted to 50 ml with water. A blank solution was prepared.

Five 0.5000-g portions of the SU-1 sulfide ore were transferred to 100-ml

polytetrafluoroethylene vessels with covers; 5.0 ml of concentrated hydrofluoric, 4.0 ml of hydrochloric and 4.0 ml of nitric acids were added, and the vessels were covered and kept on the boiling water bath for 2 h. The covers were removed, 2.0 ml of sulfuric acid were added and the contents were evaporated to dryness on a hot plate. The residues were dissolved by adding 5 drops of sulfuric acid and water, and the solutions were diluted to 50 ml with water. A blank solution was prepared.

For determinations of silver and bismuth, decompositions were made as follows.

Five 0.5000-g portions of the SU-1 and of the ASK sulfide ores were attacked in polytetrafluoroethylene-lined aluminium bombs with a mixture of 6.5 ml of concentrated hydrofluoric, 5.0 ml of hydrochloric and 5.0 ml of nitric acids; after heating for 1 h at $110 \pm 5°$, 0.5 ml of sulfuric acid was added, and the contents were evaporated to dryness. The residues were dissolved by adding 4.0 ml of sulfuric acid and water, and the solutions were diluted to 50 ml with water. A blank solution was prepared.

Measurement procedures

The settings of the spectrophotometer were adjusted as prescribed in the instrument manual. The measurements were made at the wavelengths: silver 328.1 nm, bismuth 223.1 nm, and cadmium 228.8 or 326.1 nm.

During all measurements with the furnace, the deuterium-arc background corrector was employed. The deuterium and hollow-cathode lamps were heated for 1 h before the measurements started.

A 1–10-mg portion of the solid sample, the sample–carbon mixture or the standard was weighed in a small tantalum scoop (produced by Perkin-Elmer), and the contents were placed in the middle of the graphite tube by means of a home-made adjustable insertion device; the scoop was reweighed, and the furnace was moved into its preadjusted position.

Preliminary investigations had shown that better signals from silver and bismuth were obtained by mixing the samples with equal amounts of carbon. In these analyses, 100 mg of pulverized sample and 100 mg of carbon were ground and intimately mixed in an agate mortar. Cadmium was determined without preliminary mixing with carbon.

Two slightly different standardization procedures were employed in the analysis of solid samples with solid standards. When a solid sample was measured against a natural standard, varying amounts of the latter were atomized to ensure that the element gave a straight or nearly straight calibration curve; five sets of measurements of integrated peak areas were then made, each set consisting of one measurement of the sample and one of the standard.

In those instances where the solid sample was measured against a solid synthetic standard, five sets of determinations were also made; each set consisted of one atomization of the sample and two of the standard, one to give a slightly higher, and the other a somewhat lower integrated peak area than the sample.

With the use of syringes, 5–25 μl of the liquid samples or standard solutions were introduced through the radial opening of the furnace. It is recommended that the determinations should be based on the principle of adding

a constant volume of sample or standard solution, the volume of which should not exceed 25 μl. When the standard addition technique was applied, the mixtures were prepared before being introduced into the furnace.

The drying and atomization procedures for solid samples were as follows: for silver, drying at 60 V (250°) for 90 s, atomization at 240 V (1850°) for 60 s, cleaning of the graphite tube at 260 V (1950°) for 60 s; for bismuth, no drying, atomization at 180 V (1400°) for 60 s, cleaning as for silver; for cadmium, drying as for silver, atomization at 220 V (1700°) for 45 s, cleaning as for silver.

For solutions, the furnace procedures were as follows: for silver, evaporation at 40 V (120°) for 90 s, and further procedure as above; for bismuth, evaporation at 40 V for 90 s and at 70 V (350°) for 90 s, atomization at 210 V (1650°) for 60 s, cleaning as above; for cadmium, evaporation as for silver, and the further procedure as for the solid samples.

The analyses by the boat technique were made by pipetting 0.40 ml of the sample or the appropriate standard solution into the boat and evaporating and atomizing in the acetylene–air flame. The standardization was based on plotting a calibration curve.

Silver in the SU-1 sulfide ore, and cadmium in the ASK sulfide ore, were also determined by conventional flame techniques.

RESULTS

Tables I–III give the data obtained from the analysis of various sulfide ores. The Canadian reference sulfide ore SU-1 is representative of the Sudbury

TABLE I

DETERMINATION OF SILVER IN ORES

(Five analyses of each sample were made. Results are given in p.p.m.)

Sample	*By direct atomization in the furnace*			*By atomizing the sample solution*					
				in the furnace			*in the flame*		
	$\bar{x}$[a]	s	s_r	$\bar{x}$	s	s_r	$\bar{x}$	s	s_r
SU-1 sulfide ore	4.1	0.3	7	4.1	0.6	15	5	1	20
ASK sulfide ore	17.5[b]	—	—	19	2	8	16	1	6
Ore 1									
Olavsgruben, Røros	6.7	0.2	3	—	—	—	—	—	—
Ore 2									
Killingdal	24.3	0.7	3	—	—	—	—	—	—
Ore 3									
Mofjell	9.4	0.7	7	—	—	—	—	—	—
Ore 4									
Sulitjelma	7.8	0.2	3	—	—	—	—	—	—
Ore 5									
Løkken	7.9	0.3	4	—	—	—	—	—	—

[a] $\bar{x}$, average; s, standard deviation; s_r, relative standard deviation.

[b] The ASK sulfide ore was employed as the solid standard.

nickel–copper ores, its major sulfide minerals being pyrrhotite, pentlandite and chalcopyrite. The Nordic reference sample ASK and the other five samples are representative Norwegian sulfide ores containing pyrite, pyrrhotite, chalcopyrite, sphalerite and galena.

The ASK sulfide ore was used as the solid standard, when the content of silver was determined in SU-1 and the five Norwegian ores by the direct-atomization technique. The value 17.5 p.p.m. silver in the ASK standard is the average of results from atomizing sample solutions in the flame and in the furnace; this mean was also supported by values (ranging from 16 to 25 p.p.m., average 19 p.p.m.) obtained by different methods in various Nordic laboratories[3].

For the SU-1 ore the value 4.1 p.p.m. silver by the direct-atomization technique compares favourably with the results from the two other methods used in the present investigation, and with previous data[4] (4 p.p.m. as the average of 17 values, mainly by optical spectrography).

It is interesting to note that the precision of the direct-atomization method for silver is the same as, or better than, that of the two other methods employed here.

The determinations of bismuth were based on the use of the ASK sample as the solid standard. The bismuth in the ASK sulfide ore could also be determined by atomizing sample solutions in the furnace; the concentrations were too low to permit determinations by the conventional flame technique; however, analysis by the boat technique was possible.

The two series of analyses of solutions of the ASK sulfide ore gave the average 32 p.p.m. Bi (previous values[3] of 27,40 and 34 p.p.m. were obtained by X-ray spectrography, mass spectrometry and optical spectrography, respectively).

TABLE II

DETERMINATION OF BISMUTH IN ORES

(Five analyses of each sample were made. Results are given in p.p.m.)

Sample	*By direct atomization in the furnace*			*By atomizing the sample solution*					
				in the furnace			*by the boat technique*		
	$\bar{x}^a$	s	s_r	$\bar{x}$	s	s_r	$\bar{x}$	s	s_r
SU-1 sulfide ore	2.8	0.2	7	—	—	—	5.0	0.7	14
ASK sulfide ore	32[b]	—	—	31	4.5	15	33	2	7
Ore 1 Olavsgruben, Røros	3.0	0.2	6	—	—		—	—	—
Ore 2 Killingdal	35	2.2	6	—	—	—	—	—	—
Ore 3 Mofjell	5.2	0.3	5	—	—	—	—	—	—
Ore 4 Sulitjelma	3.9	0.1	3	—	—	—	—	—	—
Ore 5 Løkken	3.0	0.1	3	—	—	—	—	—	—

[a,b] See Table I.

The value for the content of bismuth in the SU-1 sulfide ore obtained by direct atomization is not in good agreement with the result obtained by the boat technique; however, both fall within the range of previous data[4]. It should be noted that in the present analyses the boat technique was used near its detection limit; as can be seen from Table II, this resulted in a relatively poor precision.

TABLE III

DETERMINATION OF CADMIUM IN ORES

(Results are given in p.p.m. Five analyses of each sample were made, except where mentioned)

Sample	*Methods of atomization and standardization*	*Results*		
		$\bar{x}^a$	s^a	$s_r{}^a$
SU-1 sulfide ore	by atomizing the solid sample and the solid standard ASK schist in the furnace[b]	1.6	0.2	14
	by atomizing the solid sample and a solid synthetic standard in the furnace[c,d]	1.8	0.8	42
	by atomizing solutions in the furnace according to the standard addition technique	2.1	0.3	16
	by atomizing separately the solid sample and cadmium standard solutions in the furnace[c]	1.7	0.7	40
ASK sulfide ore	by atomizing the solid sample and a solid synthetic standard in the furnace[c,d]	286	47	16
	by atomizing sample and standard solutions in the flame[c,e]	297	2.8	0.9

[a] See Table I.
[b] Content of cadmium in the solid standard, 0.87 p.p.m. Sample ground to pass a 270-mesh sieve.
[c] Sample not ground (for screen tests, *vide supra*).
[d] Measured at the less sensitive cadmium line at 326.1 nm, all other measurements were made at 228.8 nm.
[e] 9 analyses were done.

As is apparent from Table III, cadmium was only determined in the samples SU-1 and ASK; the concentration of the metal in the SU-1 ore was too low to permit a determination by the flame technique. Various atomization and standardization methods were applied, these also involving the use of a solid synthetic standard.

The four standard deviations and the four averages tabulated for SU-1 were compared by statistical methods, the χ^2-test and analysis of variance, respectively. The tests demonstrated that the differences originated from random errors. From the four averages for SU-1, a weighted mean (based upon the inverse value of the variances) was calculated to be 1.75 p.p.m. Cd; the standard deviation of this weighted mean was found to be ± 0.08 p.p.m. Cd.

As is apparent from a compilation of previous data[4], the agreement between earlier values for cadmium in the SU-1 sulfide ore is less than satisfactory. The present values for cadmium in the ASK sulfide ore are somewhat lower than previous data[3], which were in the range 304–350 p.p.m.

SUMMARY

Atomic absorption spectrometric determinations of silver, bismuth and cadmium in the Canadian SU-1 and the Nordic ASK reference sulfide ores, and in a series of Norwegian sulfide ores of technical importance, were carried out by atomizing the elements directly from the solid state. Atomizations were made in a high-frequency induction-heated graphite furnace. For comparison purposes, samples were also decomposed, and analyses made by atomizing sample solutions in the furnace and in the flame.

RÉSUMÉ

Des dosages spectrométriques par absorption atomique ont été effectués pour l'argent, le bismuth et le cadmium dans des minerais sulfurés, par atomisation des éléments, directement de l'état solide. Un four de graphite chauffé par induction à haute fréquence est utilisé pour les atomisations. Pour comparaison, les échantillons ont également été analysés avec mise en solution et atomisation dans le four et dans la flamme.

ZUSAMMENFASSUNG

Atomabsorptionsspektrometrische Bestimmungen von Silber, Wismut und Cadmium in den Canadian SU-1 und den Nordic ASK Vergleichs-Sulfiderzen sowie in einer Reihe norwegischer Sulfiderze von technischer Bedeutung wurden ausgeführt, indem die Elemente direkt aus dem festen Zustand atomisiert wurden. Hierzu diente ein Graphitofen, der durch Hochfrequenzinduktion erhitzt wurde. Für Vergleichszwecke wurden die Proben auch aufgelöst und durch Atomisierung der Lösungen im Ofen und in der Flamme analysiert.

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ON-STREAM ATOMIC-ABSORPTION DETERMINATION OF ZINC AND MANGANESE IN FLOTATION LIQUORS CONTAINING CALCIUM SULPHATE*

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In flotation plant practice there is a need to find suitable control parameters so that reagent additions can be controlled to give optimal results. For example, zinc sulphate is sometimes added to depress zinc sulphide minerals (sphalerite and marmatite) during lead flotation, and copper sulphate is almost universally used to activate sphalerite and marmatite for satisfactory flotation. These reagents are relatively expensive, and in present practice there is no convenient way of determining the minimal or optimal addition. It is common to keep the addition rate at a predetermined constant level, although control algorithms have been used at Tennessee Copper Corporation[1].

When copper sulphate (as either a solid or a solution) is added to an aqueous suspension of sphalerite, copper deposits on the mineral surface and zinc goes into solution[2]. It has been found that with marmatite from Broken Hill, N.S.W., Australia, the addition of copper sulphate liberates manganese, as well as zinc, even in alkaline solution[3]. It was found by analysis of spot samples from an operating plant that the concentration of zinc (0.3–0.6 mg l^{-1}) and of manganese (2–7 mg l^{-1}) in solution varied throughout a shift. Different values were obtained in other plants.

It was considered desirable to develop a method for continuous monitoring of solution, because additional data might give a useful insight into the performance of the plant and might lead to development of a parameter for control of zinc sulphate or copper sulphate additions. It was thought that atomic absorption spectrometry, which had been used in the initial plant survey, could be adapted for continuous on-stream monitoring.

Flotation plant liquors are commonly very high in dissolved salts (up to 15 g l^{-1}) and are often saturated with calcium sulphate. This paper describes the development of a technique to cope with interference caused by calcium sulphate present in the solution.

EXPERIMENTAL

Equipment

A Varian-Techtron Model AA1000 spectrometer was used. Zinc absorbance

* An extended abstract of this paper was presented at the 4th International Conference on Atomic Spectroscopy, Toronto, Canada, October 1972

was measured at 213.9 nm, and manganese absorbance at 275.9 and 403.1 nm. Absorbances were recorded on a Leeds & Northrup Speedomax H recorder.

An air–acetylene flame (9 l min^{-1} of air and 1 l min^{-1} of acetylene) was used for zinc and manganese determinations. Acetylene was supplied from 5.6-m^3 cylinders in laboratory and in field trials. After about 24 h of continuous operation, or when the cylinder pressure dropped below 400 kPa, the cylinder was changed as a routine precautionary measure to avoid any signal enhancement caused by acetone pick-up.

The air supply line was fitted with two pressure-reducing valves in series to eliminate the effect of variable air pressure in the line. This was satisfactory in the plant as well as in the laboratory. A line filter was included to remove any solids in the air.

A Varian-Techtron high-solids burner was used. In some early work it was found that salt bridging tended to occur more readily at points where there were machining grooves on the inside of the burner slot. These grooves were removed by polishing and this gave less, and more random, bridging. Care was taken not to enlarge the slot width much above the installed value of 0.68 mm, to avoid possible burn-back of the acetylene.

A Varian-Techtron variable nebulizer was used in some of the work, but a variable nebulizer[4] supplied by Dr. J. B. Willis (CSIRO Division of Chemical Physics) was found to be more satisfactory. An uptake rate of 4 ml min^{-1} was used for most of the runs.

A spray chamber of improved design, machined from solid Teflon, was supplied by Varian-Techtron during the work. This chamber had a 5° slope and had been sand-blasted to improve its drainage characteristics.

In long-term laboratory runs, and in plant trials, apparatus was assembled for continuous addition at a constant rate of an EDTA–sodium hydroxide solution to a constant flow of the test solution, as shown in Fig. 1.

Test solutions were obtained from a small on-stream filter in the plant, or from a large bottle in the laboratory, and were pumped to a constant head tank. In the plant the overflow from this tank went to waste; in the laboratory the overflow was pumped back to the head tank by a Gorman-Rupp oscillating pump, but as this tended to warm the solution during constant circulation, the recycled solution was cooled in a condenser.

The height of the constant head tank above the mixing vessel was adjusted so that 20 ± 0.1 ml min^{-1} of liquor gravitated to the mixing vessel. Alkali–EDTA solution of the required strength (*e.g.* 190 g l^{-1} for EDTA·Na_2 and 20 g l^{-1} for sodium hydroxide) was pumped to the mixing vessel at the rate of 0.7 ml min^{-1}. The dilution of 3% caused was ignored. Test liquor and alkali–EDTA solution were mixed by a magnetic stirrer and the overflow from the mixing vessel gravitated to a feed cell in which was inserted the capillary pick-up tube from the a.a.s. unit. Overflow from this cell went to waste. All vessels were made from Pyrex glass and were connected by Pyrex or PVC tubing.

Reagents and solutions

Plant liquors were used in the initial and final stages of the work. However, at other times it was convenient to use solutions made from AR reagents. For

some of the work a synthetic solution was made up to the following analysis to approximate an actual plant solution: Ca^{2+}, 770 mg l^{-1}; Mg^{2+}, 210; Na^{+}, 408; Cl^{-}, 1500; SO_4^{2-}, 1540. Various amounts of zinc sulphate and manganese sulphate were added to give the desired concentrations of manganese and zinc.

Commercial grade samples of sodium hexametaphosphate, Cyquest 3223 (an acrylic polymer, termed an anti-precipitant, from American Cyanamid Co.), and Teepol (a surface-active agent from Shell Chemical (Australia) Pty Ltd) were used in some runs.

Procedures

Experimental procedure for the study of the stability of manganese and zinc absorbance was usually relatively simple. After warm-up and adjustment of the spectrometer, the desired solution was aspirated either from a beaker or from the feed cell G shown in Fig. 1, and the absorbance was recorded. If the recorder trace was a straight line, the run was continued for an appropriate period. If the trace showed periodic variations or was erratic, indicating incipient nebulizer blockage (as explained later), the run was simply continued until sufficient data were obtained, or the nebulizer blocked completely. If complete blockage occurred, the nebulizer was cleaned.

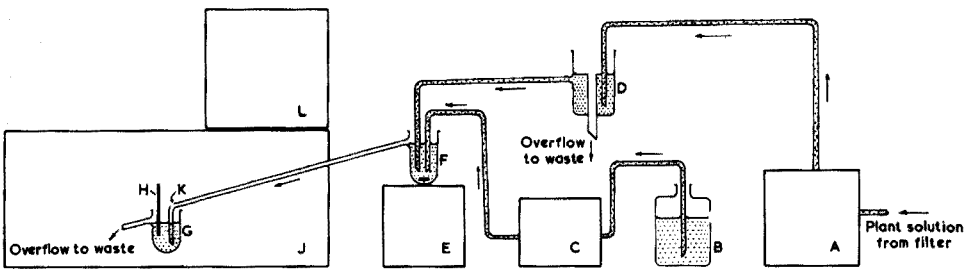

Fig. 1. Schematic diagram of equipment and technique for addition of EDTA·Na_2–NaOH solution to plant solution for continuous a.a.s. measurement. A, pump for feeding plant solution (Watson Marlow MHRE/200); B, reservoir of EDTA·Na_2–NaOH solution; C, pump for metering EDTA·Na_2–NaOH solution (Scientific Instruments No. 403); D, constant head tank; E, magnetic stirrer; F, mixing vessel; G. a.a.s. feed cell; H. a.a.s. uptake capillary; J. atomic absorption spectrometer (Varian Techtron AA1000); K. air vent to ensure continuous flow; L. Leeds & Northrup Speedomax H recorder.

Salting-up of the burner slot occurred in some runs. The salt deposit could usually be removed by scraping with a long-handled stainless steel scraper while the flame was still burning, but sometimes the burner was removed for scrubbing with a stiff bristle brush.

For plant solutions, and other solutions of unknown composition, the "total hardness", *i.e.* the calcium, magnesium, zinc, and manganese present, was determined by a variation of the method of West and Sykes[5]. An aliquot of the test solution in an ammonium hydroxide–ammonium chloride buffer was titrated with standard EDTA·Na_2 solution, using as an indicator 0.2 g of a uniformly ground dispersion of 0.5 g of eriochrome black T and 0.2 g of dimethyl yellow in 100 g of sodium chloride.

Because additions of EDTA·Na_2 to a calcium sulphate solution reduce the

pH, it was necessary in some work to know the amount of alkali required to return the solution pH to its original value. This amount was determined by adding the required amount of EDTA·Na_2 and back-titrating with standard sodium hydroxide solution to a potentiometric end-point.

A plant trial was conducted in the lead–zinc flotation concentrator of New Broken Hill Consolidated Ltd (NBHC) at Broken Hill, Australia. The spectrometer unit and most auxiliary equipment were air-freighted to the plant; services and some equipment were provided by NBHC. The equipment was located near the sampling point to reduce the time lag between sampling and measuring to about 1 min. Clear solution for analysis was obtained from an on-stream filter.

Calibration curves were prepared over the following ranges of metal concentration: 0–200 mg l^{-1} for manganese at the 403.1-nm line; 0–15 mg l^{-1} for manganese at the 279.5-nm line; 0–5 mg l^{-1} and 0–50 mg l^{-1} for zinc at the 213.9-nm line. For the latter zinc curve, the burner was angled at 45°, but otherwise it was kept parallel to the light path.

For standardization of the atomic-absorption spectrometer for the particular field trial reported here, solutions containing 10 mg of manganese and 2 mg of zinc per litre were prepared with EDTA present. For manganese standardization the manganese solution was aspirated into the flame, and the horizontal position of the burner was adjusted so that the absorbance was 0.5. For zinc standardization the burner position was adjusted so that the absorbance was 0.4. This was found to be a convenient way of obtaining reproducible absorbances, and enabled the recorder to be direct-reading over at least half the scale, which was more convenient than the more usual practice of maximizing the absorbance reading for a given metal concentration.

RESULTS AND DISCUSSION

Dilute solutions of zinc or manganese sulphate in water could be aspirated

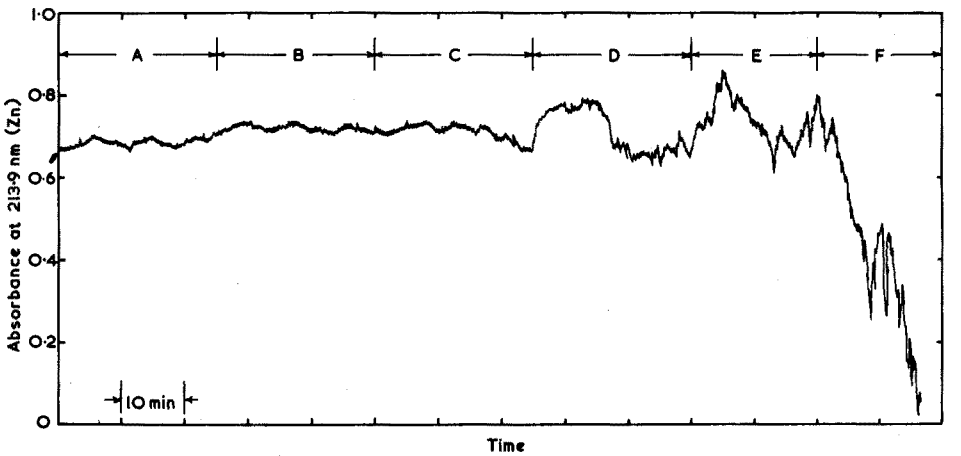

Fig. 2. Effect of various sulphate additions on absorbance at 213.9 nm of a solution containing 23 mg Zn l^{-1} (as sulphate) and 1200 mg Ca l^{-1} (as chloride), with the burner set at about 45° to the light path. Mole ratios SO_4^{2-}:Ca^{2+} in the sections of the diagram as follows: A, 0.028:1; B, 0.083:1; C, 0.167:1; D, 0.25:1; E, 0.333:1; F, 0.833:1.

for long periods, and steady absorbance readings were obtained. The recorder trace, even with minimal damping from the spectrometer circuit, was typically constant within ± 0.02 absorbance units.

When actual or synthetic plant solution was aspirated the recorded absorbance was extremely variable or erratic, as in Fig. 2, section F, and after a few minutes the absorbance dropped to zero. No solution was then being aspirated, and air was forced out of the capillary uptake tube.

Examination of the nebulizer showed that a white deposit had blocked the orifice. This deposit could not be removed with the fine wire supplied with the spectrometer. It could be at least partly removed by screwing the nebulizer adjustment screw in and out several times. The nebulizer then aspirated water at the initial uptake rate, but on re-aspirating plant solution the uptake rate again became zero within a few minutes.

The deposit could not be removed by aspirating dilute or concentrated hydrochloric acid or organic solvents such as alcohol. It was difficult to remove also by scraping, without damaging the nebulizer, and the amount obtained in this way was too small for positive identification. However, it was considered that the material was probably mainly calcium sulphate.

Effect of calcium sulphate

In order to check the effect of calcium sulphate or the sulphate:calcium ratio, the solutions listed in Table I were used, *i.e.* three calcium levels and a range of sulphate concentrations. Zinc sulphate was used to provide a convenient absorbance, and the burner was angled at about 45° to the light path to give an absorbance about mid-scale, *i.e.* 0.4–0.6 absorbance. Part of the recorded absorbances are shown in Fig. 2.

It is evident from Table I that although straight recorder traces were obtained for 400 and 800 mg Ca l^{-1} at very low sulphate concentrations, as the amount of sulphate was increased the quality of the signal deteriorated. At the 400 mg Ca l^{-1} level, the nebulizer was liable to become blocked at sulphate: calcium molar ratios above 1:1. At higher calcium levels, the nebulizer was liable to become blocked at sulphate:calcium ratios above about 0.3:1, but the time required was variable.

Although the recorder trace was often erratic, it was possible, by visual observation of the needle on the read-out meter of the spectrometer for the few seconds normally employed, to obtain an estimate of absorbance within the limits of accuracy normally expected. That is, for an observation period of 3–4 s, only normal needle "flutter" was observed. However, the recorder trace shows that even under the best conditions serious errors would be involved because a normal observation period could give an absorbance value almost anywhere on the recorder trace shown.

These results did not completely delineate the concentration regions in which calcium sulphate caused blockage of the nebulizer, but it was evident that for on-stream application to plant solutions, the interference would have to be eliminated.

Effect of complexing calcium

It was thought that the effect of calcium sulphate could possibly be

eliminated by complexing the calcium, or by retarding the rate of precipitation, or by using a surface-active agent. Results obtained with these types of reagents are summarized in Table II.

TABLE I

TYPES OF RECORDER TRACE OBSERVED FOR VARIOUS Ca^{2+} AND SO_4^{2-} CONCENTRATIONS AND RATIOS

(23 mg Zn l^{-1} present as sulphate)

Ca^{2+} (mg l^{-1})[a]	*SO_4^{2-} (mg l^{-1})*[a]	*Molar ratio SO_4^{2-}:Ca^{2+}*[a]	*Observed recorder trace*
400	33	0.035:1	straight
400	273	0.285:1	erratic
400	513	0.535:1	erratic
400	753	0.785:1	erratic
400	1470	1.54:1	very erratic
400	2430	2.54:1	very erratic
400	4830	5.04:1	very erratic
800	33	0.018:1	straight
800	273	0.143:1	erratic
800	513	0.268:1	erratic
800	753	0.393:1	very erratic
800	993	0.518:1	very erratic
800	2430	1.27:1	very erratic
800	4830	2.52:1	very erratic
1200	33	0.012:1	cycling
1200	273	0.092:1	cycling
1200	513	0.182:1	cycling
1200	753	0.262:1	erratic
1200	993	0.342:1	very erratic
1200	2430	0.842:1	very erratic

[a] Calculated assuming 100% purity for $CaCl_2$, Na_2SO_4 and $ZnSO_4 \cdot 7H_2O$.

TABLE II

EFFECT OF VARIOUS ADDITION REAGENTS ON STABILIZATION OF ABSORBANCE READINGS

(23 mg Zn l^{-1} as sulphate with 1200 mg Ca l^{-1} as chloride)

Reagent	*Reagent type*	*Reagent concentration*	*Effect on absorbance reading*
EDTA·Na_2	chelating	1:1 molar[a]	stabilized
EDTA·Na_4	chelating	1:1 molar[a]	stabilized
$(NaPO_4)_6$	complexing	1:1 molar[a]	not stabilized
Cyquest 3223	anti-precipitant	0.005%	not stabilized
Teepol	surface-active (wetting)	0.005%	not stabilized

[a] Ca^{2+}:reagent = 1:1 molar.

The disodium and tetrasodium salts of EDTA were effective in chelating the calcium and giving stable absorbance readings, whereas the other reagents were not effective under the conditions used. Because EDTA·Na_2 is more readily available than EDTA·Na_4, it was used in further work.

West and Sykes[5] reported that the optimal pH for formation of the calcium–EDTA complex is 7.5, although some complexing takes place at lower or higher pH values. Skewes[6] used EDTA to complex calcium in cyanide solutions at pH 11, because calcium or calcium carbonate was thought to hinder free atomization in the spray chamber. In the present work it was necessary to avoid raising the pH much above the original value because a precipitate formed, *i.e.* a metal hydroxide or MnO_2.

Figure 3 shows the effect of various addition ratios of EDTA at various pH values to a synthetic plant solution. With no EDTA, at the natural pH of 6.4, the nebulizer became partly blocked in 10 min, as shown by the decrease in absorbance (Fig. 3A), and would have become completely blocked if the run had not been stopped.

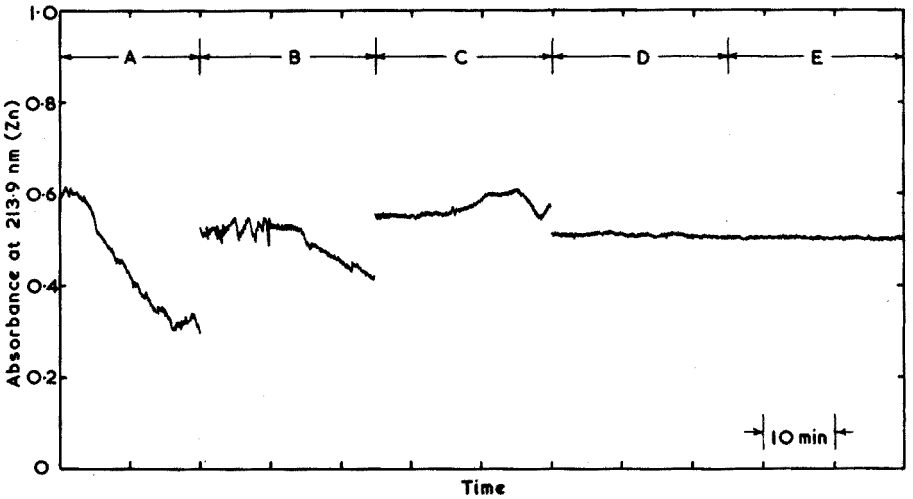

Fig. 3. Effect of EDTA·Na_2 additions on absorbance at 213.9 nm of a synthetic plant solution containing 23 mg Zn l^{-1} (as sulphate) and 1200 mg Ca l^{-1} (as chloride) with the burner set at about 45° to the light path. Mole ratios EDTA·Na_2:Ca^{2+}, and pH in the sections of the diagram as follows: A, 0:1 (pH 6.4); B, 1:1 (pH 3.6); C, 2:1 (pH 3.6); D, 1:1 (pH 6.5); E, 0.5:1 (pH 7.0).

Because addition of EDTA alone would have been simplest for on-stream runs, a trial was run with a 1:1 molar addition ratio of EDTA:Ca+Mg. This reduced the pH to 3.6, so only partial chelation occurred and the results were not satisfactory (Fig. 3B). Doubling the EDTA addition at pH 3.6 gave temporary improvement, but then the signal quality deteriorated (Fig. 3C).

By adding EDTA to give a molar ratio of EDTA:Ca+Mg of 1:1, and returning the pH to 6.5 with sodium hydroxide, a steady absorbance was obtained over a 1.5-h period (only 25 min are shown in Fig. 3D). After 1.5 h, salt deposition began to occur in the burner slot because of the relatively high total salt concentration.

With the aim of minimizing salt deposition, the EDTA·Na_2 addition was decreased to a molar ratio of EDTA:Ca + Mg of 0.5:1 and the pH was maintained at 7.0 with sodium hydroxide. This gave a steady recorder trace for 2 h (a 25-min period is shown in Fig. 3E), and was effective in reducing salting-up of the burner slot.

Effect of EDTA additions to plant solution

For a plant solution, or other solution of unknown composition, it was found that the "total hardness" determination mentioned earlier gave a satisfactory estimate of EDTA requirements for complexing the metals. The amount to be added to the solution was then half that determined by titration. This was obviously much more convenient than analysing for all metals present and calculating the EDTA requirement. The alkali requirement was determined by the method noted previously.

Additions of the reagent quantities determined in this way to a sample of plant solution enabled the solution to be aspirated successfully. In a long-term trial, in which continuous plant operation was simulated by means of an apparatus similar to that shown in Fig. 1, a steady recorder trace was obtained for a 6-h period. From time to time, salt deposition in the burner gave a ragged flame and irregular absorbance, but this was rectified by scraping the burner slot while still aspirating solution into the flame.

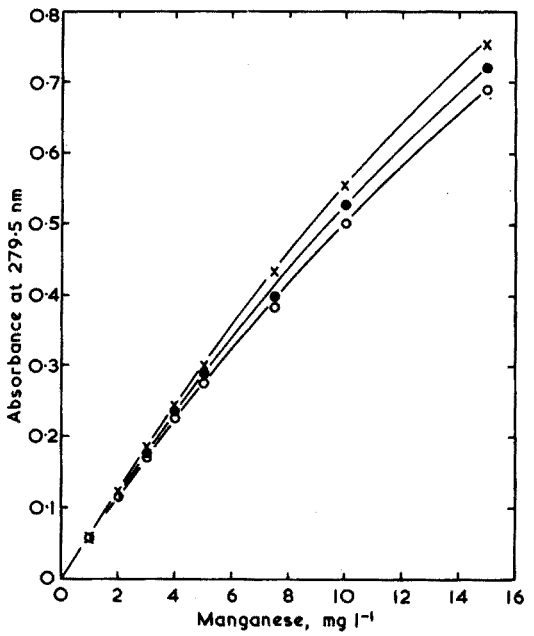

Fig. 4. Calibration curves for absorbance at 279.5 nm of solutions of manganese (as sulphate). (×) In water; (○) in synthetic plant solution with an EDTA addition equivalent to half the molar concentration of Ca + Mg, and NaOH to give pH 7; (●) in synthetic plant solution with an EDTA addition equivalent to twice the molar concentration of Ca + Mg, and NaOH to give pH 7.

Effect of EDTA on absorbance

Angino and Billings[7] have reported conflicting results for various impurities on the determination of zinc and manganese by atomic absorption spectrometry. In order to check the effect, if any, of calcium, magnesium, sulphate and EDTA on absorbance values, various calibration curves were determined.

Figure 4 shows the curve for 0–15 mg Mn l^{-1} in water, together with a curve for manganese in synthetic plant solution containing the normal addition of EDTA (*i.e.* half the molar equivalent of the manganese, calcium, and magnesium present) plus sodium hydroxide to adjust the pH, and points for manganese in synthetic solution containing four times the normal EDTA solution (*i.e.* twice the molar equivalent of the metals present). A curve for manganese in synthetic plant solution alone could not be prepared, of course, because of nebulizer blockage. The results in Fig. 4 show that straight-line calibrations were obtained up to 5 mg Mn l^{-1} and that only small errors were involved in drawing a straight line up to 10 mg Mn l^{-1}. The addition of EDTA to a plant solution attenuated the absorbance signal slightly, but there was not much difference between additions equivalent to 0.5 and 2.0 times the molar concentration of calcium. However, it was desirable for plant trials to standardize the system with manganese solutions containing EDTA.

Figure 5 shows the results of similar calibrations for 0–5 mg Zn l^{-1}.

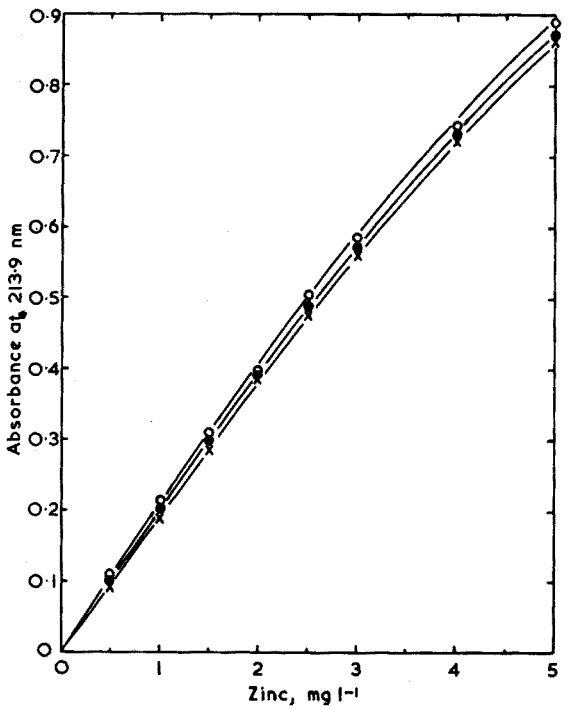

Fig. 5. Calibration curves for absorbance at 213.9 nm of solutions of zinc (as sulphate). (×) In water; (○) in synthetic plant solution with an EDTA addition equivalent to half the molar concentration of Ca + Mg, and NaOH to give pH 7; (●) in synthetic plant solution with an EDTA addition equivalent to twice the molar concentration of Ca + Mg, and NaOH to give pH 7.

Straight lines can be drawn up to about 3.5 mg Zn l^{-1}. The addition of EDTA enhanced the absorbance signal. Similar types of curve were obtained for solutions containing 0–200 mg Mn l^{-1} (at 403.1 nm) and for 0–50 mg Zn l^{-1} (with an angled burner). These results showed that manganese and zinc could be determined satisfactorily in plant solutions in the presence of EDTA.

Results of plant trial

The equipment was set up in the NBHC concentrator and, at different times, continuous samples were obtained from the final flotation cell in the lead section (termed deleaded tailing) and from the first conditioner before zinc flotation *i.e.* after the addition of copper sulphate to the pulp. Sufficient alkaline EDTA solution was prepared to cover the estimated period of determination. Typically, to 20 ml min^{-1} of clear plant liquor an addition of 0.7 ml min^{-1} of a solution which was 187.5 g l^{-1} in EDTA·Na_2 and 20 g l^{-1} in sodium hydroxide was made.

The system shown in Fig. 1 operated satisfactorily without constant attention under the non-ideal plant conditions which included a floor subject to vibration and an unprotected area. The short chimney on the spectrometer unit was found to be adequate in shielding the flame from draughts. Although the calcium concentration of the samples probably varied by about 5% throughout the period of measurement, as shown in previous work[3], and the pH of the plant sample varied from 6.9 to 8.8, the alkaline EDTA addition coped satisfactorily with these variations.

Salt bridging of the burner occurred after about 3 h of operation. By regular attention, this deposit could sometimes be removed by scraping the slot while continuing the operation. At other times it was found desirable to remove the burner and scrub it with a brush. On reaspirating solution, it was found that the absorbance of the standard manganese solution was identical with that at the start of the run.

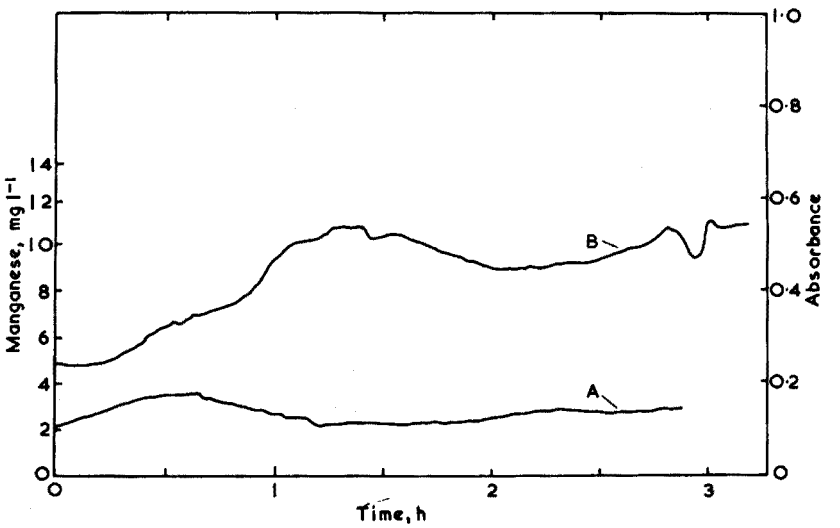

Fig. 6. Recorded concentration of manganese in solution at New Broken Hill Consolidated Ltd. A, in deleaded tailing (recorded 1972-06-28); B, in first zinc conditioner (recorded 1972-06-29).

Measurements of manganese and zinc were made, and some preliminary results were obtained for copper. Manganese measurements were most informative, and part of the results are shown with a condensed time scale in Fig. 6. It can be seen that the manganese concentrations in solution are much higher in the zinc conditioner than at the end of lead scavenging. At both locations the concentrations changed with time. Changes in deleaded tailing were gradual, whereas those in the zinc conditioner were more rapid and of greater magnitude. Similar results were obtained at these locations at different times.

These changes were caused by changes in the material being processed or by changes in operation of the plant, and were broadly similar to those reported previously[3] for grab samples, thus confirming the earlier work. However, more reliance can be placed on the results from continuous measurement.

CONCLUSIONS

This work has shown that manganese and zinc, and probably other metals, can be determined continuously by atomic absorption spectrometry in a plant liquor containing calcium sulphate provided that EDTA is added at controlled pH. At least 3 h of continuous operation can be obtained before burner salt-up occurs, but efforts can be directed to increase this time of operation.

Because the technique can give continuous determination of metals in solutions high in calcium sulphate and other dissolved salts, it should be valuable in plant and laboratory pulp chemistry investigations, particularly in activation and depression studies.

The authors would like to thank Dr. J. B. Willis of CSIRO Division of Chemical Physics for his advice and for lending them a variable nebulizer. They would also like to thank the management and staff of New Broken Hill Consolidated Ltd for their help during the field trial and for permission to publish the plant results obtained.

SUMMARY

Flotation studies require continuous monitoring of metal concentrations in solution, *e.g.* manganese in the 2–7 mg l^{-1} range and zinc in the 0.3–0.6 mg l^{-1} range in plant solutions that may contain up to 15 g l^{-1} of dissolved salts and be saturated with calcium sulphate. With this calcium sulphate, the nebulizer of an atomic absorption spectrometer quickly becomes completely blocked; the effect is worst at high calcium concentrations (1200 mg l^{-1}) and at high sulphate:calcium molar ratios (1:1). By adding EDTA · Na_2 at controlled pH (about 7) to complex the metals present, precipitation is prevented and zinc and manganese can be determined. EDTA has little effect on the absorbance values. In on-stream application, with continuous addition of alkaline EDTA solution to flotation plant liquor, continuous monitoring for manganese and zinc is possible for at least 3 h, before salt-bridging of the burner occurs.

RÉSUMÉ

La flottation de plantes nécessite un contrôle continu des concentrations

métalliques en solution, par example: 2–7 mg l^{-1} pour le manganèse et 0.3–0.6 mg l^{-1} pour le zinc dans des solutions de plantes pouvant contenir jusqu'à 15 g l^{-1} de sels dissous et saturées en sulfate de calcium. En présence de telles quantités de sulfate de calcium, le nébuliseur du spectromètre d'absorption atomique se bouche très rapidement. On peut éviter cette précipitation de sulfate de calcium, par addition d'EDTA·Na_2 à pH contrôlé (environ 7) pour complexer les métaux présents. Le zinc et le manganèse peuvent alors être dosés, l'EDTA n'ayant que peu d'influence sur les valeurs d'absorption. Il est possible de contrôler ainsi en continu les teneurs en manganèse et en zinc, pendant au moins 3 h.

ZUSAMMENFASSUNG

Flotationsuntersuchungen erfordern eine kontinuierliche Kontrolle der Metallkonzentrationen in Lösung, z.B. Mangan im Bereich 2–7 mg l^{-1} und Zink im Bereich 0.3–0.6 mg l^{-1} in Betriebslösungen, die bis zu 15 g l^{-1} an gelösten Salzen enthalten und mit Calciumsulfat gesättigt sein können. Dieses Calciumsulfat blockiert den Zerstäuber eines Atomabsorptionsspektrometers schnell; der Effekt ist besonders schlimm bei hohen Calciumkonzentrationen (1200 mg l^{-1}) und bei hohen Molverhältnissen Sulfat:Calcium (1:1). Durch Zugabe von EDTA·Na_2 bei kontrolliertem pH-Wert (etwa 7) werden die vorliegenden Metalle komplexiert und dadurch eine Fällung verhindert, so dass Zink und Mangan bestimmt werden können. EDTA hat nur einen geringen Einfluss auf die Extinktionswerte. Bei Anwendung auf strömende Lösungen mit kontinuierlicher Zugabe alkalischer EDTA-Lösung zu der Flüssigkeit der Flotationsanlage ist eine ununterbrochene Kontrolle der Mangan- und Zinkkonzentrationen für mindestens 3 h möglich, bevor der Brenner durch Salz verstopft wird.

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INTERFERENCE BY ACIDS IN THE DETERMINATION OF MOLYBDENUM BY ATOMIC ABSORPTION SPECTROMETRY

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Conflicting statements appear in the literature regarding interference effects in the atomic absorption determination of molybdenum. Various workers have found interference, varying in degree from almost negligible effects to severe enhancement or depression of molybdenum absorption, from many anions, cations and acids. The object of this study was to re-examine the somewhat confusing situation which exists in the literature on the interference by acids in the atomic absorption of molybdenum. Perhaps some confusion is understandable, when sample materials are of such diversity as ferrous alloys, fertilizers, lubricants and geological specimens, for which the solvents chosen include one or more of six common acids.

The first paper describing the determination of molybdenum by atomic absorption spectrometry recognized the interference caused by acids. In this paper, David[1] reported a detailed investigation of the determination of molybdenum in an air–acetylene flame, and many of his findings concerning interference have been substantiated. Thus, for example, the critical nature of the flame type and the position of the flame relative to the light beam were then well recognized. Of the acid effect, David commented on the depressed absorption of molybdenum in the presence of a mixture of hydrochloric (0.06 *M*) and nitric (0.82 *M*) acids and attributed this, not to viscosity or surface-tension effects, but to the presence of nitric acid imposing oxidizing conditions on a flame which for this determination needs to be distinctly reducing in nature. From the limited tests made with other acids, phosphoric acid caused a small enhancement whereas hydrochloric and sulphuric acids led to a depression of the molybdenum absorption.

Among others who were primarily concerned with the use of nitric and hydrochloric acids, Mostyn and Cunningham[2] described the depression by nitric acid of the molybdenum absorption, and the large depression caused by aluminium (2000 p.p.m.) and nitric acid (4% by volume) in admixture. Kirkbright *et al.*[3] used a mixture of nitric and hydrochloric acids (20 ml + 2 ml, respectively) as solvent for steels, and directly aspirated the resulting diluted solution. Standard molybdenum solutions were prepared in the same acid mixture for preparation of the calibration curve. Apparently, by matching the standard solutions no problem of interference was encountered.

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In a later paper dealing with the analysis of superphosphate for molybdenum, David[4] recommended the use of dilute hydrochloric acid as the solvent. The necessity of excluding nitrate ion from solution was again stressed, but the opposing effects of sulphate and phosphate ions[1] were only mentioned.

With regard to sulphuric acid, Julietti and Wilkinson[5] developed a method for the determination of molybdenum in which an alkaline fusion followed by dissolution of the melt in sulphuric acid was followed simply by aspiration of the acidic solution into the flame. Standard solutions were carefully matched by adding identical amounts of both reagents. Evidently, this use of sulphuric acid presented no difficulties, and the authors claimed the complete exclusion of nitrate ion as an advantage of the method.

Ramakrishna *et al.*[6] working with aqueous molybdate solutions (20 p.p.m. level) found considerable variation in the effects of acids (200 p.p.m. level). Both perchloric and acetic acids enhanced the absorption to a marked degree (30%), whereas sulphuric acid had the reverse effect; hydrochloric and nitric acids caused little change in the absorption. Kerbyson and Ratzkowski[7] presented distinctly opposing evidence concerning the effects of acids: sulphuric, perchloric and hydrochloric acid at 1% concentrations caused marked increases in molybdenum absorption and this position changed further as the acid concentration was increased to 10%. As the concentration rose, sulphuric acid produced a depression, whereas hydrochloric acid merely led to a reduction in the enhancement of the molybdenum absorption. The reason for this result with sulphuric acid was attributed to a viscosity change, but this seems unlikely.

The use of perchloric acid as a solvent was advocated by Thomerson and Price[8] for the elimination of interference in the determination of chromium and molybdenum in steels. It was argued that none of the earlier methods[1–4] proposing the addition of one or more salts was completely effective for the whole range of elements found in steels. They could not substantiate the report by Ramakrishna *et al.*[6] that perchloric acid enhanced the molybdenum absorption. Some mention was made of interferences arising from sulphuric, phosphoric, hydrochloric and nitric acids but details were not presented. It would appear, in fact, that with some residual sulphuric and phosphoric acid in the final solution, some interference could be anticipated in the second method proposed by Thomerson and Price for molybdenum where the tungsten concentration is high (>0.5%).

For the determination of small amounts of molybdenum in geological and related materials, Hutchison[9] recommended extraction with α-benzoinoxime in a pre-concentration step before finally aspirating the molybdenum in a solution consisting of ammonium chloride (0.5% w/v) and perchloric acid (1% v/v obtained by diluting 60% w/w acid). Data were given to show that perchloric acid at 1% and 2% concentrations (v/v) has no effect on the absorption of molybdenum (25 p.p.m.); with 10% perchloric acid, the depression of absorption was about equal to that imposed by 1% sulphuric acid.

In recent work, Van Loon[10] stated that hydrochloric and phosphoric acids caused an enhancement in molybdenum (10 p.p.m.) absorption dependent on the concentration of each acid. Purushottam *et al.*[11] found no interference from hydrochloric, nitric and sulphuric acids below 2 *N* concentration, or perchloric acid below 6 *N* concentration on the molybdenum (10 p.p.m.) absorption. At

higher concentrations a depression of the absorption was observed. The use of phosphoric acid to overcome the effects of certain cations was advocated.

The present paper describes a study in which several common acids, in a concentration range of practical interest, were employed as the potential sources of interference. Foreign metal ions were excluded in an effort to simplify interpretations. Two reducing flame types, air–acetylene (A–A) and nitrous oxide–acetylene (N–A) were compared.

EXPERIMENTAL

Preparation of solutions

Molybdenum stock solutions (500 p.p.m.) were prepared by dissolving (a) molybdenum trioxide in acid medium [0.3750 g of Specpure MoO_3 in concentrated ammonia–water (2 ml + 5 ml), water (100 ml), concentrated nitric acid–water (1 + 3, 200 ml) and final dilution with water to 500 ml]; or (b) ammonium molybdate in water (0.9205 g of analytical reagent-grade ammonium molybdate tetrahydrate in water and diluted to 1 l).

Further dilutions were made with nitric acid (10% v/v) or water to give solutions containing 5–20 p.p.m. molybdenum.

Stock solutions of acids (100,000 p.p.m.) were made by diluting concentrated acids of analytical reagent-grade with distilled water. The solutions prepared were nitric acid (103 ml l^{-1}), hydrochloric acid (277 ml l^{-1}) sulphuric acid (55.5 ml l^{-1}) and phosphoric acid (65.5 ml l^{-1}). Further dilutions gave the desired concentration range of 500–96,000 p.p.m.

Instrumental conditions

A Varian Techtron Model AA5 atomic absorption spectrophotometer was employed. The most sensitive molybdenum absorption line at 313.3 nm was used, and the operational settings were as shown in Table I.

The dilute solutions were aspirated into the flames of distinctly reducing types in both cases, after the burner had been allowed to operate for 10–15 min. In this way, thermal equilibrium was attained before any final adjustment was made to the burner height, gas flows or amplifier gain. Only then, with the instrument set for maximum molybdenum sensitivity, were readings noted.

TABLE I

OPERATIONAL SETTINGS

	Air–acetylene	*Nitrous oxide–acetylene*
Lamp current (mA) (ASL type lamp)	5	5
Slit width (μm)	100	100
Burner	AB40	AB40
Burner height	7.5	5
Fuel pressure (p.s.i.g.)	4	8.25
Support gas pressure (p.s.i.g.)	6.25	6
Scale expansion (approx.)	5	5
Damping	C	C

All absorbance values are the average of ten readings recorded successively from the digital readout accessory. The figures shown in the Tables represent, for convenience, absorbance readings (A) multiplied by 1000 (actually, of course, 5 A $\times$ 1000).

RESULTS AND DISCUSSION

The results showing the effects of hydrochloric, sulphuric and phosphoric acids at concentrations as high as 50,000 p.p.m. for three concentrations of molybdenum in 10% nitric acid are given in Table II. For hydrochloric acid, it is evident that there is no interference in the air–acetylene flame although at the highest concentration (10,000 p.p.m.) of acid, there is a small enhancement of *ca.* 10% in the nitrous oxide–acetylene flame. Phosphoric acid causes a small enhancement, and sulphuric acid a severe depression of the molybdenum absorption. These two effects are uniform for the concentration range studied and are more pronounced for the nitrous oxide–acetylene flame.

TABLE II

EFFECT OF VARIOUS ACIDS ON THE ABSORPTION OF MOLYBDENUM IN AIR–ACETYLENE AND NITROUS OXIDE–ACETYLENE FLAMES

Interfering species	*Molybdenum concn. (p.p.m.)*[a]	*Flame type*	*Molybdenum absorption*[b] *with added interfering species (p.p.m.)*								
			0	*10*	*100*	*500*	*1000*	*5000*	*10,000*	*50,000*	
HCl	5	A–A	90	86	93		88		89		
		N–A	121	126	122		129		130		
	10	A–A	187	188	188		187		187		
		N–A	252	248	250		252		270		
	20	A–A	372	371	371		373		370		
		N–A	509	502	511		502		550		
H_2SO_4	5	A–A	96			85	79	85	84	82	9(
		N–A	112			85	81	62	47	39	11
	10	A–A	200			187	185	191	190	175	19
		N–A	223			184	180	144	113	71	22(
	20	A–A	410			386	388	370	397	365	40
		N–A	450			353	369	315	273	122	45
H_3PO_4	5	A–A	55			63		62		66	5
		N–A	123			109		126		145	12(
	10	A–A	112			123		124		126	12
		N–A	241			237		254		289	25
	20	A–A	227			246		247		246	23
		N–A	473			515		519		568	46(

[a] Solutions were prepared from the stock solution containing MoO_3 and nitric acid. Final dilutions were made v nitric acid (10% by volume).

[b] Readings: absorbance $\times$ 5000.

In Table III are presented the data showing the effects of hydrochloric, sulphuric, nitric and phosphoric acids added to an aqueous molybdenum solution in amounts as high as 96,000 p.p.m. (*ca.* 27, 10, 5 and 6% acids by volume,

TABLE III

EFFECT OF ACIDS ON THE ABSORPTION OF AQUEOUS SOLUTIONS OF MOLYBDENUM IN AIR–ACETYLENE AND NITROUS OXIDE–ACETYLENE FLAMES

Interfering species	*Flame type*	*Molybdenum absorption[a] with added interfering species (p.p.m.)*								
		0	*100*	*500*	*1000*	*5000*	*10,000*	*50,000*	*96,000*	*0*
HCl	A–A	405	400	397	396		403	381	359	399
	N–A	548	537	545	540		548	509	486	563
H_2SO_4	A–A	399	366	358	356		345	291	211	386
	N–A	563	504	467	454		340	161	146	544
HNO_3	A–A	399	394	393	397		412	415	405	405
	N–A	554	532	529	507		515	499	495	548
H_3PO_4	A–A	211		217		223		231		209
	N–A	491		499		528		598		494

[a] Molybdenum concentration for all data is 20 p.p.m. Solutions were prepared from the aqueous molybdate stock solution. Readings: absorbance × 5000.

respectively). These results were obtained in order to expose any difference between a nitric acid medium (data of Table II) and an aqueous system as solvent for the molybdenum. The effect of the ammonium ion at the dilutions used is assumed to be negligible. The results are not unlike those of Table II in that sulphuric and phosphoric acids behave consistently, as before. Hydrochloric acid, however, shows a depressive effect at concentrations greater than 10,000 p.p.m. and does so independently of the flame type.

The data point to nitric acid as the most suitable solvent. Only a very small elevation of the absorption is observed in the air–acetylene flame for concentrations in excess of 1000 p.p.m. With the nitrous oxide–acetylene flame, the effect is reversed and a depression of the molybdenum absorption is observed from the lowest concentrations of nitric acid. It is noteworthy in a comparison of the data of Tables II and III that there is a reduction of the interference by sulphuric acid when nitric acid is also present. For example, the depression caused by sulphuric acid at 50,000 p.p.m. is reduced from 27% in aqueous medium to 11% in the nitric acid medium for the air–acetylene flame.

Table IV shows the effect of hydrochloric and sulphuric acids when added to a matrix consisting of 10% nitric acid. Again, the trends of the earlier data are evident.

These results can be seen, therefore, to agree with some of the published data, but are in conflict with others, for reasons which are not clear. However, the question of interference by acids in atomic absorption spectrometry is not restricted to observations with molybdenum. For example, recent work points to similar problems with copper, chromium, manganese and nickel[12,13], and tin[14,15]. Again, the effect is minimal with hydrochloric[12] and nitric[13,14] acids. Barnett[12] has highlighted some features of interference which are equally applicable in the case of molybdenum. In particular, the air–acetylene flame is preferred, because it is more interference-free (compare Tables II and III) and it gives more precise readings (see Table V).

TABLE IV

EFFECT OF ACID MIXTURE ON THE ABSORPTION OF MOLYBDENUM IN AIR ACETYLENE AND NITROUS OXIDE–ACETYLENE FLAMES

Interfering species	*Flame type*	*Molybdenum absorption[a] with added interfering species (p.p.m.)*					
		0	*1000*	*5000*	*10,000*	*86,000*	*0*
HCl in	A–A	388	388	383	378	340	382
10% HNO_3	N–A	567	513	568	593	602	583
H_2SO_4 in	A–A	386	346	359	366	310	374
10% HNO_3	N–A	544	396	358	317	144	547

[a] Molybdenum concentration for all data is 20 p.p.m. Solutions were prepared from the aqueous molybdate stock solution. Readings: absorbance × 5000.

TABLE V

THE INFLUENCE OF FLAME TYPE ON THE PRECISION OBTAINED FOR MOLYBDENUM

Flame type	*Readings[a]*					*Mean (M)*	*Range (R)*	*R/M × 100*
A–A	396	399	396	394	399			
	402	400	402	398	400	399	8	2
N–A	547	555	554	557	560			
	571	564	572	578	570	563	31	5.6

[a] Molybdenum concentration 20 p.p.m. Readings: absorbance × 5000.

It is of interest to consider briefly the causes of interference. It is possible to identify such features as solute-vaporization interference contributing to depression of the signal with sulphuric acid and a surprising elevation of the signal in the data for phosphoric acid. The absence of an over-riding depression of signal for rising concentrations of phosphoric acid suggests that viscosity cannot be a major factor. There is no absorption by the respective solvents.

Although the flame chemistry involved may be a prime cause of the interference, the possibility that interference reflects features of instrumental design cannot be overlooked. Van Loon[10], who appears to be the only investigator to have compared two different instruments, found molybdenum absorption to be markedly different for each instrument. Curiously, with one exception, enhancement was observed with one instrument (Instrumentation Laboratory Model 153) and depression of molybdenum absorption with the other (Perkin-Elmer Model 303). Again, Barnett[12], working with a Perkin-Elmer Model 403 instrument, found the single-slot burner head superior to the three-slot type which gave markedly greater interference in work with copper, chromium, manganese and nickel. Perhaps other features such as nebulizer efficiency and nebulizer operation (contrast the Perkin-Elmer 303 and the Varian Techtron AA4 models), flame velocities, flame temperatures, burner slit widths, lengths and designs (premix or total consumption) may require closer examination.

In the literature, there is frequent reference to the critical nature of the flame, whether air–acetylene or nitrous oxide–acetylene, necessary for molybdenum absorption to occur. In the nitrous oxide–acetylene flame, it has been argued[16] that the red zone of the fuel-rich flame is the critical region whose temperature and reducing properties control the metal atom population. Maximum temperature is attained[17] when sub-stoichiometric amounts of air or nitrous oxide are used. If it is assumed that the CN species is a key factor in the fuel-rich nitrous oxide–acetylene flame, some of the observations with acids may be explained as the result of a scavenging of the reducing CN species by acid products in the flame, *e.g.* oxidizing species derived from hydrochloric, nitric or sulphuric acids. The marked depression by sulphuric acid may also be due to a decreased flame temperature. According to this hypothesis, phosphoric acid must enhance the formation of the reducing species sufficiently to over-ride the lowering of the flame temperature caused by the introduction of the acid.

The results presented are of practical importance. For essentially pure solutions of molybdenum, dilute hydrochloric and nitric acids and the air–acetylene flame should be suitable. Otherwise, only very close matching of standards and samples can be expected to be satisfactory; such procedures, of course, complicate any simple approach based on direct aspiration or dilution of sample solutions in order to reduce interference, and are not readily achieved when samples are of varied composition. There seems to be a need for a highly selective scheme for the separation of molybdenum from its matrix when low concentrations are involved.

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SUMMARY

The nature of the acid environment in the determination of molybdenum by atomic absorption spectrometry is shown to be of considerable importance. The most favourable conditions are provided by dilute hydrochloric acid and especially nitric acid. Sulphuric and phosphoric acids are not recommended because of their marked but opposite effects. The air–acetylene flame gives more reproducible results than the nitrous oxide–acetylene flame.

RÉSUMÉ

Lors du dosage du molybdène par spectrométrie d'absorption atomique la nature de l'acide utilisé joue un rôle important. Ce sont l'acide chlorhydrique dilué et spécialement l'acide nitrique qui conviennent le mieux. Les acides sulfuriques et phosphorique ne sont pas recommandés. La flamme air–acétylène fournit des résultats plus reproductibles que la flamme protoxyde d'azote–acétylène.

ZUSAMMENFASSUNG

Die Art der vorliegenden Säure ist bei der Bestimmung von Molybdän

mittels Atomabsorptionsspektrometrie von erheblicher Bedeutung. Die günstigsten Bedingungen ergeben sich bei verdünnter Salzsäure und besonders Salpetersäure. Schwefelsäure und Phosphorsäure werden wegen ihrer merklichen, entgegengesetzten Einflüsse nicht empfohlen. Die Luft–Acetylen-Flamme führt zu besser reproduzierbaren Ergebnissen als die Lachgas–Acetylen-Flamme.

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RELATIVE EFFICIENCY OF SEVERAL ATOMIZATION AND SAMPLE INTRODUCTION SYSTEMS IN ANALYTICAL ATOMIC SPECTROMETRY

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The chemical flame has found wide acceptance as an atomization system for analytical atomic spectrometry. Flame atomic emission spectrometry has been used for many years for analytical purposes, and is still widely used to determine easily excited elements. In this application, the flame serves for both the atomization and excitation processes. For the many elements with resonance lines below about 300.0 nm, atomic absorption spectrometry is widely used for the determination, and in this process, the flame serves merely as an atomization cell. Chemical flames are also widely used as atomization cells for atomic fluorescence spectrometry.

Flames have many advantages as atomization systems: convenience, relatively low cost, simplicity of operation, relatively high atomization efficiency for some elements, and good analytical sensitivity and minimum detectable quantities in the μg to pg range in many cases. There are also problems associated with the use of chemical flames for analytical purposes. Most of these are a result of one or more of the flame properties, such as a rather limited temperature range, relatively poor atomization efficiency in many cases, a very reactive (and poorly understood) chemical environment, background emission, and relatively low atomic concentration.

Significant efforts have been made towards improving the chemical flame as an atomization system, primarily through burner design, sample introduction systems, flame gas combinations, and so forth. Similar efforts are currently being applied to the development of non-flame atomization systems, such as plasmas and resistively heated devices.

Rann and Hambly[1] and Chakrabarti *et al.*[2] have studied the atomic distributions of several elements in various flames, and have found that the individual atomic absorption profiles depend on flame stoichiometry, anions present, solvent properties, burner design and temperature. Several investigations[3–8] have studied the efficiency of atomization for various elements in several flames. These studies indicate that the atomization efficiency varies widely with the element, type of flame, stoichiometry, temperature, and other factors. It would appear that most elements are not completely atomized in commonly used flames, and only a few easily atomized elements, such as Cu, Ag, Au, etc., which do not form highly stable

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compounds at elevated temperatures, appear to be completely atomized in most flames.

Flame atomic emission and atomic fluorescence measurements are frequently made with "total-consumption" aspirator burners or Meker-type burners with indirect sample introduction. The geometry of these flames is considered to be less than optimal for atomic absorption measurements, owing to the relatively short absorption path length. Therefore, in atomic absorption spectrometry one usually employs an elongated slot type burner with indirect sample introduction. These burners are generally difficult to operate safely with gas mixtures like oxy-hydrogen and oxy-acetylene, which have high temperature and high burning velocity and the air–acetylene mixture, which has lower temperature and lower burning velocity, is frequently employed. For many elements, higher temperatures than those attainable with air–acetylene are needed for more efficient atomization. Willis[9] proposed the use of nitrous oxide and acetylene, which has a low burning velocity and a temperature comparable to oxy-acetylene. This flame has found wide acceptance in atomic absorption spectrometry and has significantly increased the number of elements which can be determined in the $\mu g\ ml^{-1}$ concentration range and below.

Additional burner types which appear to offer improvements in minimum detectable concentrations include long-path flame cells[10], capillary burners[11], and separated flames[12]. Separated flames have been especially successful in atomic emission and fluorescence measurements, owing to their low background emission.

Various nebulization and sample introduction systems have been developed. The "total-consumption" systems are not completely efficient since rather large aerosol droplets are produced, resulting in much of the sample not being atomized in the flame. With the indirect spray-chamber systems frequently employed in atomic absorption slot burners, much of the sample never reaches the flame. Sample introduction efficiency of these systems is frequently in the 3–6% range; "efficiency" here means the portion of the analyte element aspirated that actually reaches the flame. Ultrasonic nebulization[13], as opposed to the more common pneumatic systems, is one possible means of increasing the fraction of sample solution reaching the flame. Heated chamber pneumatic systems[14–16] have been shown to increase analytical sensitivity by about an order of magnitude, owing to their high efficiency of sample introduction.

Non-flame atomization systems, especially the heated graphite type[17–21], have received considerable attention recently as atomization cells for atomic absorption and atomic fluorescence spectrometry. Low background emission, ability to analyze very small samples, and high analytical sensitivity are the principal features of these devices. Some matrix problems occur, some elements react with the hot graphite, and when samples in the μl range are used, reproducibility is somewhat poorer than with flame atomization systems. Nevertheless, absolute detection limits about 2 orders of magnitude better than flame atomization are reported for many elements, indicating that these non-flame atomization systems have considerable potential.

In the flame atomic emission, absorption and fluorescence literature of the past several years, many different detection limits have been reported for the elements. It is often difficult or impossible to compare these values directly, because of the considerable differences in apparatus employed and conditions used. Detec-

tion limits are determined by the signal-to-noise (S/N) ratio obtained in the measurement. The signal magnitude depends on many variables, such as monochromator dispersion, slit width and aperture, the external optical system, the detector, the source intensity, the amplification system, atomization efficiency, etc. The noise (fluctuations) magnitude likewise depends on many parameters, such as source flicker, detector-readout noise, time constant of the readout system, frequency bandwidth, fluctuations in the atomization system, etc. Consequently, one will frequently make measurements with a particular system under a particular set of conditions, and find that the detection limits obtained are better or worse than someone else's results, where a different system and conditions were used. In some cases, it is even found difficult to reproduce the results of others under presumably identical conditions.

The objective of this investigation was to attempt to compare, under controlled conditions and using the same apparatus, several flame atomization systems, both commercially available designs and versions constructed in this laboratory. Both atomic emission and atomic absorption measurements were made and the basis of comparison was to be the minimum amount of an easily determined element that could be detected. Several types of chemical flames and burner designs were investigated, employing direct pneumatic, indirect pneumatic, ultrasonic, and solventless sample introduction systems. A heated graphite rod atomization system was also investigated, with both direct placement of small sample volumes on the rod and continuous sample introduction.

EXPERIMENTAL

Apparatus

The basic instrumental set-up used for all of the measurements consisted of the items shown in Table I. These were used in the conventional manner.

TABLE I

INSTRUMENTAL COMPONENTS USED FOR ALL MEASUREMENTS

Apparatus	*Description*
Monochromator	0.5-m Ebert; $f/8$ aperture; 1.6 nm/mm dispersion; 1180 grooves/mm grating, blazed for 300.0 nm; curved entrance and exit slits, adjustable between 5–400 μm width and 0–20 mm height
Detector	EMI type 9783B multiplier phototube, powered by 0–3100 V high-stability d.c. power supply. 649 kΩ anode load resistor
Amplifier	Model HR-8 with Type D pre-amplifier (Princeton Applied Research, Princeton, N.J.) Analog voltage recorder output divided by 1000
Recorder	24 cm, 10 mV, <1 s response time
Source[a]	Westinghouse hollow-cathode discharge lamp; quartz window; powered by current-regulated d.c. supply
Modulation	Source[a] and flame[b] radiation modulated at 330 Hz with mechanical chopper having equal "on" and "off" times

[a] For absorption.
[b] For emission.

External optics consisted of two fused silica lenses, 25 mm diam. × 100 mm f.l., one used to focus an unmagnified image of the flame on the entrance slit and the other used to focus an unmagnified image of the source in the flame.

Atomization systems. Two total-consumption type burners and two different premixed type burners were used with various sample introduction systems and fuel–oxidant combinations. In addition, a heated graphite rod atomization system was also employed, with both discrete-small-volume and continuous sample introduction. These systems are described in Table II.

TABLE II

ATOMIZATION AND SAMPLE INTRODUCTION SYSTEMS USED

Designation	*System*
B-OH	Beckman oxy-hydrogen. Medium-bore, total-consumption, aspirator–burner (Beckman Instruments, Fullerton, Calif.)
B-OA	Beckman oxy-acetylene. Same as above, with hydrogen fuel jacket replaced with acetylene fuel jacket
Slot	Slot-type burner. Air–acetylene, 10 cm slot, pneumatic nebulization, chamber-type indirect sample introduction (Perkin-Elmer, Norwalk, Conn.)
POA-P	Premixed oxy-acetylene, pneumatic nebulization. A welding-type, single orifice burner tip mounted on a premixing tube and operating on premixed oxygen and acetylene. Sample introduction system consisted of pneumatic nebulizer spraying into heated chamber, followed by water-cooled condenser. Similar to unit described previously (ref. 14) except condenser modified with additional external water jacket
POA-U	Premixed oxy-acetylene, ultrasonic nebulizer. Same as POA-P, but ultrasonic nebulization employed (Tomorrow Enterprises, Portsmouth, Ohio)
C-Rod	Heated graphite rod system, 6 mm O.D. nitrogen-sheathed, resistively-heated graphite rod with 25 mm heated zone. Principal features described previously (ref. 21). Three sample introduction systems used: (a) discrete, 10 μl samples placed in 2 mm diam. × 2 mm deep cavity in rod; (b) continuous sample introduction around central 4 mm of rod with POA-P system; and (c) same with POA-U system.

Measurements

All of the measurements were obtained with the same basic apparatus, to permit direct comparison of the data. Each of the atomization systems was optimized, *i.e.*, the gas flow ratio, spectral bandwidth, detector gain, region viewed, etc., which resulted in the best S/N ratio were determined. The sample introduction systems differ in the rate at which sample solution is aspirated, and even more significant, they differ in efficiency of sample introduction. For example, all of the solution aspirated by the Beckman burners is sprayed directly into the flame, while only a fraction of the sample aspirated by the indirect systems reaches the flame. In addition to this consideration, the portion of the sample aerosol reaching the flame may or may not be completely atomized, depending on the droplet size, temperature, flame conditions, flame geometry, etc. Perhaps the best way to compare the detection limits obtained with the various systems is on an absolute basis, that is, on the basis of the smallest amount of analyte detectable. This could be expressed as a weight, a number of atoms or moles, or

as a concentration, provided that a volume is specified in the latter case. Since most spectroscopists are familiar with concentration units such as $\mu g\ ml^{-1}$, this unit was selected as the basis for comparison of detection limits.

In this case, it is then necessary to specify the minimum volume of sample solution required for a measurement. Since detection limits can frequently be improved by trading time for an improved S/N ratio (e.g., with increased time constant), a readout system time constant of 10 s was selected. That is, in the authors' opinion, about the longest practical time constant that can be used in these types of measurements. A 10-s time constant requires that the sample signal reading be made continuously over a period of about 1 min. Several of the systems employed in this study had aspiration rates of about 2 ml min^{-1}, so the detection limits are based on this volume (2 ml) of sample solution and a 10-s time constant.

It is important to note that this is 2 ml of sample solution *aspirated* in a 1-min period, and not necessarily the rate at which sample solution reaches the flame. The latter is also a function of the efficiency of the sample introduction system. Using this criterion, one can then compare directly the detection limits, in absolute terms, obtained with the various atomization/sample introduction systems.

Copper was chosen for the comparison element, and the 324.7-nm line was used for all emission and absorption measurements. The two principal reasons for this choice are that (a) copper is readily atomized in most chemical flames and exhibits little compound formation, resulting in high atomization efficiencies[3–8], and (b) the 324.7-nm resonance line for copper is near the "crossover point" between atomic absorption and flame atomic emission. That is, elements with primary resonance lines much below 300.0 nm are usually more sensitive analytically by atomic absorption, while those with resonance lines much above 300.0 nm are usually more sensitive by flame atomic emission. Consequently, copper might be expected to exhibit comparable analytical detection limits by either technique.

RESULTS AND DISCUSSION

The solution uptake rates under the optimal conditions used for the measurements, the efficiency of the sample introduction system and the corresponding net rate at which analyte sample solution reached the flame are shown in Table III.

TABLE III

SOLUTION ASPIRATION RATES, EFFICIENCIES AND NET ASPIRATION RATES FOR SAMPLE INTRODUCTION SYSTEMS

System	*Solution aspiration rate (ml min^{-1})*	*Overall efficiency (%)*	*Net aspiration rate (ml min^{-1})*
B-OH	1.9	—	(1.9)
B-OA	1.9	—	(1.9)
Slot	5.1	4.1	0.21
POA-P	2.0	34	0.78
POA-U	4.0	6.1	0.24

With the B-OH and B-OA burner systems, the solution aspiration rate is identical, since the same oxygen flow rate was used in each case, and since the inner (oxygen) portion of the burner was the same for both. Only the outer fuel jackets were interchanged. With the slot-burner system, the nebulizer supplied with the burner was used, with air as the nebulizing gas. The heated chamber/condenser pneumatic system utilized oxygen (or argon, in the case of the carbon-rod system) at 40 p.s.i. and 3.5 l min^{-1} flow rate for the nebulizer. The ultrasonic nebulizer was supplied with sample solution at a rate of 4.0 ml min^{-1} from a motor-driven pump which is part of the system and was operated at maximum power. Oxygen was supplied separately at a rate of 3.5 l min^{-1} for the POA-U system (argon used with carbon-rod system). With the carbon-rod atomizer, the POA-P and POA-U sample introduction systems were used, in addition to discrete 10-μl samples deposited on the rod, as described earlier.

The overall efficiencies of the atomization systems, as indicated in Table III, were measured indirectly because of the difficulty of completely trapping the aerosol particles issuing from the devices. This was done by aspirating 10 ml of a 10 μg ml^{-1} lithium solution into the clean system, collecting the condensate, combining this with rinsings of the entire system interior, and diluting to a volume of 1 l. This solution was then analyzed for lithium by flame atomic emission spectrometry and the efficiency of the system was calculated from these data. The relatively low overall efficiency of the ultrasonic system was due to the fact that the aerosol was not generated directly within the heated chamber. Instead, it was produced in an external room temperature chamber and swept into the heated chamber. Consequently, much of the sample solution was not utilized. If the transducer cooling is sufficient to allow operation within the heated chamber, the overall efficiency should be greatly improved, although this possibility was not investigated, for fear of damaging the transducer.

Each of the 8 atomization/sample introduction combinations were optimized for the maximum S/N ratio obtainable for both atomic emission and absorption (absorption only for the carbon-rod systems) at the 324.7-nm copper line. Some general trends and effects of the various parameters such as detector voltage, slit width and flame stoichiometry are worth noting. For example, in the emission measurements, optimization of the S/N ratio was achieved by varying the fuel/oxidant flow ratio, detector voltage and slit width. For each flame atomization system, these parameters were varied between stoichiometric and 4/3, 600–900 V, and 25–400 μm, respectively. For each combination of conditions, the relative background emission intensity, the peak-to-peak noise, the net signal from copper, and the S/N ratio were determined. This involved over 100 measurements per atomization system. To allow direct comparison of the results, the values were appropriately corrected to the same basis, namely, an aspiration rate of 2 ml min^{-1} of a 10-μg ml^{-1} copper solution, and a 3-s amplifier time constant. Comparing these data, it was usually obvious where the optimal analytical conditions lay.

For all of the atomization systems, the detector voltage had the smallest effect on the S/N ratio. Between 600 and 900 V, the S/N ratio changed by less than a factor of 2 with all of the atomization systems. The middle of this range, about 750 V, usually proved to be optimal.

Fuel/oxidant flow ratios were found to have a greater effect on the S/N ratio and were related to the observed background emission intensity, which is also a function of the spectral bandwidth. With the B-OH system, the optimum S/N ratio was about an order of magnitude greater at a fuel/oxidant ratio of 1 than the "stoichiometric" flame (ratio of 0.5). With the B-OA system, the optimum S/N ratio was approximately the same at flow ratios of 2/3 and 1, except that the optimal slit width was smaller in the latter case, presumably because of the higher background emission intensity. Similar trends were generally observed with the other systems. The optimal viewing region was also examined, and found to be non-critical, *i.e.*, the observed maximum S/N ratio was not found to depend very strongly on the region of the flame viewed. The optimization of this parameter was about as easy as optimization of the detector voltage.

In many experiments, one frequently has a preconceived idea of the results, based on experience and intuition. If asked to predict in advance the outcome of this study, one might have expected the B-OH and B-OA systems to be the least effective systems, owing to the short absorption path length and/or large aerosol droplets produced. One might also expect the slot system to be rather insensitive in emission due to the relatively low flame temperature and relatively inefficient sample introduction system. One might also expect that the POA-P and POA-U systems would be superior, especially for emission measurements, while the carbon-rod system might prove best for absorption.

In any analysis, the detection limit is defined as the smallest amount of analyte that can be detected. The term "detected" does not always mean the same to all, but it is generally accepted as the amount of analyte required to produce a reading with a S/N ratio of 2.

In comparing the atomization systems used in this investigation, the authors have assumed that a readout system time constant of 10 s is the practical upper limit and that 2 ml of sample solution are required for a determination. With these criteria, the emission and absorption results shown in Table IV were obtained for the various atomization systems. These are expressed as concentrations, and are all on the basis of 2 ml of sample solution required and a solution aspiration rate of 2 ml min^{-1}.

The data in Table IV indicate that all of the flame atomization systems

TABLE IV

RELATIVE DETECTION LIMITS FOR SEVERAL ATOMIZATION AND SAMPLE INTRODUCTION SYSTEMS

System	*Detection limit ($\mu g\ ml^{-1}$)*	
	Emission	*Absorption*
B-OH	0.012	0.030
B-OA	0.030	0.010
Slot	0.054	0.012
POA-P	0.019	0.012
POA-U	0.10	0.054
C-rod (P)	—	0.0015

shown result in detection limits by atomic emission and atomic absorption spectrometry that are well within an order of magnitude. With the carbon-rod atomizer, and the continuous sample introduction system used with POA-P, absorption detection limits of about an order of magnitude lower than the flame systems were obtained. When discrete 10-μl samples were deposited on the rod, 7.5×10^{-11} g of copper could be detected. In 2 ml, this would correspond to a detection limit of 0.000038 μg ml^{-1}, or over 2 orders of magnitude lower than the flame systems. Copper atomic emission at 324.7 nm was not observed under the conditions used with the carbon-rod system.

These results, while certainly not conclusive for all flame systems, conditions and instrumental arrangements, suggest that improvements of much over an order of magnitude in absolute detection limits through burner design would not be expected. They also indicate that far greater potential improvements could be expected with non-flame systems, such as the heated graphite atomizers, for atomic absorption spectrometry. While these non-flame systems may prove in time to have significant limitations such as matrix effects and the number of elements to which they may be successfully applied, a very real potential exists for greatly improved detection limits in many cases.

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SUMMARY

Flame atomic emission and atomic absorption detection limits are compared on an absolute basis for the easily determined element, copper. Remarkably similar detection limits are obtained with a variety of atomization and sample introduction system combinations. Heated graphite atomizers offer significantly lower minimum detectable quantities.

RÉSUMÉ

Les limites de détection obtenues par émission atomique et absorption atomique de flamme sont comparées, pour un élément facilement dosable, le cuivre. Des valeurs remarquablement similaires sont obtenues pour diverses combinaisons d'atomisation et de systèmes d'introduction d'échantillon. Les atomiseurs de graphite chauffés fournissent les meilleures sensibilités.

ZUSAMMENFASSUNG

Die Nachweisgrenzen bei der Flammen-Atomemission und Atomabsorption werden für das leicht bestimmbare Element Kupfer auf einer absoluten Grundlage miteinander verglichen. Bemerkenswert ähnliche Nachweisgrenzen werden mit zahlreichen Kombinationen von Systemen der Atomisierung und der Probenzuführung erhalten. Die Nachweisgrenzen sind bei Atomisatoren mit erhitztem Graphit bedeutend niedriger.

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Analytica Chimica Acta, 69 (1974) 305–311

SPECTROPHOTOMETRIC DETERMINATION OF MOLYBDENUM(VI) WITH 2-MERCAPTOBENZO-γ-THIOPYRONE AND AMMONIUM THIOCYANATE

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Many methods have been suggested for the colorimetric determination of molybdenum[1–5]. Of these, the most extensively investigated is the thiocyanate–tin(II) chloride method which has the disadvantage that for full colour development a small amount of iron is required. Dick and Bingley[6] observed that the minimal quantity of iron required to give maximal colour development is proportional to the amount of molybdenum, and that when iron is absent the colour intensity decreases by about 35%. It was also observed that the isoamyl alcohol extract of the molybdenum–thiocyanate complex formed in the absence of iron(II) slowly darkens on standing. During the present investigations, it was observed that the sensitivity and stability of the thiocyanate method for molybdenum(VI) can be enhanced considerably by using ammonium thiocyanate and the potassium salt of 2-mercaptobenzo-γ-thiopyrone as mixed ligands followed by extraction with acetophenone. These reagents formed an orange-red acetophenone-soluble complex with molybdenum(VI) in hydrochloric acid medium in the presence of tin(II) chloride. Based on this colour reaction, a selective spot test and a spectrophotometric method were developed for molybdenum. 2-Mercaptobenzo-γ-thiopyrone gives a pink colour with molydenum(VI), in 1.5 *M* hydrochloric acid medium, which can be extracted with chloroform and has an absorption maximum at 495 nm. The colour, however, fades completely in about 30 min at room temperature and even more quickly on heating. The colour system remains stable for about 45 min in the presence of potassium perchlorate.

EXPERIMENTAL

Preparation of 2-mercaptobenzo-γ-thiopyrone (potassium salt)

The reagent was prepared as follows. Shake a mixture of acetophenone (80 ml) and carbon disulphide (100 ml) with a saturated solution of potassium hydroxide (50 ml) for 10 min in a 500-ml round-bottomed flask, reflux over a steam bath for about 1 h, and then distil at reduced pressure (water pump) to remove carbon disulphide. Crystallize the product from a chloroform–ether mixture. Analysis: found, 27.5% S and 16.8% K; calc. for $C_9H_5OS_2K$, 27.5% S and 16.9% K.

Apparatus, reagents and solutions

A Beckmann DU2 spectrophotometer with 1-cm quartz cells was used for absorbance measurements.

Standard molybdenum solution. Dissolve AnalaR ammonium molybdate in a known volume of distilled water containing a few drops of ammonia. The metal content was determined gravimetrically by the 8-hydroxyquinoline method[7].

Reagent solutions. Prepare aqueous 1% (w/v) solutions of 2-mercaptobenzo-γ-thiopyrone and 5% (w/v) ammonium thiocyanate.

Tin(II) solution. Dissolve 11.5 g of AnalaR tin(II) chloride dihydrate in 17 ml of AnalaR concentrated hydrochloric acid and dilute to 100 ml with water.

Solutions of diverse ions were prepared from AnalaR chemicals.

Spot test for molybdenum

Procedure. In a micro test tube, place 0.1 ml of 10 *M* hydrochloric acid, 0.10 ml of molybdenum(VI) solution, 0.05 ml of ammonium thiocyanate solution and 0.05 ml of the γ-thiopyrone reagent solution followed by 0.5 ml of acetophenone. Shake for 1 min. A stable deep orange-red acetophenone layer indicates the presence of molybdenum.

The limit of identification was found to be 0.1 μg of molybdenum with a dilution limit of 1:1,000,000.

Effect of variables. Maximal colour intensity was found in 1.5–3.6 *M* hydrochloric acid solution. The complex was stable for over 24 h at room temperature and variation in temperature from 15 to 55° had no effect. Slight variations in the amounts of reagents also had no effect on the stability and colour formation.

Interference of various ions. The spot test described was highly selective. No interference was found with Mn^{2+}, Zn^{2+}, Mg^{2+}, Be^{2+}, Fe^{2+}, Pb^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Sn^{2+}, Al^{3+}, Cr^{3+}, Sb^{3+}, La^{3+}, Ce^{4+}, Th^{4+}, Zr^{4+}, Ta^{5+}, Nb^{5+} and V^{5+} even in 1000-fold amounts. Iron(III) was tolerated in 200-fold amounts in the presence of tin(II) chloride. Cobalt(II) could be masked in 500-fold amounts by complexing with citrate. Interferences of Ni^{2+}, Cu^{2+}, Ag^{+} and Co^{2+} were avoided by complexing these ions with the γ-thiopyrone reagent at pH 6–10 and extracting with ethyl acetate or chloroform. Of the anions tested, tartrate, oxalate, citrate, hydrogencarbonate, carbonate, nitrate, sulphate, chloride, bromide, iodide, phosphate and EDTA did not interfere even in 1000-fold excess, but fluoride and thiosulphate interfered.

Spectrophotometric determination of molybdenum

Place an aliquot of molybdenum(VI) solution containing 0.25–9.41 μg of the metal in a separatory funnel and add 2 ml of 10 *M* hydrochloric acid, 2 ml of 5% ammonium thiocyanate solution, 2 ml of the 1% γ-thiopyrone reagent solution and 2 ml of tin(II) chloride solution followed by 3 ml of acetophenone. Shake vigorously for 2 min. Transfer the deep orange-red acetophenone layer into a small beaker containing 0.5 g of anhydrous sodium sulphate. Extract twice more with 2-ml portions of acetophenone and collect in the same beaker. Transfer the combined extract to a 10-ml volumetric flask and dilute to the mark with the solvent. Measure the absorbance at 470 nm against a reagent blank prepared similarly.

RESULTS AND DISCUSSION

Absorbance spectra

The absorbance of the reagent blank continuously decreases from 400 nm to 520 nm, whereas the absorbance of the complex shows a maximum at 470 nm (Fig. 1).

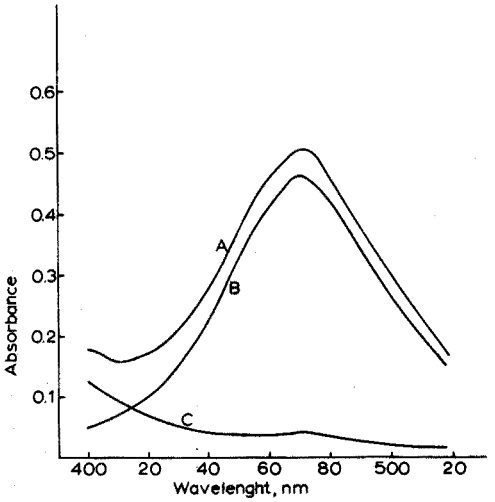

Fig. 1. Absorption spectra. (A) 2.36 p.p.m. molybdenum *vs.* acetophenone; (B) 2.36 p.p.m. molybdenum *vs.* reagent blank; (C) reagent blank *vs.* acetophenone.

Optimal conditions

To obtain maximal colour intensity, a 1.46–3.6 *M* hydrochloric acid medium is required. Sulphuric and nitric acid were found to decrease sensitivity. For maximal colour development, 1.5–3.0 ml of 5% (w/v) thiocyanate and 1.5–2.5 ml of tin(II) chloride were required (Fig. 2). Further variations in their amounts decreased the colour intensity. Of the various solvents tried for the extraction, acetophenone proved to be the most suitable because of its insolubility in water

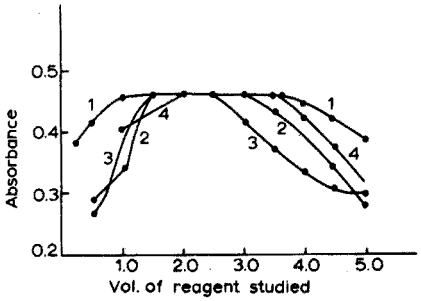

Fig. 2. Effect of concentrated hydrochloric acid, ammonium thiocyanate, 2-mercaptobenzo-γ-thiopyrone and tin(II) chloride. (1) Effect of concentrated hydrochloric acid (10 *M*); (2) effect of 5% (w/v) ammonium thiocyanate; (3) effect of 1% (w/v) 2-mercaptobenzo-γ-thiopyrone; (4) effect of tin(II) chloride.

and very low volatility. The complex was stable at room temperature for over 24 h. Change in temperature from 15 to 35° had no marked effect on the colour system. The colour system obeyed Beer's law from 0.25 to 9.41 p.p.m. of molybdenum at 470 nm. The optimal concentration range according to Ringbom's[8] method was 0.75–8.5 p.p.m. The relative analytical error per 1% absolute photometric error calculated from Ayres equation[9] was 1.675%. The photometric sensitivity of the method as defined by Sandell[1] was 0.005 μg Mo cm^{-2}. The Sandell sensitivity of the thiocyanate–tin(II) chloride method is 0.01 μg Mo cm^{-2}.

Interferences

Most of the common ions are tolerated in more than 1000-fold amounts. Table I summarizes the amounts of cations tolerated. The effects of various interfering ions are the same for the spectrophotometric method as for the spot test described above.

TABLE I

NON-INTERFERING IONS

Group	*Ions*
I	Na^+, K^+ (200 mg each)
II	Be^{2+}, Mg^{2+}, Cd^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Zn^{2+} (200 mg each) Hg^{2+} (50 mg)
III	Al^{3+}, Ce^{4+}, La^{3+} (200 mg each)
IV	Sn^{2+}, Pb^{2+}, Ti^{4+}, Zr^{4+} (300 mg each)
V	As^{3+}, Sb^{3+} (50 mg each)
VI	Cr^{3+} (100 mg)
VII	Mn^{2+} (50 mg)
VIII	Fe^{2+} (50 mg)

Precision and accuracy

A statistical study was made to estimate the precision. Ten independent determinations on solutions containing 2.36 μg Mo ml^{-1} gave a mean absorbance value of 0.466 and a standard deviation of 0.003 absorbance units. A similar statistical study of ten independent determinations of the reagent blank solution gave a mean absorbance of 0.026 and a standard deviation of 0.0018.

APPLICATIONS

Spectrophotometric determination of molybdenum in steel

Dissolve an appropriate weight of the sample (1 g for 0.5% of molybdenum, 0.1–0.3 g for 6% of molybdenum) in 50 ml of 3 *M* sulphuric acid in a 150-ml Erlenmeyer flask, and oxidize with concentrated nitric acid. Cover the flask with a small funnel and boil to reduce the volume to about 25 ml. Cool, add 25 ml of water and boil for a few minutes. Cool, filter if necessary, add 0.5 g of iron(II) sulphate to reduce vanadium(V) and chromium(VI), transfer to a 100-ml volumetric flask and dilute to the mark. Take an aliquot of this solution, add 1 ml of saturated ascorbic acid and determine the molybdenum content by the procedure given above.

Results for three analyses of two steels are given in Table II.

TABLE II

DETERMINATION OF MOLYBDENUM IN STEELS AND SYNTHETIC MIXTURES

Sample	*Composition*	*Mo recovered*	*% Error*
High speed steel[a]	4.95% Mo, 4.55% Cr, 1.99% V	4.90–5.04%	−0.67 to +1.18
Alloy steel[b]	0.43% Mo, 2.59% Ni, 0.75% Cr	0.41–0.45%	−5.00 to +5.00
Synthetic mixture	1.86 μg Mo + 1.1 mg Co	1.84 μg	−1.10
Synthetic mixture	1.86 μg Mo + 2.5 mg Co	1.85 μg	−0.50
Synthetic mixture	1.86 μg Mo + 2.1 mg Ag	1.86 μg	0.00
Synthetic mixture	1.86 μg Mo + 1.2 mg Ni	1.83 μg	−1.50
Synthetic mixture	46.52 μg Mo + 2.5 mg Cu + 7.6 mg Ni + 7.2 mg Co	45.86 μg	−1.50
Synthetic mixture	93.04 μg Mo + 9.46 mg Cu + 5.98 mg Ni	94.08 μg	+1.10
Synthetic mixture	465.21 μg Mo + 25.46 mg Cu + 32.72 mg Ni + 72.06 mg Co	456.58 μg	−1.60

[a] Bureau of Analysed Samples Standard 64B.
[b] Bureau of Analysed Samples Standard 60B.

Determination of molybdenum in synthetic mixtures

A synthetic mixture containing molybdenum and associated elements was taken in a 250-ml beaker and treated with aqueous 2% (w/v) 2-mercaptobenzo-γ-thiopyrone reagent with constant stirring until an excess of reagent was present to ensure complete precipitation. The precipitate was digested at about 45–60° and allowed to stand for about 45 min. After filtration through a quantitative filter paper, and washing with aqueous 0.05% (w/v) reagent solution, the filtrate was made up to a predetermined volume. An aliquot of this solution was used to determine the molybdenum content by the proposed method. The results are given in Table II.

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SUMMARY

Molybdenum(VI) in 1.4–3.6 *M* hydrochloric acid medium forms an aceto-

phenone-extractable orange-red complex with the potassium salt of 2-mercapto-benzo-γ-thiopyrone and ammonium thiocyanate in the presence of tin(II) chloride. The limit of identification of the spot test based on this reaction is 0.1 μg of molybdenum (dilution limit, $1:1 \cdot 10^6$). The spectrophotometric method is fairly selective, the sensitivity being 0.005 μg Mo cm^{-2} at 470 nm. The colour system obeys Beer's law; the optimal concentration range is 0.75–8.5 μg Mo ml^{-1}, the relative photometric error being 1.675%. The complex is stable for over 24 h. Common ions can be tolerated in amounts greater than 1000-fold. Interferences of Co^{2+}, Ni^{2+}, Cu^{2+} and Ag^{+} are avoided by complexing these ions with 2-mercaptobenzo-γ-thiopyrone at pH 6–10 and extracting with ethyl acetate or chloroform. The proposed method is applied to the determination of molybdenum in steel and in artificial mixtures.

RÉSUMÉ

Le molybdène(VI) en milieu acide chlorhydrique 1.4–3.6 *M* forme un complexe rouge orange, extractible dans l'acétophénone, avec le sel de potassium du mercapto-2-benzo-γ-thiopyrone et le thiocyanate d'ammonium, en présence de chlorure d'étain(II). La limite d'identification du test à la touche, basé sur cette réaction, est de 0.1 μg de molybdène (limite de dilution $1:1 \cdot 10^6$). La méthode spectrophotométrique est très sélective; la sensibilité est de 0.005 μg Mo cm^{-2} à 470 nm. La loi de Beer s'applique de 0.75 à 8.5 μg Mo ml^{-1}, l'erreur photométrique relative étant 1.675%. Le complexe est stable. Les interférences de Co^{2+}, Ni^{2+}, Cu^{2+} et Ag^{+} sont évitées par extraction de leur complexe mercapto-2-benzo-γ-thiopyrone, à pH 6–10, dans l'acétate d'éthyle ou le chloroforme. Cette méthode a été appliquée au dosage du molybdène dans l'acier et dans des mélanges artificiels.

ZUSAMMENFASSUNG

Molybdän(V) bildet in 1.4–3.6 *M* salzsaurem Medium mit dem Kaliumsalz von 2-Mercaptobenzo-γ-thiopyron und Ammoniumthiocyanat in Gegenwart von Zinn(II)-chlorid einen mit Acetophenon extrahierbaren rotgefärbten Komplex. Die Identifizierungsgrenze der auf dieser Reaktion beruhenden Tüpfelprobe ist 0.1 μg Molybdän (Verdünnungsgrenze $1:1 \cdot 10^6$). Die spektrophotometrische Methode ist leidlich selektiv, die Empfindlichkeit ist 0.005 μg Mo cm^{-2} bei 470 nm. Das System befolgt das Beersche Gesetz; der optimale Konzentrationsbereich ist 0.75–8.5 μg Mo ml^{-1}, wobei der relative photometrische Fehler 1.675% beträgt. Der Komplex ist 24 h lang stabil. Die üblichen Ionen können in mehr als 1000-facher Menge toleriert werden. Störungen durch Co^{2+}, Ni^{2+}, Cu^{2+} und Ag^{+} können durch Komplexierung dieser Ionen mit 2-Mercapto-γ-thiopyron bei pH 6–10 und durch deren Extraktion mit Äthylacetat oder Chloroform vermieden werden. Die vorgeschlagene Methode wird auf die Bestimmung von Molybdän in Stahl und in künstlichen Gemischen angewendet.

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Analytica Chimica Acta, 69 (1974) 313–320

SPECTROPHOTOMETRIC DETERMINATION OF MOLYBDENUM IN ROCKS WITH THIOCYANATE

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An increasing need has arisen for a rapid, accurate method of determining microgram amounts of molybdenum in rocks. The great disparity over the years in molybdenum values[1,2] for the U.S. Geological Survey standard rocks emphasizes this need. As molybdenum content in many rocks is in the 0.5–5 p.p.m. range, atomic absorption spectrometry and most spectrochemical methods lack sufficient sensitivity. Good results have been achieved recently with neutron activation analysis[3], but such procedures are not practical for routine rock analysis.

Many spectrophotometric methods using a wide variety of reagents have been described for the determination of molybdenum. Probably the oldest and most widely used reagent for molybdenum has been thiocyanate[4–7]; however, in recent years, dithiol has received much application in silicate rock analysis[8,9]. Although both thiocyanate and dithiol methods for molybdenum are sufficiently sensitive for most rock analyses, many problems have been associated with their use. The molybdenum thiocyanate color has been notorious[5,7,8] for its lack of stability and reproducibility and for difficulties involved in developing its maximal intensity. Both dithiol and thiocyanate methods are subject to many interferences, often necessitating separation techniques based on precipitations[8] or ion-exchange[9], which make their routine use tedious.

In the method described in this paper, 0.25–3 μg of molybdenum in rock samples is easily and effectively separated from all interfering elements by a series of solvent extractions. It is then reacted with thiocyanate in the presence of copper(I) chloride and ascorbic acid, extracted into a small volume of amyl alcohol, and determined spectrophotometrically. The color obtained is stable, reproducible, and of maximal intensity. For maximal accuracy and precision, the use of ^{99}Mo tracer to determine chemical yields is recommended.

EXPERIMENTAL

Reagents

Standard molybdenum solution. (100 μg Mo ml^{-1}). Dissolve 25.0 mg of molybdenum metal in 10 ml of 1 *M* nitric acid. Dilute to 250 ml in a volumetric flask with 0.1 *M* hydrochloric acid.

TBP solution (20% v/v). Dilute 40 ml of tributyl phosphate to 200 ml with xylene.

α-Benzoinoxime solution. (0.25% w/v). Dissolve 0.25 g of α-benzoinoxime in 100 ml of ethanol. Prepare fresh daily.

Copper(I) chloride solution. (0.05 *M*). Dissolve 0.50 g of copper(I) chloride in 100 ml of hydrochloric acid. Oxidation to copper(II) is unimportant.

Tracer solution. Dilute commercially available carrier-free ^{99}Mo with 2 *M* sodium hydroxide so that 0.01 ml yields about 100,000 counts min^{-1}. The half-life of ^{99}Mo is 67 h and, with this initial counting rate, the tracer will be usable for several weeks.

Apparatus

Counting apparatus. A 3 × 3 in. NaI(Tl) detector coupled to a single-channel analyzer was used to count ^{99}Mo. The window of the analyzer was adjusted to pass the combined 0.78–0.74 MeV γ-rays of the ^{99}Mo and to exclude the 0.14 MeV γ-ray of the ^{99}Tc daughter product.

Solvent extraction apparatus. Extractions were performed by mixing the phases in a separatory funnel with an air stream as described by Greenland and Campbell[10]. To facilitate discarding the upper phase after extraction was complete, a vacuum pump was connected through glass reservoirs to a glass tube (2 mm I.D.) with flexible Tygon tubing. The tip of the glass tube was placed slightly above the interface of the two phases and the upper phase drawn into the discard reservoir. The lower phase was drained when necessary in the normal fashion by opening the funnel stopcock. With this apparatus, all separations of 18 samples (operated concurrently) were completed in less than 2 h.

Procedure

Weigh 500 mg of rock sample containing 0.25–3 μg of molybdenum into a Teflon beaker. Pipet aliquots of standard molybdenum solution to give 0.5, 1.0, 2.0, and 3.0 μg of molybdenum into separate Teflon beakers. If chemical yield corrections are to be made, add enough tracer to samples, standards, and a counting vial to yield at least 10,000 counts min^{-1}. Dilute the tracer in the counting vial to 10 ml with water for a counting standard. Add 10 ml of concentrated nitric acid, 10 ml of 40% hydrofluoric acid, and 1 ml of 72% perchloric acid to the beakers; evaporate overnight on a hot plate at a surface temperature of 110°. Add 15 ml of 4 *M* hydrochloric acid and heat gently to dissolve the salts.

Transfer the solutions to separatory funnels and extract with 10 ml of TBP solution for 2 min. Discard the aqueous phase and wash the organic phase with 5 ml of 3 *M* hydrochloric acid. Add 10 ml of chloroform and 5 ml of water to the funnel and extract for 2 min; discard the organic phase. To the aqueous phase add 5 ml of 4 *M* hydrochloric acid and 5 ml of the α-benzoinoxime solution and mix well. Extract with 10 ml of chloroform for 2 min and discard the aqueous layer. Wash twice with water. Strip the chloroform with 5 ml of conc. hydrochloric acid and discard the organic phase. Wash the aqueous phase with 5 ml of chloroform. Add 10 ml of water, 1.5 ml of freshly prepared aqueous 5% (w/v) ascorbic acid solution, 0.1 ml of the copper(I) solution, and 1.5 ml of aqueous 40% (w/v) potassium thiocyanate solution (prepared weekly), mixing after each addition. Extract with 4.5 ml of amyl alcohol and discard the aqueous

phase. Wash the alcohol sequentially with 5 ml of (1+49) hydrofluoric acid solution, with 5 ml of 0.02 *M* EDTA (disodium salt) solution, and finally with 5 ml of 5% (w/v) ascorbic acid solution, discarding the aqueous phase each time. Add 0.5 ml of ethanol and read the absorbance at 465 nm in a 5-cm cell against water. If no corrections for chemical yield are made, plot the amount, in micrograms, of molybdenum taken for the standard solution against its corresponding absorbance value to obtain a calibration curve. If corrections for yield are made, transfer the solution from the cell to a counting vial and dilute to 10 ml with ethanol. Count samples, standard solutions, and the counting standard.

Calculations for yield corrections

Calculate the mass X in micrograms of molybdenum present in the final standard solution, by the following equation:

$$X = \frac{(R_1 - R_0)}{(R_2 - R_0)} m_1$$

where m_1 is the original mass of molybdenum taken, R_1 is the counting rate of the standard solution, R_2 is the counting rate of the counting standard, and R_0 is the background counting rate. Plot absorbance values against their corresponding values of X to obtain a calibration curve.

Calculate the mass Y in micrograms of molybdenum present in each sample, as follows:

$$Y = \frac{(R_2 - R_0)}{(R_3 - R_0)} m_2$$

where m_2 is the mass of molybdenum present in the final sample solution as obtained from the calibration curve, and R_3 is the counting rate of the sample solution.

RESULTS AND DISCUSSION

Separations

Interference has always been a problem in molybdenum thiocyanate methods, especially when trace amounts are being determined. The greatest sources of interference are from iron, titanium, vanadium, tungsten, and niobium. The series of separations described in this paper effectively isolates molybdenum from these and most other elements commonly found in rocks.

Molybdenum in hydrochloric acid forms chloro complexes that can be extracted into oxygenated solvents and back-extracted into water[11,12]. According to Sandell[11], tributyl phosphate is the best extractant for molybdenum, with extraction coefficients of 4–65 in 1–2 *M* hydrochloric acid. It was found here that the amount of molybdenum extracted into a 50% solution of tributyl phosphate in xylene increases rapidly with hydrochloric acid concentration, with greater than 90% extraction at a hydrochloric acid concentration of 2.5 *M*. By adjusting the hydrochloric acid and tributyl phosphate concentrations, conditions can be set so that molybdenum will be separated from most other elements.

At the concentrations finally chosen—4 *M* hydrochloric acid and 20%

tributyl phosphate—*ca.* 95% of the molybdenum is extracted, while many elements that ordinarily would interfere with the molybdenum thiocyanate color are either not extracted or only partially extracted. By washing with 3 *M* hydrochloric acid, these interfering elements are removed to an even greater extent, effectively separating molybdenum from titanium, niobium, tungsten, and, to a lesser degree, iron and vanadium.

Extraction of molybdenum and tungsten α-benzoinoximates into chloroform as a means of separation from other elements is widely used[6,11,12]. The previous removal of tungsten during the tributyl phosphate extraction makes the α-benzoinoxime extraction selective for molybdenum. Approximately 90% of the molybdenum is extracted under the conditions specified in this method. Previous colorimetric methods[6,11] using the α-benzoinoxime extraction of molybdenum required a tedious decomposition of the chloroform layer to recover the molybdenum. Peng and Sandell[6] noted that α-benzoinoxime was unstable in strongly acidic solutions. An attempt, therefore, was made to strip the chloroform layer with concentrated hydrochloric acid; it was found that *ca.* 95% of the molybdenum was recovered.

Development of the molybdenum thiocyanate color

Many problems have been associated with the molybdenum thiocyanate color: stability, reproducibility, and the development of the color itself. Most authors agree that the color formed is due to a pentavalent molybdenum complex with thiocyanate and that the key to color development is the proper reduction of Mo(VI) to Mo(V)[5–7,11,12]. Most methods use tin(II) chloride alone[4,7], or in conjunction with either copper(II)[5] or iron(II)[6,11,12], to accomplish this reduction. A solvent extraction study[13] of molybdenum chloride and molybdenum thiocyanate complexes suggests that the function of reducing agents in the production of the colored molybdenum thiocyanate species is to catalyze a color isomerization reaction of hexavalent molybdenum rather than to stabilize pentavalent molybdenum.

In any case, it was found that the use of strong reducing agents, such as tin(II) chloride, invariably led to problems in the determination of microgram amounts of molybdenum. The use of iron(II) was also unsatisfactory because it merely introduced a source of interference.

The use of weak reducing agents, such as copper(I) chloride or ascorbic acid, seems to give the best color, although the mechanisms involved in the color development differ depending on which reducing agent is used[13]. Unlike ascorbic acid, the color development with copper(I) chloride is dependent upon acid concentration. This dependence probably is due to reactions between copper(I) and thiocyanate rather than due to a dependence of the colored species itself, because the species formed by ascorbic acid and that formed by copper(I) chloride were found to be identical, absorbing at the same wavelength and having the same molar absorptivities. In both cases, maximal absorbance was obtained at thiocyanate concentrations greater than 3%.

Copper(I) chloride was found to give a more reproducible color than ascorbic acid. However, at the acid concentration necessary to obtain a reproducible maximal color with copper(I) chloride—6 *M* or greater—the thiocyanate

itself gave a colored blank. It was found that by combining ascorbic acid and copper(I) chloride, a stable, reproducible color of maximal intensity could be obtained in a hydrochloric acid concentration of 4 *M* or less without interference from thiocyanate. Absorbance values for 3 μg of molybdenum on eleven different days averaged 0.581 ± 0.023, giving an analytical error from day to day of about 4.0%. The mean molar absorptivity was 18,600, and the color did not change over a 24-h period. Amyl alcohol was chosen to extract and concentrate the molybdenum thiocyanate because of its low volatility, so that a final dilution to volume was unnecessary.

The final washes of the alcohol solution are a precaution against any interferences that might have survived the separations. The hydrofluoric acid wash removes niobium, titanium, and tungsten; the EDTA wash complexes iron and vanadium; and the ascorbic acid wash removes any yellow iron(III)–EDTA complex and reduces any remaining traces of iron(III).

Analysis of rocks

The precision of this method, with and without yield correction, was tested by analyzing replicates from each of three bottles of seven of the U.S. Geological Survey standard rocks. The various aliquots were randomized and analyzed over a period of several weeks to maximize random error and to avoid any correlation of errors. The results of these analyses are given in Table I. A statistical study of the results, with and without yield corrections, is shown in Table II.

LE I

LYSES OF U.S. GEOLOGICAL SURVEY STANDARD ROCKS

k	*Molybdenum (μg g^{-1})*[a]	
	Yield corrections	*No yield corrections*
1, Quartz latite	(2.4, 2.5, 2.5)(2.3, 2.4, 2.4)(2.5, 2.4, 2.3)	(2.3, 2.3, 2.5)(2.1, 2.3, 2.4)(2.3, 2.3, 2.1)
-1, Basalt	(1.5, 1.5, 1.3)(1.5, 1.5, 1.4)(1.5, 1.3, 1.3)	(1.5, 1.4, 1.2)(1.4, 1.5, 1.2)(1.3, 1.3, 1.3)
-1, Nephiline syenite	(5.1,.5.1, 5.1)(5.0, 5.2, 4.6)(5.0, 4.9, 4.8)	(4.7, 4.4, 5.0)(4.8, 5.0, 4.2)(4.9, 4.7, 4.8)
-1, Granodiorite	(0.29, 0.40, 0.41)(0.29, 0.32, 0.36) (0.33, 0.49, 0.42)	(0.28, 0.48, 0.58)(0.34, 0.37, 0.48) (0.30, 0.55, 0.43)
-1, Rhyolite	(2.5, 2.4, 2.4)((2.4, 2.4, 2.6)(2.4, 2.5, 2.4)	(2.5, 2.3, 2.3)(2.3, 2.3, 2.1)(2.3, 2.3, 2.4)
O-1, Basalt	(1.0, 0.97, 0.95)(0.97, 0.94, 0.95) (0.92, 0.94, 0.90)	(0.98, 0.84, 1.0)(0.95, 0.80, 0.82) (0.77, 0.93, 0.89)
-1, Andesite	(2.2, 2.0, 1.9)(2.0, 2.2, 2.0)(2.1, 2.0, 1.8)	(2.1, 2.1, 1.8)(1.8, 2.0, 2.1) (1.8, 1.6, 1.7)

its of given bottle in parenthesis.

The relative error, calculated from the analyses by a one-way analysis of variance, is less than 10% for all rocks except GSP-1 (which contains less than 0.5 p.p.m. Mo). The error when yield corrections are omitted is only slightly greater than when they are included.

For maximal accuracy and precision, the use of ^{99}Mo tracer to determine chemical yields is recommended. However, for routine silicate rock analysis, the use of tracer is probably unnecessary. Although total losses in the procedure are as great as 20%, they are very consistent from sample to sample. The mean percent

TABLE II

STATISTICAL STUDY OF MOLYBDENUM ANALYTICAL DATA FOR U.S. GEOLOGICAL SUR STANDARD ROCKS

Rock	*Mean molybdenum content ($\mu g\ g^{-1}$)*		*Standard deviation*		*Error (%)*	
	Yield correction	*No yield correction*	*Yield correction*	*No yield correction*	*Yield correction*	*No yie correct*
QL-1, Quartz latite	2.41	2.29	0.075	0.13	3.1	5.6
BCR-1, Basalt	1.42	1.34	0.10	0.12	7.0	9.3
STM-1, Nephiline syenite	4.98	4.72	0.18	0.30	3.7	6.4
GSP-1, Granodiorite	0.37	0.42	0.063	0.12	17	29
RGM-1, Rhyolite	2.44	2.31	0.082	0.10	3.3	4.3
BHVO-1, Basalt	0.95	0.89	0.020	0.084	2.2	9.5
AGV-1, Andesite	2.02	1.89	0.14	0.14	7.0	7.7

yield and standard deviation of the 63 analyses in Table I were 82.39 ± 4.40. For 48 standard solutions over the same time period, the mean percent yield and standard deviation were 86.80 ± 3.05. For ordinary analysis, these variations in yield are not great enough to cause significant error. The small systematic error can be obviated by using the U.S. Geological Survey standard rocks to prepare the standard curve, and the random error can be reduced by preparing a new standard curve with each batch of samples.

To determine the accuracy of the method, the mean values, with and without yield corrections, for five of the U.S. Geological Survey standard rocks were compared with those obtained by other methods. Values for molybdenum quoted by Fleischer[1] and Flanagan[2] vary to such an extent that any attempt to determine the accuracy of the method based on them is impossible. Table III shows, however, that the present molybdenum values agree very well with the recent values of Kawabuchi and Kuroda[9], obtained spectrophotometrically with dithiol after ion-exchange separation, and with those of Steinnes[3], obtained by epithermal neutron activation analysis.

TABLE III

COMPARISON OF MOLYBDENUM CONTENT VALUES ($\mu g\ g^{-1}$) IN U.S. GEOLOGICAL SURVEY STANDARD ROCKS WITH VALUES DERIVED FROM OTHER METHODS

Author	*Method*	*G-1*	*G-2*	*BCR-1*	*AGV-1*	*GSP-1*
This work	Photometric (Yield corrections)	5.2	<0.2	1.4	2.0	0.37
This work	Photometric (No yield corrections)	5.0	<0.3	1.3	1.9	0.42
Kawabuchi and Kuroda (ref. 9)	Photometric	5.45	0.15	1.21	1.66	0.30
Steinnes (ref. 3)	Epithermal neutron activation analysis	—	0.13	1.15	1.71	0.27

SUMMARY

A rapid procedure for the determination of microgram amounts of molybdenum in rocks is described. After acid decomposition, molybdenum is extracted from a hydrochloric acid solution into xylene with tributyl phosphate. After back-extraction with water, molybdenum is extracted as the α-benzoinoximate into chloroform, stripped into hydrochloric acid, extracted as the thiocyanate into amyl alcohol, and determined spectrophotometrically. The molybdenum thiocyanate color produced is stable, sensitive, and reproducible. Results of analyses of several of the U.S. Geological Survey standard rocks are given.

RÉSUMÉ

On décrit une méthode de dosage rapide du molybdène dans les roches, en quantité de l'ordre du microgramme. Après décomposition acide, le molybdène est extrait, en milieu acide chlorhydrique, dans le xylène, au moyen de tributylphosphate. Après extraction en retour dans l'eau, le molybdène est extrait dans le chloroforme sous forme de benzoinoximate, strippé dans l'acide chlorhydrique, extrait dans l'alcool amylique sous forme de thiocyanate et dosé par spectrophotométrie; la coloration de thiocyanate de molybdène produite est stable, sensible et reproductible. Les résultats d'analyse obtenus pour plusieurs roches standard de l'U.S. Geological Survey sont donnés.

ZUSAMMENFASSUNG

Es wird ein schnelles Verfahren für die Bestimmung von Mikrogramm-Mengen Molybdän in Gesteinen beschrieben. Nach der Zersetzung durch Säure wird das Molybdän aus salzsaurer Lösung mit Tributylphosphat-Xylol extrahiert. Nach Reextraktion mit Wasser wird das Molybdän als α-Benzoinoximat mit Chloroform extrahiert, wieder reextrahiert mit Salzsäure, schliesslich als Thiocyanat mit Amylalkohol extrahiert und spektrophotometrisch bestimmt. Die Molybdänthiocyanat-Färbung ist beständig, empfindlich und reproduzierbar. Die Analysenergebnisse von verschiedenen U.S. Geological Survey-Standardgesteinen werden vorgelegt.

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THE SPECTROPHOTOMETRIC DETERMINATION OF ANIONS BY SOLVENT EXTRACTION WITH METAL CHELATE CATIONS

PART L. SPECTROPHOTOMETRIC DETERMINATION OF IODIDE BY SOLVENT EXTRACTION AS BIS(NEOCUPROINE)COPPER(I) TRI-IODIDE*

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Systematic studies have already been reported on the colorimetric[2,3] or atomic-absorption spectrometric[4] determination of various anions by solvent extraction of ion-pairs with ferroin[2] or its analogs such as tris(2,2'-bipyridine)-iron(II)[3], bis(neocuproine**) copper(I)[4,5] and bis(cuproine***)copper(I)[5], etc. In the case of the copper chelate, the copper(II) ion should be reduced to copper(I) in the aqueous phase before extraction. Iodide ion could not be extracted in the copper(I) system because the anion reacts with copper(I) to form a copper iodide precipitate, even when present in trace amounts. Hall *et al.*[6], however, isolated the bis(neocuproine)copper(I) tri-iodide salt from aqueous medium by reaction of bis(neocuproine)copper(II) with iodide anion. It therefore seemed feasible to extract this ion pair of bis(neocuproine)copper(I)tri-iodide into an organic solvent and to determine the amount of iodide by measuring the absorbance of the organic phase. The absorbance of the tri-iodide ion at 370 nm is about three times higher than that arising from bis(neocuproine)copper(I) at 455 nm. The method proposed here has the advantage of being not only consistent and relatively sensitive, but also free from interference by other halide ions.

There are several methods for the spectrophotometric determination of trace amounts of iodide in aqueous systems through the catalytic action of iodide[7,8]. However, these procedures tend to be rather delicate because of the poised reactions involved. A different ion-pair extraction method has been based on the use of cationic dyestuffs such as neutral red[9].

EXPERIMENTAL

Apparatus

The spectrophotometric measurements were made with a Hitachi Model 139 spectrophotometer with 10-mm quartz cells. The atomic-absorption measurements

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* For Part XLIX see ref. 1.

** Neocuproine: 2,9-dimethyl-1,10-phenanthroline.

*** Cuproine: 2,2'-biquinoline.

were made with a Nippon Jarrell-Ash Model AA-1 EW atomic-absorption spectrophotometer equipped with a Hamamatsu TV Model L 233 copper hollow-cathode lamp (324.8 nm). γ-Ray counting of ^{131}I was performed with an Aloka Model PSM 801 γ-ray spectrometer fitted with a 1.5×1-in. NaI(Tl) crystal. The pH values were measured with a Hitachi-Horiba Model H-5 pH meter. An Iwaki Model KM shaker was used for extraction.

Reagents

Bis(neocuproine)copper(II) sulphate solution. A $1.0 \cdot 10^{-2}$ *M* solution was prepared by dissolving 0.625 g of copper sulphate pentahydrate and 1.15 g of neocuproine (recrystallized from water–ethanol mixture; m.p. 159–162°) and diluting to 250 ml with water. The resulting solution contains neocuproine in 0.2 mol excess to 1.0 mol of copper to prevent appreciable dissociation of the chelate cation. This solution should be stored in a refrigerator.

Standard iodide solution. A $1.5 \cdot 10^{-2}$ *M* stock solution was prepared by dissolving 0.6225 g of potassium iodide and diluting to 250 ml with water. Solutions of suitable concentration were prepared by further dilution.

Radioactive iodide solution. A radioactive iodide solution was prepared with NaI–^{131}I (Japan Radioisotope Association).

Buffer solution. The phosphate buffer was 0.25 *M* in potassium dihydrogenphosphate and was adjusted to pH 4 with 0.1 *M* sulphuric acid.

Unless stated otherwise, all chemicals were of reagent grade and the water used was passed through a monobed ion-exchange resin.

Recommended procedure

Take 1 ml of the bis(neocuproine)copper(II) sulphate solution ($1.0 \cdot 10^{-2}$ *M*), 10 ml of the buffer solution and 2 ml of iodide solution ($3.0 \cdot 10^{-4}$ *M*) into a 50-ml separating funnel. Dilute the mixture to 30 ml with water and shake the funnel for 5 min with 10 ml of chlorobenzene. After 5-min standing, separate the organic phase. Measure the absorbance at 370 nm (or 455 nm) against a reagent blank used as reference.

RESULTS AND DISCUSSION

Absorption spectra

Absorption spectra of the extract and the reagent blank are shown in Fig. 1. When iodide ion is present in the aqueous phase, the spectrum of the extract has a strong absorbance maximum at 370 nm and a shoulder at about 455 nm; the latter band corresponds to the charge transfer spectrum of bis(neocuproine)-copper(I)[3,5]. The absorption spectrum of bis(neocuproine)copper(I) perchlorate salt dissolved in chlorobenzene is also given in Fig. 1 for comparison. The strong absorption band centered at 370 nm can be assigned to the absorption of tri-iodide anion as will be discussed later. In this work, absorbances were measured at both 370 and 455 nm for comparison.

Effect of pH

The influence of pH on the degree of extraction was studied by extracting

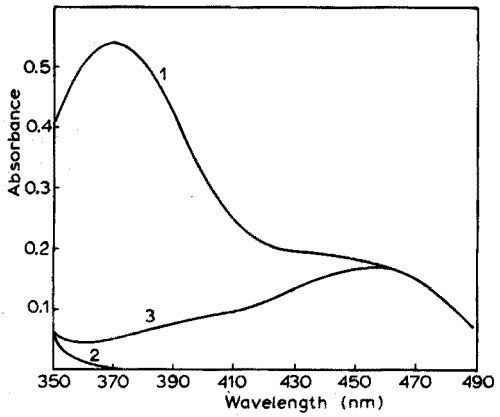


Fig. 1. Absorption spectra. (1) Chlorobenzene extract with $2.0 \cdot 10^{-5}$ *M* iodide; (2) chlorobenzene extract without iodide (reagent blank); (3) $2.0 \cdot 10^{-5}$ *M* bis(neocuproine)copper(I) perchlorate in chlorobenzene solution. Reference: (1), reagent blank; (2) and (3), chlorobenzene.

the iodide from a series of aqueous solutions buffered in the pH range 2–8. The buffer solutions used were prepared by mixing 0.25 *M* potassium dihydrogenphosphate or 0.25 *M* disodium hydrogenphosphate with a dilute sulphuric acid or sodium hydroxide solution. As Fig. 2 shows, the absorbances at both 370 and 455 nm are maximal and constant over the pH range 3–5. In more acidic or more alkaline solutions, each absorbance tends to decrease.

Effect of bis(neocuproine)copper(II) sulphate concentration

With other variables constant, different amounts of bis(neocuproine)copper(II) sulphate were added to an aqueous solution of iodide and extraction was done as described above. As can be seen in Fig. 3, the chelate should be present in at least 5-fold molar concentration relative to iodide to obtain the maximal constant color response. Therefore, the chelate concentration was usually kept at $3.3 \cdot 10^{-4}$ *M*.

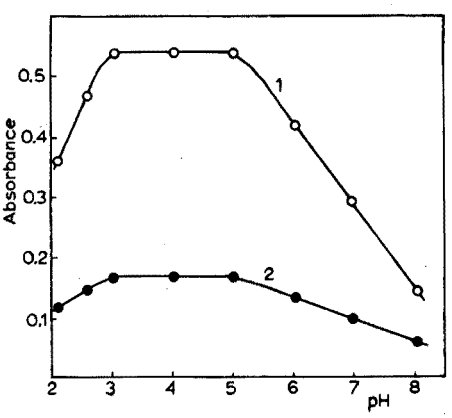


Fig. 2. Effect of pH on net absorbance for $2.0 \cdot 10^{-5}$ *M* iodide. (1) 370 nm, (2) 455 nm.

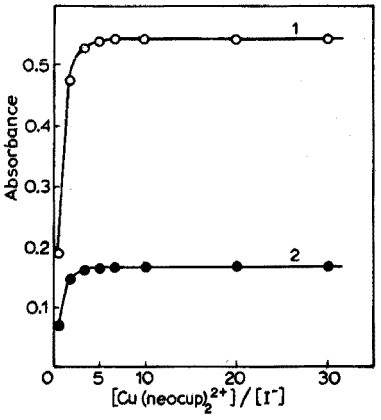


Fig. 3. Effect of concentration of bis(neocuproine)copper(II) on net absorbance for $2.0 \cdot 10^{-5}$ *M* iodide. (1) 370 nm, (2) 455 nm.

Solvent

The behavior of various solvents in the extraction was investigated. Solvents could be classified into the following three categories:

(1) Those for which the presence of iodide leads to a considerable increase in the extraction. The order of relative extractability is as follows (the values in parentheses are the absorbance ratios relative to chlorobenzene): chlorobenzene (1.00), 1,2-dichloroethane (0.93), *o*- and *m*-dichlorobenzene (0.87), 1,1-dichloroethane (0.83), chloroform (0.80), benzene (0.63), methyl isobutyl ketone (0.45), benzonitrile (0.33), and 1,1,1-trichloroethane (0.32).

(2) Those which do not extract even in the presence of iodide: ethyl acetate, *n*-butyl acetate, isobutyl acetate or isoamyl acetate.

(3) Those with which the chelate cation is extractable even without iodide: tetrachloroethane, *n*-amyl alcohol, isoamyl alcohol, *n*-hexyl alcohol or cyclohexyl alcohol.

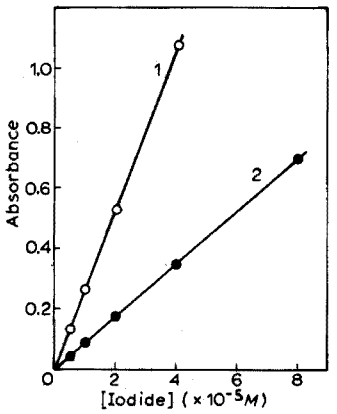
The extracted species are not stable in chloroform or in some chloro-substituted ethanes. In this work, chlorobenzene was selected as the suitable solvent.

Effect of shaking time

The shaking time for the extraction was varied from 1 to 30 min. Extraction was quantitative with 2 min of shaking. Continued shaking up to 30 min produced no further change of absorbance.

Calibration curve

Figure 4 shows the calibration curves obtained by the procedure described above. The absorbance of the organic phase followed Beer's law in the tested concentration range $5 \cdot 10^{-6}$–$4 \cdot 10^{-5}$ *M*. The sensitivity at 370 nm was about three times higher than that at 455 nm. The precision was estimated from twelve results for $2.0 \cdot 10^{-5}$ *M* iodide. The relative standard deviations were 1.0 and 1.1% for measurements at 370 and 455 nm, respectively.



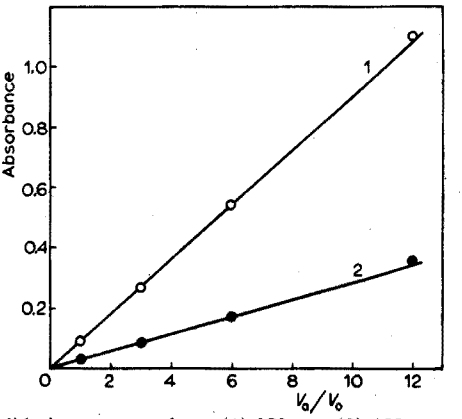


Fig. 4. Plots for net absorbance *vs.* concentration of iodide in aqueous phase. (1) 370 nm, (2) 455 nm.

Fig. 5. Effect of phase-volume ratio on net absorbance for $1.0 \cdot 10^{-5}$ *M* iodide. Organic phase: 10 ml, (1) 370 nm, (2) 455 nm.

Effect of phase volume ratio on sensitivity

The effect of the volume ratio of aqueous phase to organic phase (V_a/V_0) was investigated by extracting iodide ($1.0 \cdot 10^{-5}$ *M*) from various amounts of aqueous solution with 10 ml of chlorobenzene. The results are shown in Fig. 5. As expected, the apparent sensitivity was enhanced linearly with increase in the volume ratio V_a/V_0.

Interference

Table I shows the effect of diverse ions on the recovery of iodide.

TABLE I

EFFECT OF DIVERSE IONS

(Anions added as potassium salt, cations as sulfate, except where indicated)

Ion	*[Ion]/[I⁻]*[a]	*Recovery (%)*		*Ion*	*[Ion]/[I⁻]*[a]	*Recovery (%)*	
		370 nm	*455 nm*			*370 nm*	*455 nm*
F^-	2000	100	100	Mn(II)	500	103	102
Cl^-	2000	100	104	Fe(III)	200	101	98
Br^-	50	99	119	Fe(II)[c]	0.1	90	95
ClO_4^-	1	123	239	Co(II)	500	100	100
	10	112	268	Ni(II)	500	100	99
CN^-	1	92	99	Mg	500	101	100
SCN^-	1	105	136	Ca	100	98	96
NO_3^-	10	102	113	Al[d]	500	101	103
IO_3^-	100	103	104	Pb[e]	50	99	96
SiO_3^{2-}[b]	2000	104	142	Ag(I)	1	0	0
Zn	100	97	100	Hg(II)	1	0	0
Cd	5	98	97				

[a] $[I^-]$: $2.0 \cdot 10^{-5}$ *M*.
[b] Sodium salt.
[c] Mohr's salt.
[d] Potash alum.
[e] Acetate salt.

When measurements are made at 370 nm, fluoride and chloride have no effect if added in 2000-fold molar amounts relative to iodide. Bromide in 50-fold amounts compared to iodide is tolerable; large amounts of silicate, sulphate and phosphate do not interfere. Cyanide and thiocyanate react with copper to form a precipitate and therefore interfere when they are present in equimolar concentration levels. Perchlorate, one of the most extractable species in the system with the metal chelate cation including ferroin, gave a considerable positive effect when the absorbance measurement was done at 455 nm, but a less positive effect at 370 nm. As for cations, silver, mercury(II) and cadmium, which react with iodide, have a large negative effect even if they are present in extremely low concentrations. These results show that the interference of diverse ions is much less at 370 nm than at 455 nm. Hence, measuring the absorbance at 370 nm should normally be used for the determination.

Percentage extraction

The percentage extraction of iodide was determined by the radioactive tracer method with an ^{131}I-labelled iodide solution. One milliliter of each phase was taken after extraction, and the γ-activities were measured with a scintillation counter. The results are shown in Table II together with the distribution ratio (log D) derived from each extraction percentage. The data indicate that the extraction of iodide is essentially quantitative. It was confirmed that such trace amounts of iodide ion were not appreciably extracted into chlorobenzene when the bis(neocuproine)copper(II) cation was absent in the aqueous phase.

Composition of extracted species

In order to examine the composition of the extracted species, continuous variation plots were first made in two series of extraction. As for the determination of the chelate formation ratio of neocuproine to copper, the concentration of iodide and the total concentration of copper and neocuproine were kept constant, and then the extraction was made with varying mol ratios of copper to neocuproine. The resulting curves show that the ratio in the extract balances at 1:2 (Fig. 6, A). By similar plots, the ratio of iodine to copper was determined to be

TABLE II

PERCENTAGE EXTRACTION AND DISTRIBUTION RATIO

Iodide (M)	$V_a:V_0$	*E (%)*	*log D*
$2\cdot10^{-5}$	1:1	97.0±0.8	32.4±0.1
	3:1	90.4±0.6	
	6:1	87.7±0.4	
$4\cdot10^{-5}$	1:1	97.2±0.8	35.1±0.1

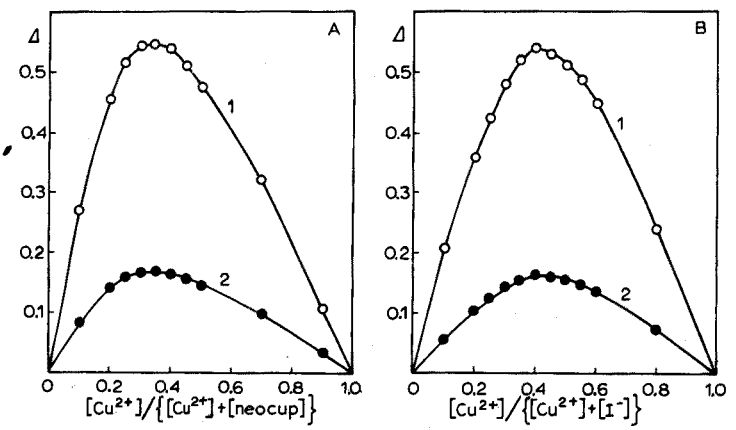


Fig. 6. Continuous variation plots at pH 4. (A) For neocuproine–copper(II): total concentration of neocuproine and copper(II), $3.0\cdot10^{-4}$ M; concentration of iodide, $1.0\cdot10^{-3}$ M. (B) For copper(II)–iodide: total concentration of bis(neocuproine)copper(II) and iodide, $3.0\cdot10^{-4}$ M; aqueous phase 10 ml, organic phase 10 ml; (1) 370 nm, (2) 455 nm, neocup = neocuproine.

3:2 (Fig. 6, B). From these results, the reaction and extraction mechanism can be proposed as follows:

$$2\,Cu(neocup)_2^{2+} + 3\,I^- \rightarrow Cu(neocup)_2^+ \cdot I_3^- + Cu(neocup)_2^+$$

Thus the ion-pair of $Cu(neocup)_2^+ \cdot I_3^-$ may be extracted into an organic (chlorobenzene) phase and one mol of bis(neocuproine)copper(I) cation per one mol of the ion-pair remains in the aqueous phase. To confirm such a mechanism to be correct, the concentration of copper in the organic phase after extraction was determined by atomic-absorption spectrometry; bis(neocuproine)copper(I) perchlorate salt was used as a standard material for the calibration of the concentration. It was found that the amount of copper transferred into organic phase was equal to one-third of the amount of iodide in the initial aqueous phase. The results, together with those for the percentage extraction of iodide, lead to the same conclusion for the extraction mechanism as that suggested by the continuous variation method.

Another experiment was carried out as follows. Sodium perchlorate was added to an aqueous phase separated after the extraction of iodide, and then extraction was done again with 10 ml of chlorobenzene. The absorbance of the extract at 455 nm in the second extraction was nearly equal to that in the first extraction, while the absorbance at 370 nm was much lower than that in the first extraction. This shows that in the second extraction, perchlorate ion was transferred to chlorobenzene as the ion-pair with the bis(neocuproine)copper(I) which remained in the aqueous phase after the first extraction. When only perchlorate ion was added to bis(neocuproine)copper(II) solution, no reduction of the bivalent chelate cation occurred and therefore perchlorate ion was not extracted. The absorption spectra of the extracts obtained for the iodide extraction system have a strong maximum at 370 nm as described above, while this absorption band is not observed for the spectrum of a chlorobenzene solution of bis(neocuproine)-copper(I) perchlorate. On the other hand, the spectrum of tri-iodide ion in aqueous solution shows the same absorption profile in the u.v. region as those obtained by this extraction.

From these facts, it was concluded that the extraction mechanism is acceptable, and the absorption band of the extract at 370 nm is assigned to the charge-transfer spectrum due to tri-iodide ion of the ion-pair extracted.

SUMMARY

A spectrophotometric method is proposed for the determination of small amounts of iodide. The method is based on the reduction of bis(neocuproine)-copper(II) to the monovalent copper chelate cation in the aqueous phase by iodide ion and subsequent solvent extraction into chlorobenzene of the ion-pair formed between bis(neocuproine)copper(I) cation and the tri-iodide anion. At least a 5-fold molar amount of the copper(II) chelate cation, relative to iodide, is needed, and the optimal pH range is 3–5. The absorbance of the extract at 370 nm is a linear function of iodide concentration in the aqueous phase over the range $5 \cdot 10^{-6}$–$4 \cdot 10^{-5}$ *M* (*ca.* 0.6–5 p.p.m.). The relative standard deviation was 1.0%. Large amounts of fluoride and chloride (2000-fold molar) and bromide (50-fold) did not appreciably affect the determination of iodide. The extraction mechanism is elucidated.

RÉSUMÉ

Une méthode spectrophotométrique est proposée pour le dosage de faibles quantités d'iodure. Elle est basée sur la réduction du bis(néocuproïne)cuivre(II) en cation chélate cuivre monovalent, par l'ion iodure; on procède ensuite à une extraction dans le chlorobenzène. La gamme de pH optimale va de 3 à 5: L'absorption mesurée à 370 nm est une fonction linéaire de la concentration en iodure, en phase aqueuse, de $5 \cdot 10^{-6}$ à $4 \cdot 10^{-5}$ *M* (env. 0.6–5 p.p.m.), avec une déviation standard relative de 1.0%. De grandes quantités de fluorure et de chlorure (2000 fois molaires) et de bromure (50 fois) ne présentent pas d'interférences appréciables. Une explication est donnée du mécanisme d'extraction.

ZUSAMMENFASSUNG

Es wird eine spektrophotometrische Methode für die Bestimmung kleiner Mengen Jodid vorgeschlagen. Die Methode beruht auf der Reduktion von Bis-(neocuproin)kupfer(II) zu dem einwertigen Kupferchelat-Kation in wässriger Phase durch Jodidionen und der anschliessenden Extraktion des zwischen Bis(neocuproin)-kupfer(I)-Kation und dem Trijodid-Anion gebildeten Ionenpaars mit Chlorbenzol. Es wird eine mindestens 5-fache molare Menge des Kupfer(II)-Chelat-Kations in Bezug auf Jodid benötigt, und der optimale pH-Bereich ist 3–5. Die Extinktion des Extraktes bei 370 nm ist eine lineare Funktion der Jodidkonzentration in der wässrigen Phase im Bereich $5 \cdot 10^{-6}$–$4 \cdot 10^{-5}$ *M* (*ca.* 0.6–5 p.p.m.). Die relative Standardabweichung war 1.0%. Grosse Mengen von Fluorid und Chlorid (2000-fach, molar) und Bromid (50-fach) beeinflussten die Jodidbestimmung nicht merklich. Der Extraktionsmechanismus wird erläutert.

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CHELATE VON β-DICARBONYLVERBINDUNGEN UND IHREN DERIVATEN

TEIL XXXVI.* DIE EXTRAKTIONSPHOTOMETRISCHE BESTIMMUNG VON CADMIUM MIT THIODIBENZOYLMETHAN IM VERGLEICH MIT ANDEREN SPEZIALREAGENZIEN

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Als Methode der Wahl zur photometrischen Cadmium-Bestimmung gilt allgemein die Bestimmung mit Dithizon[2]. Die grosse Störanfälligkeit dieses hochselektiven und empfindlichen Verfahrens führte jedoch, in den letzten Jahren verstärkt, zur Suche nach in Selektivität und Empfindlichkeit gleichwertigen aber deutlich weniger störanfälligen Methoden zur photometrischen Bestimmung von Cadmium. Unter diesem Aspekt wurde die Brauchbarkeit einer Reihe spezieller Reagenzien für die Cadmiumanalyse untersucht. Um unmittelbar vergleichbare Ergebnisse zu erhalten, führten wir die Testung unter einheitlichen Bedingungen, wie sie im wesentlichen von Gottschalk[3] vorgeschlagen wurden, durch. Für die Cadmium-Bestimmung erstmalig wurden neben Thiodibenzoylmethan Calmagit[4] und Benzthiazolyl-(2-azo-1′)-2′-naphthol (BTAN)[4] eingesetzt. Zum Vergleich untersuchten wir auch die Analysenverfahren mit Dithizon[5] und Pyridylazoresorcin (PAR)[6] sowie die Bestimmung mit 6-Brombenzthiazolyl-(2-azo-1′)-2′-naphthol (BBTAN)[7] die von Schkrobot und Bakinowskaja[8] beim Vergleich mit den Reagenzien Cadion, Cadion IREA, Kristallviolett und Rhodamin C als dies vorteilhafteste befunden wurde.

DIE EXTRAKTIONSPHOTOMETRISCHE CADMIUM-BESTIMMUNG MIT THIODIBENZOYLMETHAN

Auf die Anwendung von Thiodibenzoylmethan zur Bestimmung von Metallspuren wurde bereits mehrfach hingewiesen[9–12]. Thiodibenzoylmethan bildet bevorzugt mit thiophilen Metallen extrahierbare Chelate. Die hohen molaren Extinktionskoeffizienten im sichtbaren Spektralbereich, die Schwerlöslichkeit der Chelate in Wasser, ihre gute Löslichkeit in inerten organischen Lösungsmitteln und die grosse Beständigkeit ermöglichen die Verwendung von Thiodibenzoylmethan als extraktionsphotometrisches Reagens.

* Siehe Literatur 1 für Teil XXXV.

Cadmium kann mit Thiodibenzoylmethan in einfacher Weise und hochselektiv bestimmt werden, wenn störende Fremdmetalle durch Maskierung in der wässrigen Phase zurückgehalten werden. Dabei hat sich der Einsatz von EDTA und Cyanid als Maskierungsmittel bei anschliessender selektiver Demaskierung des Cadmiums durch Chloralhydrat bewährt[13–15], Anstelle von Chloralhydrat wird auch häufig Formaldehyd eingesetzt, welcher jedoch schlechter handhabbar ist[16]. Das Arbeiten mit Chloralhydrat ist insofern vorteilhaft, als die Substanz leicht in reiner Form erhältlich ist, nicht polymerisiert und jederzeit Lösungen hoher Wirksamkeit hergestellt werden können. Die photometrische Cadmiumbestimmung wird bei dieser Arbeitsweise nur durch vorhandenes Zink gestört. Durch mehrfaches Behandeln des organischen Extraktes mit Alkalien lässt sich diese Störung in ähnlicher Weise wie überschüssiger Ligand beseitigen.

Geräte und Reagenzien

Zur Durchführung der photometrischen Messungen stand das Spektralkolorimeter Spekol mit Zusatzverstärker ZV (VEB Carl Zeiss Jena) zur Verfügung. Die pH-Messungen wurden am pH-Meter OP 204 mit Einstab-Glaselektrode (Fa. Radelkis, Budapest) durchgeführt. Die Dosierung kleiner Flüssigkeitsmengen erfolgte mit Hilfe von Kolbenbüretten. Für die statistische Auswertung der Analysenergebnisse kam der Digitalrechner SER 2 zum Einsatz.

Als Cadmiumstandardlösung diente eine $2 \cdot 10^{-5}$ *M* Cadmiumsulfat-Lösung, die aus $CdSO_4 \cdot 8/3\ H_2O$ bereitet wurde. Die Formelmasse der Standardsubstanz ergab sich durch mehrfache komplexometrische Titration (gegen Methylthymolblau) zu 256.52 ± 0.12.

Thiodibenzoylmethan (Schmp. 83–84°)[17] kam in Form einer $3 \cdot 10^{-3}$ *M* Lösung in *p.a.* Benzol zum Einsatz. Für alle weiteren Zusätze wurden entsprechende Lösungen von p.a. Chemikalien in zweifach destilliertem Wasser verwendet. Die Reagenslösung ist bei Aufbewahrung in dunklen Flaschen drei Tage verwendbar. Unter dem Einfluss des Sonnenlichtes bildet sich bei längerem Stehen das Disulfid des Thiodibenzoylmethans, welches die Extinktion deutlich beeinflusst. Bei Lösungen des Cadmiumchelates in Benzol konnte im Verlauf von 10 Stunden keine Änderung der Extinktion festgestellt werden.

Messlicht

Der Cadmiumkomplex mit Thiodibenzoylmethan ist von intensiver gelber Farbe. Sein Absorptionsspektrum ist zusammen mit dem des freien Liganden in Fig. 1 dargestellt. Danach ist das Absorptionsmaximum bei 400 nm ($\varepsilon_\lambda \approx 30000$) für die photometrische Auswertung gut geeignet, jedoch muss überschüssiger Ligand vor der Messung mit Alkali aus der organischen Phase reextrahiert werden.

Abhängigkeit der Extraktion von pH-Wert, Reagenskonzentration und Schüttelzeit

Zur Untersuchung des pH-Einflusses auf die Cadmiumextraktion wurden jeweils 5 ml Cadmium-Standard-Lösung mit 10 ml Reagenslösung und 10 ml Pufferlösung nach Schwabe[18] (pH = 1 bis pH = 12) 3 Minuten geschüttelt. Die wässrige Phase der jeweiligen Probe wurde verworfen und die verbleibende benzolische Lösung zweimal eine Minute mit je 10 ml 3 *M* Kalilauge extrahiert. Nach dem Zentrifugieren wurde die Extinktion der organischen Schicht bei 400 nm und 1

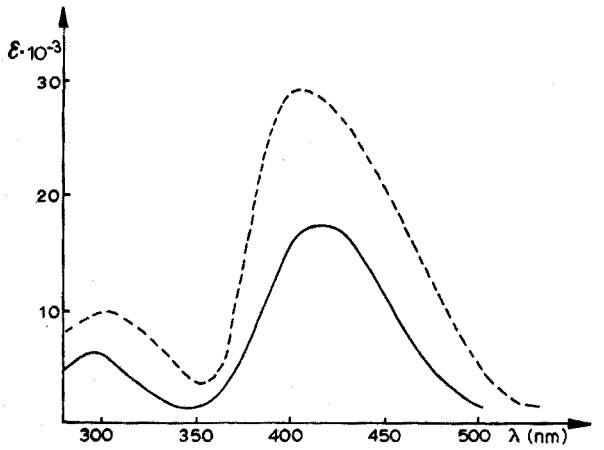

Fig. 1. Absorptionsspektren von Thiodibenzoylmethan (——) und seinem Cadmiumchelat (---).

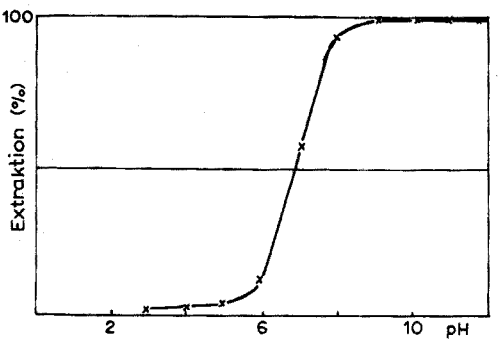

Fig. 2. pH-Abhängigkeit der Extraktion von Cadmium mit Thiodibenzoylmethan.

cm Schichtdicke gegen eine analog behandelte Blindlösung gemessen. Die erhaltenen Ergebnisse sind in Fig. 2 dargestellt. Es ist ersichtlich, dass die Extraktion oberhalb pH 8 maximal ist.

Um Störungen durch Zink auszuschalten, empfiehlt es sich, von vornherein im alkalischen Milieu zu arbeiten. Das Cadmiumchelat des Thiodibenzoylmethans wird durch Alkalien geringfügig zersetzt. Bei erhöhter Konzentration an Ligand kann aber dieser Einfluss weitgehend zurückgedrängt werden. Zur Extraktion des Cadmiums aus schwach alkalischer Lösung reicht in der Regel ein einfacher Reagensüberschuss aus. Im Extrakt liegt, wie mit Hilfe der Jobschen Methode bestätigt werden konnte, Bis(thiodibenzoylmethanato)-cadmium vor. Zur maximalen Extraktion des Cadmiums mit Thiodibenzoylmethan reicht eine Schüttelzeit von 3 Minuten aus, wenn bei pH >8 gearbeitet wird.

Testung des Verfahrens im Arbeitsbereich 0.02–0.2 μ mol Cd

Zur Durchführung der Eichmessungen wurde eine vorgelegte Menge der Cadmium-Standard-Lösung (1, 2, 4, 6, 8 bzw. 10 ml) nacheinander mit 2 ml 0.1 *M* EDTA-Lösung, 3 ml 0.1 *M* Kaliumcyanid-Lösung, 4 ml gesättigter Chloralhydrat-Lösung und 10 ml 6 *M* Kalilauge versetzt, danach 10 ml Reagenslösung

zugegeben und 2 Minuten geschüttelt. Nach dem Abtrennen der wässrigen Phase wurde die organische Schicht zweimal 1 Minute mit je 20 ml 3 *M* Kalilauge extrahiert, zentrifugiert und in 1 cm-Küvetten bei 400 nm gegen eine analog behandelte Cd-freie Blindlösung photometriert.

Die Messergebnisse ergaben, dass für den Arbeitsbereich 0.02–0.2 μmol Cd das Lambert-Beersche Gesetz erfüllt ist. Der Gehalt einer Probe errechnet sich nach der Beziehung

$$c = E[\omega] + c_A$$

mit $[\omega] = 0.033$ μmol cm^{-3} und $c_A = 0.0002$ μmol cm^{-3}.

Zur Untersuchung des Einflusses von Fremdionen wurden Lösungen ihrer *p.a.*-Salze zu Cadmiumlösungen der unteren (b_u) und oberen (b_o) Arbeitsbereichsgrenze zugesetzt und dann die Cadmium-Bestimmung durchgeführt. Das Fremdion stört, wenn das Ergebnis der Bestimmung ausserhalb des absoluten Fehlerbereiches T ($S = 99\%$) liegt. Über die erhaltenen Resultate informiert Tabelle I.

TABELLE I

EINFLUSS VON FREMDMETALLEN AUF DIE PHOTOMETRISCHE BESTIMMUNG VON CADMIUM MIT THIODIBENZOYLMETHAN

Störverhältnis	*keine Störung*
1:10000	Erdalkali- und Alkalimetalle, B, Al, Ga, In, Mn, Fe, Cr
1:1000	As, Sb, Sn, Pb, Ni, Co, Cu, Bi, Pd
1:100	Tl, Hg, Zn, Ag

TABELLE II

VERGLEICH DER ZUR CADMIUMBESTIMMUNG VERWENDETEN REAGENZIEN

	Dithizon	*Thiodi-benzoyl-methan*	*Calmagit*	*PAR*	*BTAN*[a]	*BBTAN*[b]
Arbeitsbereich						
b_u bis	0.0003[c]	0.002[c]	0.002	0.0004	0.0006	0.0006
b_o (μmol ml^{-1})	0.0030	0.02	0.020	0.0040	0.0060	0.0060
Lösungsmittel	Chloroform	Benzol	Wasser	Wasser	Wasser	Wasser
λ (nm)	515	406	530	495	590	595
d (cm)	3.000	1.000	2.995	2.995	2.995	3.012
ε (cm^2 μmol^{-1})	80.4	30.0	12.0	84.0	56.9	63.5
S (μmol ml^{-1})	±0.00003	±0.00018	±0.00011	±0.00003	±0.00005	±0.00005
V_{bo} (rel. %)	±1.0	±0.9	±0.55	±0.3	±0.8	±0.8
V_{bu} (rel. %)	±10.0	±9.0	±5.50	±7.5	±8.3	±8.3

[a] Benzthiazolyl-(2-azo-1')-2'-naphthol.

[b] 6-Brombenzthiazolyl-(2-azo-1')-2'-naphthol.

[c] Bezogen auf die organische Phase, d.h. ohne Berücksichtigung der möglichen anreichernden Extraktion.

VERGLEICH VERSCHIEDENER REAGENZIEN

In Tabelle II sind einige Kenngrössen der von uns getesteten photometrischen Cadmium-Bestimmungen mit verschiedenen Spezialreagenzien zusammengestellt. Von ihnen wurden Dithizon (0.0018–0.018 μ mol ml^{-1}), Calmagit (0.01–0.10 μ mol ml^{-1}), PAR (0.0024–0.024 μ mol ml^{-1} und BTAN (0.0036–0.036 μ mol ml^{-1}) auch in den dabeistehenden Arbeitsbereichen (Schichtdicke $d=0.5$ cm) mit Erfolg geprüft. Diese Methoden lassen sich demnach in einem breiten Konzentrationsbereich anwenden.

Für die Bestimmung mit Dithizon erwies sich das Einfarbenverfahren als am vorteilhaftesten. Die hohe Selektivität beruht auf der Säurezersetzlichkeit und der Alkalibeständigkeit des primären Cadmiumdithizonats und wird durch eine sinnvolle Kombination aufeinanderfolgender Extraktionsprozesse erreicht. Bei Anwendung sehr verschiedener Störionen sind bis zu 12 Extraktionen erforderlich, wobei die Störanfälligkeit ausserordentlich steigt[4].

Die Bestimmungen mit Calmagit oder PAR erfordern praktisch immer Vortrennungen. Die beiden Benzthiazolylazonaphthole BTAN und BBTAN zeigen als Cadmiumreagenzien kaum Unterschiede[4], ihre Selektivität (detaillierte Angaben dazu bei Drapkina *et al.*[7]) entspricht etwa der des Thiodibenzoylmethans. Mit diesen Reagenzien ist die Cadmiumbestimmung in speziellen Fällen ohne vorherige Abtrennung möglich.

Unsere experimentellen Untersuchungen ergaben, dass von den bekannten Möglichkeiten zur Isolierung von Cadmiumspuren[19–21] die Ionenaustauschextraktion des Tetrabromocadmat-Komplexes mit Amberlite LA-2 in Xylol[20] die beste Selektivität besitzt.

ZUSAMMENFASSUNG

Thiodibenzoylmethan ist zur extraktionsphotometrischen Bestimmung von Cadmiumspuren gut geeignet. Von den untersuchten Fremdmetallen verursachen Alkali- und Erdalkalimetalle, B, Al, Ga, In, Mn, Fe, Cr bis zum 10000-fachen Überschuss, As, Sb, Sn, Pb, Bi, Cu, Co, Ni, Pd bis zum 1000-fachen Überschuss keine Störung. Ag, Hg, Tl und Zn können bis zum 100-fachen Überschuss anwesend sein. Das vorgeschlagene Verfahren wird mit einer Reihe anderer Methoden zur Cadmiumbestimmung verglichen.

SUMMARY

Thiodibenzoylmethane is a suitable reagent for the extraction and photometric determination of traces of cadmium. There is no interference from 10000-fold amounts of alkaline and alkaline earth metals, B, Al, Ga, In, Mn, Fe, Cr, and from 1000-fold amounts of As, Sb, Sn, Pb, Bi, Cu, Co, Ni, Pd; Ag, Hg, Tl and Zn are tolerated up to a 100-fold excess. The proposed method is compared with various other possibilities for cadmium determinations.

RÉSUMÉ

Le thiodibenzoylméthane est un réactif pouvant être utilisé pour l'extraction

et le dosage photométrique de traces de cadmium. Les métaux alcalins et alcalino-terreux, ainsi que B, Al, Ga, In, Mn, Fe, Cr ne présentent pas d'interférence jusqu'à des quantites 10000 fois supérièures; As, Sb, Sn, Pb, Bi, Cu, Co, Ni, Pd jusqu'à des quantites 1000 fois supérièures. Quant à Ag, Mg, Tl, et Zn, ils peuvent être tolérés jusqu'à 100 fois. Cette methode est comparée avec quelques autres dosages de cadmium possibles.

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A SOLVENT EXTRACTION STUDY OF MOLYBDENUM CHLORIDE AND MOLYBDENUM THIOCYANATE COMPLEXES

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The reaction of molybdenum with thiocyanate to form a colored complex has found wide application for the determination of small quantities of molybdenum[1–4]. Several spectrophotometric studies of the reaction mechanism have been published[1,5,6], and it is generally agreed that the colored species contains pentavalent molybdenum as the $MoO(SCN)_3$ complex. In the course of studying the solvent extraction of molybdenum from hydrochloric acid solutions, we became dubious that the mild reducing agents in common use in analytical procedures (e.g. iron(II), copper(I), ascorbic acid) actually could produce pentavalent molybdenum. In this paper, evidence is presented that the previous identification of the colored species may be incorrect; it is suggested as an alternative that the color production depends on an isomerization reaction of the $MoO_2(SCN)_2$ complex of molybdenum(VI).

The use of solvent extraction techniques to study complexes in solution is well known[7,8]; the treatment of Irving *et al.*[7] was followed here. Consider an aqueous solution saturated with an organic solvent, S, containing hydrogen ions, H, cations, M, and anions, L. After equilibration with an organic solvent, the average composition of the species in the aqueous phase can be written as $H_{mh}M_mL_{mn}(H_2O)_{mw}S_{ms}$ and the average composition of the organic phase species as $H_{m\underline{h}}M_m\underline{L}_{m\underline{n}}(H_2O)_{m\underline{w}}\underline{S}_{m\underline{s}}$, where m represents the polymerization number, h and n the number of hydrogen and ligand ions, and w and s the number of water and organic solvent molecules per metal atom. The partition coefficient p is defined as the ratio of the concentration of the ayerage organic phase species in the organic phase to its concentration in the aqueous phase. The distribution coefficient D is defined as the ratio of the total concentration of metal in the organic phase to that in the aqueous phase. From these definitions, the equation

$$\begin{aligned}\log D = {} & \log(p\underline{\beta}/\beta) + \log(\underline{m}/m) + (\underline{mh} - mh)\log[H] + \\ & + (\underline{m} - m)\log[M] + (\underline{mn} - mn)\log[L] + \\ & + (\underline{mw} - mw)\log[H_2O] + (\underline{ms} - ms)\log[S] \end{aligned} \quad (1)$$

can be derived with $\underline{\beta}$ and β representing the overall stability constants of the average organic and aqueous phase species, respectively.

Equation (1) is often applied to determine stability constants where the composition of the extracting species is known or assumed. It is well known, however, that log–log plots of the distribution coefficient and the independently-varied solution

variables can reveal the presence of species changes (produced by reduction, complexation, etc.) by changes in the partition and stability constants, number of ligand and solvent molecules bound by the extracting species, etc. In the following work eqn. (1) is used to study the effect of reducing agents on molybdenum(VI) solutions and no attempt is made to evaluate stability constants.

EXPERIMENTAL

Reagents

Reducing agents used in this study were: (1) 0.05 *M* copper(I) chloride in concentrated hydrochloric acid to which a slight excess of tin(II) chloride was added just before use to reduce copper(II); (2) 40% tin(II) chloride in concentrated hydrochloric acid (diluted with the acid as necessary to provide lower concentrations); and (3) 5% ascorbic acid in water. The molybdenum stock solution was prepared by dissolving the metal in dilute nitric acid and making the solution to volume in 0.1 *M* hydrochloric acid. The tracer solution was prepared from carrier-free ^{99}Mo, available commercially. A 40% solution of potassium thiocyanate in water was used as a source of thiocyanate ion.

Procedure

A standardized procedure, as similar as possible to recommended analytical thiocyanate methods[1,9], was adopted. Sufficient hydrochloric acid was added to a beaker to give the desired final molarity. Molybdenum carrier (10 μg Mo) and tracer were added to the acid. Where specified, reducing agent and/or thiocyanate were added. Finally, sufficient water to bring the final volume to 10 ml was added. The solution was extracted for 2 min in a separatory funnel with 10 ml of amyl alcohol or, in some cases, a mixture of amyl alcohol and cyclohexane. The aqueous phase was drained to a counting vial and counted with a single-channel analyzer coupled to a NaI(Tl) detector. The distribution ratio was calculated from the counting rate of the aqueous phase and the counting rate of an unextracted standard prepared at the same time as the experimental solution.

Preliminary experiments in which molybdenum carrier and tracer were evaporated together with aqua regia several times before the extractions showed no difference from solutions in which carrier and tracer were simply mixed in concentrated hydrochloric acid. It can be assumed, then, that isotopic exchange was very rapid and that the carrier was hexavalent.

In these experiments the aqueous and organic phases were not pre-equilibrated with each other nor was any correction made for volume changes during extraction. Neither was the ionic strength maintained constant by the addition of "inert" salts. These omissions would result in errors if equilibrium constants were to be estimated, but were of little importance here, because only relative changes due to changing molybdenum species were sought.

Activity corrections were made to the hydrochloric acid concentration. All other reagents were sufficiently dilute that activity corrections were negligible.

MOLYBDENUM SPECIES IN HYDROCHLORIC ACID

The molybdenum complexes in hydrochloric acid were first studied in the absence of thiocyanate. These results are described below.

Polymerization of molybdenum

Polymerization of molybdenum in hydrochloric acid was studied by extracting varying amounts of molybdenum (10^{-6}–10^{-5} *M*) with amyl alcohol from a constant (3.6 *M*) hydrochloric acid solution. The experiment was then repeated with the same acid in the presence of 0.004 *M*, 0.04 *M*, and 0.2 *M* tin(II) chloride.

No variation of the distribution coefficient with changing molybdenum concentration was observed in any of these experiments. This implies (from eqn. 1) that within each of the experiments the same polymerization state was present in both aqueous and organic phase complexes and thus the distribution results are independent of the molybdenum concentration.

Dependence of molybdenum extraction on hydrogen ion activity

Protonation of the organic phase species of molybdenum can be determined from eqn. 1 by varying the concentration of a non-complexing acid while maintaining the chloride concentration constant. Alternatively, if protonated species are not present, then

$$\left(\frac{\partial \ln D}{\partial \ln [\mathrm{H}]}\right)_{\mathrm{Mo,Cl,S}} = 0 \tag{2}$$

and therefore

$$\left(\frac{\partial \ln D}{\partial \ln [\mathrm{NaCl}]}\right)_{\mathrm{Mo,H,S}} = \left(\frac{\partial \ln D}{\partial \ln [\mathrm{HCl}]}\right)_{\mathrm{Mo,S}} \tag{3}$$

It was found that identical distribution curves were obtained by varying either hydrochloric acid or sodium chloride concentration in the 0–3 *M* range in solutions initially 0.3 *M* in hydrochloric acid. This implies that molybdenum is present in the organic phase as a molecular complex rather than a protonated species[8] and therefore

$$\left(\frac{\partial \ln D}{\partial \ln [\mathrm{HCl}]}\right)_{\mathrm{Mo,S}} = (\underline{n} - n) = \Lambda \tag{4}$$

where Λ is the average charge of the molybdenum species in the aqueous phase (it is assumed that the extracting species is neutral).

Effect of reducing agents on extraction of molybdenum

According to eqns. (1) and (4), a log–log plot of the distribution coefficient against the hydrochloric acid activity should yield a straight line with a slope equal to the charge of the average aqueous phase species and an intercept determined by the partition coefficient and stability constant of the oganic phase species. It is most improbable that complexes of different valence states of molybdenum would have both partition coefficients and stability constants identical (or compensating). Therefore the effect of various reducing agents on Mo(VI) solutions should be clearly apparent.

Figure 1 compares the distribution coefficient of Mo(VI) solutions with Mo(V) solutions (prepared with a silver reductor[10]) as a function of hydrochloric acid activity and shows that the average aqueous charge of Mo(V) species is less than that of Mo(VI) and that the partition coefficient and/or stability constant of the extractable Mo(V) complex is less than that of the Mo(VI). It should be noted

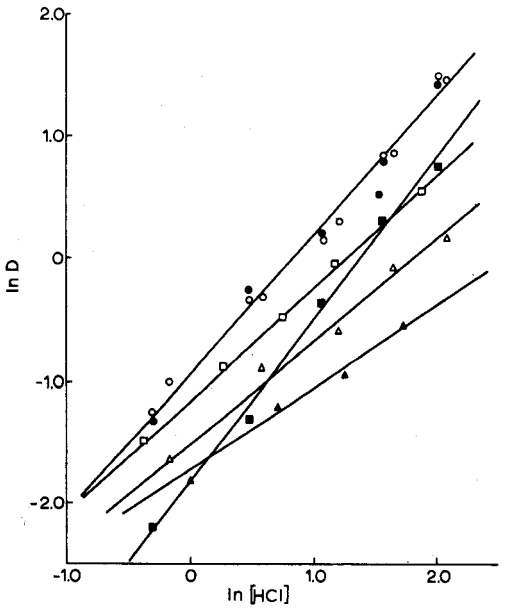


Fig. 1. Distribution coefficient of molybdenum as a function of hydrochloric acid activity in the presence of various reducing agents. Reducing agents: (○) none; (●) 0.0005 *M* Cu(I); (□) Ag (Mo(V) curve); (■) 0.004 *M* Sn(II); (△) 0.04 *M* Sn(II); (▲) 0.2 *M* Sn(II).

that the original Mo(V) solution was not assayed to determine the efficiency of the silver reductor nor were any precautions taken to prevent oxidation of Mo(V) during extraction. It is likely that the "Mo(V)" curve in Fig. 1 is actually a mixture of Mo(V) and Mo(VI) species; nevertheless, enough Mo(V) was present to show a significant difference in the extraction of Mo(VI) and Mo(V) complexes.

The data points of Fig. 1 referring to 0.0005 *M* copper(I) were obtained with a copper(I) concentration which is common to analytical procedures[1] based on the thiocyanate color of molybdenum. From this curve it is apparent that this concentration of copper(I) has no effect on the extraction of Mo(VI) and, specifically, does not reduce Mo(VI) to Mo(V). Varying the copper(I) concentration from 0.0001 to 0.01 *M*, while maintaining the final hydrochloric acid concentration constant at 3.6 *M*, resulted in no change in the molybdenum distribution coefficient, confirming the absence of reduction.

In independent experiments it was found that the molybdenum thiocyanate color could be produced with ascorbic acid as reducing agent. The extraction of molybdenum from hydrochloric acid was therefore studied in the presence of ascorbic acid. Varying the ascorbic acid concentration from 0.0003 to 0.08 *M* again resulted in no change of the molybdenum distribution coefficient in 3.6 *M* hydrochloric acid.

Tin(II) ion, unlike ascorbic acid and copper(I), does reduce Mo(VI) in hydrochloric acid solution. Figure 1 shows the variation of the distribution coefficient in the presence of three different concentrations of tin(II) chloride. The steeper slope of the distribution curve with 0.004 *M* tin(II) chloride implies the existence of a molybdenum species other than Mo(VI), Mo(V), or a mixture of

these. Higher concentrations of tin(II) chloride result in distribution curves having progressively lower slopes than the silver-reduced Mo(V) curve.

Figure 2 shows the effect on the distribution coefficient of varying the tin(II) chloride concentration from 0.000045 to 0.5 *M* at a constant hydrochloric acid concentration of 3.6 *M*. The sharp break in the distribution curve at a tin(II) chloride concentration of about 0.01 *M* is strong evidence for the stepwise reduction of Mo(VI) to two species by tin(II). Confirming the existence of these two reduced species, the extraction curves in Fig. 1 show a distinct difference in the average aqueous charges present between solutions greater than and less than 0.01 *M* in tin(II) chloride.

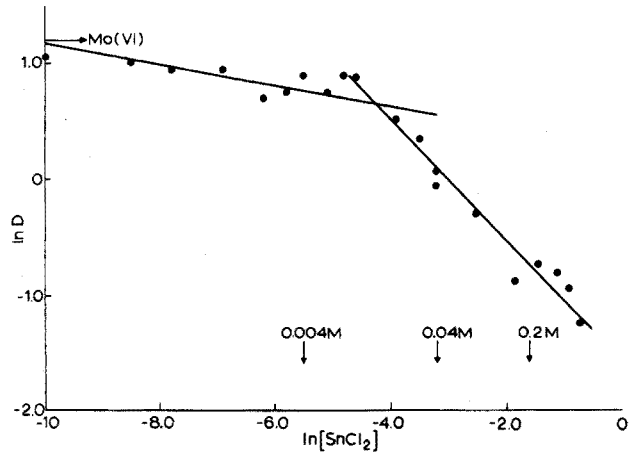


Fig. 2. Distribution coefficient of molybdenum as a function of tin(II) concentration. Extraction from 3.6 *M* hydrochloric acid.

The results shown in Figs. 1 and 2 give no direct evidence for the valence of either of the two molybdenum species produced by tin(II) reduction. It may be that neither species is Mo(V) because both have different extraction characteristics than the Mo(V) (or the Mo(V)–Mo(VI) mixture) produced by the silver reductor. However, the continuous decrease of both the distribution coefficient and slope in the 0.01–0.5 *M* tin(II) range suggests that the differences in the extraction curves of molybdenum (Figure 1) for reduction with silver, 0.04 *M* tin(II), and 0.2 *M* tin(II) can be attributed to varying Mo(VI) : Mo(V) ratios resulting from initial incomplete reduction and/or oxidation during extraction. If this is true, the species produced by low concentrations of tin(II) must be a polynuclear Mo(VI) · Mo(V) complex.

Organic solvation of molybdenum chloride complexes

The molybdenum species produced by reduction with tin(II) and with the silver reductor were studied further by determining their solvation numbers. According to eqn. 1, plotting the distribution coefficient in log–log coordinates as a function of the amyl alcohol concentration in the organic phase should give a straight line with a slope equal to the difference between the number of solvent

molecules bound in the aqueous phase and oganic phase complexes. Table I shows approximately the same solvation number for Mo(VI) alone, for silver-reduced molybdenum, or for Mo(VI) reduced by 0.04 and 0.2 *M* tin(II) chloride. A distinctly higher solvation number is obtained by reduction with 0.004 *M* tin(II) chloride; this is consistent with the stepwise reduction of molybdenum to two valence states by tin(II).

The two valence states produced by stannous chloride must be either a polynuclearMo(VI)·Mo(V) complex and Mo(V), or Mo(V) and Mo(III). The solvation number of about 3 and 2 for the low and high concentrations, respectively, of tin(II) are only compatible with the former alternative if six-coordinate species are assumed. At the highest tin(II) chloride concentration used (0.2 *M*), the solvation number never approached the 3 (or 5) necessary for a Mo(III) complex of 6 (or 8) coordination.

Molybdenum species in hydrochloric acid

Table I summarizes the data on the average charge of the aqueous phase and the solvation number in the organic phase for the various species of molybdenum.

TABLE I

SUMMARY OF RESULTS FROM HYDROCHLORIC ACID EXTRACTION OF MOLYBDENUM

Reducing agent	*Average charge of aqueous phase*	*Solvation number in organic phase*	*Species in organic phase*	*Molybdenum valence*
None	1.13	1.8	$MoO_2Cl_2 \cdot S_2$[a]	VI
Silver reductor	0.92	1.8	$MoOCl_3 \cdot S_2$	V
0.0005 *M* copper(I)	1.13	—	$MoO_2Cl_2 \cdot S_2$	VI
0.004 *M* tin(II)	1.33	2.6	$(MoO_2 \cdot MoO)Cl_5 \cdot S_3$	VI, V
0.04 *M* tin(II)	0.77	1.8	$MoOCl_3 \cdot S_2$	V
0.2 *M* tin(II)	0.73	1.7	$MoOCl_3 \cdot S_2$	V

[a] Amyl alcohol solvent molecules written as S.

Suggested species given in Table I are based on the assumptions that the extractable complex is neutral and is six-coordinate. Because amyl alcohol is appreciably soluble in the aqueous phase, the aqueous phase species are likely to be solvated to some extent by the alcohol, and it was assumed that the next higher integer is the number of solvent molecules bound by the complex in the organic phase. Neumann and Cook[11] have shown previously that MoO_2Cl_2 is the extractable species of Mo(VI) in hydrochloric acid solutions and $MoOCl_3$ in a well known Mo(V) complex.

It is difficult to reconcile the observation that neither copper(I) ion nor ascorbic acid reduces Mo(VI) to Mo(V) in hydrochloric acid solution with the analytical observation that both produce a molybdenum thiocyanate color which has been attributed to a Mo(V) complex. Tin(II) ion, on the other hand, does reduce Mo(VI) to Mo(V) (or Mo(VI)·Mo(V)) and yet gives only about half the color intensity[9], and even this color fades rapidly. If thiocyanate stabilizes Mo(V) complexes in the presence of copper(I) ion or ascorbic acid, then it must also stabilize lower-valent molybdenum complexes in the presence of tin(II) ion. An

alternative hypothesis is suggested by the molybdenum thiocyanate extraction studies described in the following section.

MOLYBDENUM THIOCYANATE EXTRACTIONS

Average charge of molybdenum in the aqueous phase

The extraction of molybdenum as a function of thiocyanate concentration in the presence of various reducing agents is shown in Fig. 3. Colored species were observed in the presence of copper(I), ascorbic acid, and, where noted below, with tin(II) chloride. No color was observed in the absence of reducing agents.

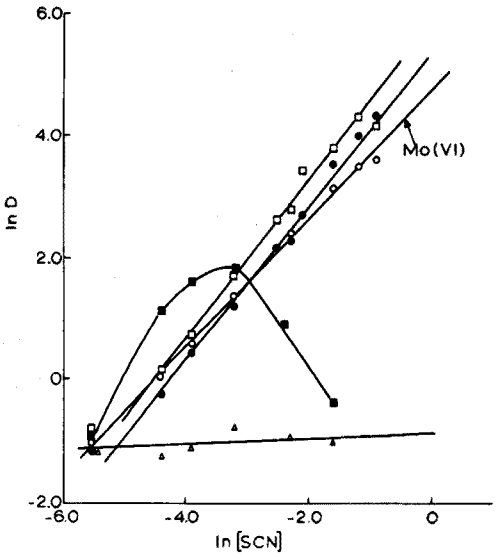


Fig. 3. Distribution coefficient of molybdenum as a function of thiocyanate concentration. Extraction from 0.3 *M* hydrochloric acid. Reducing agents: (○) none; (●) 0.0005 *M* Cu(I); (□) 0.028 *M* ascorbic acid; (■) 0.004 *M* Sn(II); (△) 0.04 *M* Sn(II).

Unlike the hydrochloric acid system, copper(I) ion and ascorbic acid have a real, albeit small, effect on the distribution coefficient of molybdenum thiocyanate. Also the average charge of the aqueous phase species, determined from the slopes of the curves in Fig. 3, is 1.25 and 1.30 (identical within experimental error) in the presence of copper(I) ion and ascorbic acid, respectively, as compared with 1.06 in the absence of reducing agents.

The difference in distribution coefficients between the colored species produced by copper(I) ion and ascorbic acid, and the colorless species produced in the absence of the reducing reagents, is no greater than that between the copper(I)-produced and ascorbic-produced colored species and therefore cannot reflect a valence difference. The difference in slope between colored and colorless species, however, implies a difference in the aqueous phase equilibria.

The addition of thiocyanate to solutions of the polynuclear Mo(VI)·Mo(V) complexes presumed to be produced by 0.004 *M* tin(II) chloride results first in a

complex which extracts well with increasing thiocyanate and then, above 0.0006 *M* thiocyanate, forms a non-extractable complex. The extractable complex is colored and may be the same as that produced by the milder reducing agents. However, the initial slope (about 1.6) of the extraction curve with tin(II) ion differs greatly from that with copper(I) and ascorbic acid, indicating that the aqueous phase species are different, and thus that different reaction mechanisms are involved in the production of colored complexes. The 0.004 *M* tin(II) extraction curve shown in Fig. 3 was obtained immediately after addition of thiocyanate and dilution; it was noted that both the color and the distribution coefficient decreased rapidly on standing and therefore the extractable colored complex appears to be a metastable intermediate reaction product.

The addition of thiocyanate to solutions of Mo(V) produced by 0.04 *M* tin(II) chloride results in neither a color nor an extractable species over the concentration range studied.

Molybdenum thiocyanate solvation numbers

Solvation numbers, given by the slopes of the curves in Fig. 4, of the molybdenum thiocyanate species in the organic phase were determined by extraction with various dilutions of amyl alcohol in cyclohexane. The solvation numbers of the thiocyanate complexes are about the same as the chloride complexes. Because acido-species usually have solvation numbers greater than two[8], it is likely that only a simple ligand-exchange reaction between chloride and thiocyanate is involved

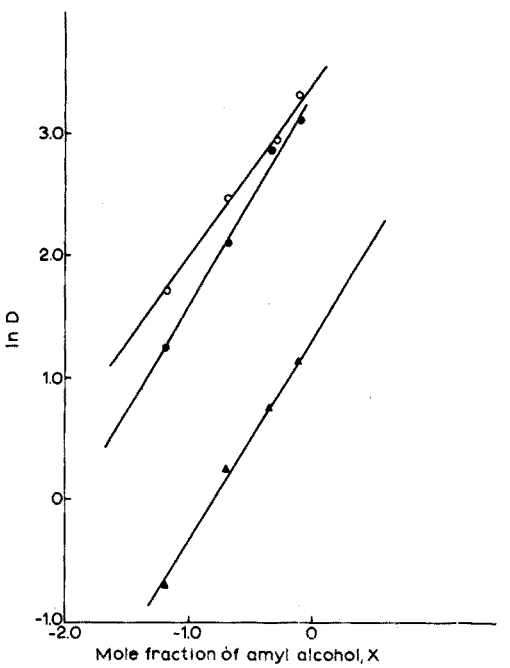


Fig. 4. Distribution coefficient of molybdenum as a function of the mole fraction of amyl alcohol in cyclohexane. (▲) 0.3 *M* HCl, 0.04 *M* SCN^-; (●) 0.18 *M* HCl, 0.20 *M* SCN^-, 0.5% ascorbic acid; (○) 0.3 *M* HCl, 0.20 *M* SCN^-, 0.0005 *M* Cu(I).

rather than a ligand-exchange plus protonation reaction.

The presence of ascorbic acid does not affect the solvation number of molybdenum(VI) thiocyanate species, although it does produce a different species as shown by the color. The sightly lower solvation number obtained in the presence of copper(I) may be a real effect.

The small differences in solvation numbers (Fig. 4) and in distribution coefficients (Fig. 3) between the species produced by copper(I) and by ascorbic acid, suggest that the two species are not identical. Consequently, the absorption spectra were examined for the colored species produced by the two reducing agents after extraction into amyl alcohol. No difference could be found in the absorbance peaks or in the molar absorptivities of the two colored species. It is probable, then, that the color-producing moiety is identical in the two species, while the extraction differences reflect isomeric differences, or possibly reaction-rate differences, owing to different reaction mechanisms in the production of colored species by copper(I) and by ascorbic acid.

Molybdenum thiocyanate species and reaction mechanisms

The reaction of Mo(VI) in hydrochloric acid with thiocyanate to produce an extractable species can be simply represented by the ligand exchange reaction:

$$MoO_2Cl_2 + 2(SCN)^- = MoO_2(SCN)_2 + 2\,Cl^-$$

In the presence of copper(I) and ascorbic acid, however, the reaction is less obvious. The analytical literature[9] generally accepts as the function of mild reducing agents the production of colored thiocyanate complexes of Mo(V) by reactions such as:

$$MoO_2(SCN)_2 + Cu^+ + (SCN)^- = MoO(SCN)_3 + CuO$$

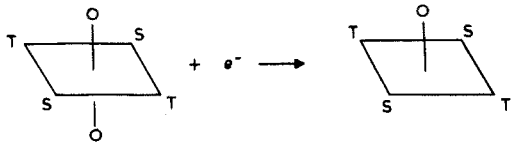
The primary evidence for such a reaction is based on the observation that the colored species can be produced by the addition of thiocyanate to Mo(V) solutions but not to Mo(VI) solutions in the absence of a reducing agent[1,5]. Also, both Hiskey and Meloche[5] and Perrin[6] concluded from spectrophotometric studies that the Mo:(SCN) ratio was 1:3, but Perrin notes that the complex studied by Hiskey and Meloche could not have been the same complex he studied and that the results of the two studies are mutually incompatible. Present attempts to determine the Mo:(SCN) ratio of the colored species by common spectrophotometric techniques yielded only ambiguous results, and it was concluded that conditions could be chosen to yield almost any desired ratio.

The extraction results described here raise several objections to the reduction hypothesis: (1) there is no evidence of any reduction of molybdenum in hydrochloric acid solutions by either copper(I) or ascorbic acid. If reduction takes place, both the stability constants and partition coefficients of Mo(VI) and Mo(V) chloride complexes must be identical (or compensating), a most implausible result; (2) the small difference in the thiocyanate distribution coefficients between colored and colorless species is no greater than the difference between that of the two colored species produced by ascorbic acid and by copper(I). Certainly, the difference is much less than that observed between Mo(VI) and Mo(V) chloride complexes (Fig. 1). Attributing the small difference in extractability between colored and colorless

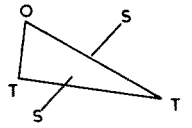
species to a molybdenum valence change leaves the difference in extractability of the two colored species inexplicable; (3) if our interpretation of the sequential reduction of molybdenum to a polynuclear Mo(VI)·Mo(V) complex and Mo(V) with tin(II) ion is correct, the extraction results with thiocyanate show that Mo(V) forms neither a colored nor an extractable thiocyanate complex if sufficient tin(II) is present to prevent oxidation to Mo(VI).

As an alternative to the reduction hypothesis, the colored species may represent an isomeric transformation of $MoO_2(SCN)_2$ and the transformation may take place through an unstable Mo(V) intermediate. It seems probable that $MoO_2(SCN)_2$ occurs as a distorted octahedral structure with the metal–oxygen bonds much shorter than the others and that the color arises from a charge transfer process after thiocyanate is substituted for oxygen at one of the short-bond positions.

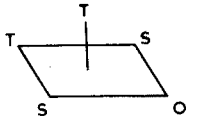
A possible mechanism for such an isomerization reaction involving a reducing agent as catalyst to provide a 5-coordinate Mo(V) intermediate can be postulated following the recent discussion by Springer[12]. Reduction of MoO_2^{2+} species in aqueous solution commonly yields a MoO^{3+} species which could occur initially as a 5-coordinate complex:



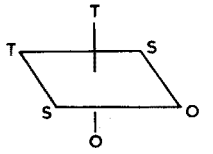
where O refers to oxygen, T to thiocyanate, and S to coordinated solvent. Rearrangement of the 5-coordinate square pyramid to a trigonal bipyramid by a 1/2 Berry pseudorotation process could give:



which could return to a square pyramid structure by another 1/2 Berry pseudorotation to give:



Oxidation to Mo(VI) with the concomitant addition of an oxygen would give an octahedral complex:



resulting in a thiocyanate on a short bond and an ionizable oxygen.

This model provides a ready explanation for most of the observations of the molybdenum thiocyanate color. The first reaction to provide a 5-coordinate inter-

mediate containing only a single oxygen atom is immediate if molybdenum is initially pentavalent and is facilitated by a reducing agent for hexavalent molybdenum; the final reaction to produce an octahedral Mo(VI) complex cannot occur in the presence of a reducing agent sufficiently strong to stabilize Mo(V) complexes. The isomerization reaction leading to an ionizable oxygen atom also accounts for the higher average charge of the aqueous phase observed when the colored species was present, because the neutral extractable complex would then be in equilibrium with both singly and doubly charged complexes depending on whether thiocyanate or oxygen were ionized from the neutral species. Such a mechanism also makes it obvious why the spectrophotometric results of Hiskey and Meloche[5] with tin(II) as a reducing agent are incompatible with Perrin's[6] results with copper(I); if this mechanism is at all correct, the assumptions of such spectrophotometric techniques are invalid and thus the results are ambiguous at best.

The isomerization reaction given above cannot be entirely correct. The differing extraction curves and solvation numbers between copper(I)-produced and ascorbic acid-produced species are evidence of a direct involvement of the reducing agents to produce specific isomers. Nevertheless, it is apparent from these extraction results that the common attribution of the molybdenum thiocyanate color to a molybdenum(V) complex is very questionable, and that considerable work remains before the color reaction can be described with any confidence.

SUMMARY

The effect of reducing agents on molybdenum(VI) solutions in hydrochloric acid was studied by a solvent extraction technique to elucidate the composition of the colored molybdenum thiocyanate complex. Neither copper(I) chloride nor ascorbic acid have any effect on the extraction of MoO_2Cl_2; it is inferred that tin(II) chloride reduces Mo(VI) stepwise to a polynuclear Mo(V)·Mo(VI) complex and then to Mo(V). The colored thiocyanate complex produced by copper(I) and by ascorbic acid differs only slightly in extraction characteristics from the uncolored Mo(VI) complex. It is suggested that the color may be produced by an isomerization reaction of $MoO_2(SCN)_2$ and thus that the colored species may be a hexavalent, rather than pentavalent, molybdenum complex.

RÉSUMÉ

Une étude est effectuée pour examiner l'influence des agents de réduction sur le molybdène(VI), en milieu acide chlorhydrique, on procède par extraction dans un solvant. Ce travail a pour but de définer la composition du complexe thiocyanate de molybdène coloré. Ni le chlorure de cuivre(I), ni l'acide ascorbique n'ont un effet sur l'extraction de MoO_2Cl_2. Le chlorure d'étain(II) réduit le molybdène(VI) en un complexe polynucléaire Mo(V)·Mo(VI), puis en Mo(V). Le complexe thiocyanate coloré produit par le cuivre(I) et l'acide ascorbique ne diffère que légèrement du complexe de Mo(VI) incolore, dans ses caractéristiques d'extraction. On peut admettre que la coloration est produite par une réaction d'isomérisation de $MoO_2(SCN)_2$, et que la substance colorée correspond à un complexe de molybdène hexavalent, plutôt que pentavalent.

ZUSAMMENFASSUNG

Der Einfluss von Reduktionsmitteln auf salzsaure Molybdän(VI)-Lösungen wurde durch ein Solventextraktionsverfahren untersucht, um die Zusammensetzung des gefärbten Molybdän-Thiocyanat-Komplexes zu ermitteln. Weder Kupfer(I)-chlorid noch Ascorbinsäure haben irgendeinen Einfluss auf die Extraktion von MoO_2Cl_2; es wird gefolgert, dass Zinn(II)-chlorid Mo(VI) stufenweise zu einem mehrkernigen Mo(V)·Mo(VI)-Komplex und dann zu Mo(V) reduziert. Der mit Kupfer(I) und mit Ascorbinsäure erhaltene gefärbte Thiocyanatkomplex unterscheidet sich in seinen Extraktionseigenschaften nur wenig von dem farblosen Mo(VI)-Komplex. Es wird angedeutet, dass die Färbung durch eine Isomerisierungsreaktion von $MoO_2(SCN)_2$ hervorgerufen werden könnte und dass damit die gefärbte Spezies eher ein sechswertiger als ein fünfwertiger Molybdänkomplex sein könnte.

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Analytica Chimica Acta, 69 (1974) 347–353

SOLVENT EXTRACTION OF LEAD, SILVER, ANTIMONY AND THALLIUM WITH ZINC DIBENZYLDITHIOCARBAMATE AND ITS APPLICATION TO THE SEPARATION OF BISMUTH FROM LARGE AMOUNTS OF LEAD

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Various derivatives of dithiocarbamate have been used as analytical reagents for metal ions[1–6]. Among these, zinc dibenzyldithiocarbamate (Zn-DBDTC) has been used for the extraction–spectrophotometric determination of copper by several workers[6–10]. In comparison with the well-known diethyldithiocarbamate, the main advantage of zinc-DBDTC is that it can be used in strongly acidic solutions. This reagent has also been used for the extraction and spectrophotometric determination of bismuth[11] and thallium[12]. However, no extensive study of this reagent for other metals has been made despite its apparent advantages.

The present paper describes a detailed investigation of the extraction of lead, silver, antimony(III) and thallium(III) with zinc-DBDTC. In addition, the extraction separation of bismuth from large amounts of lead with this reagent has been studied in detail.

Several methods such as precipitation, ion exchange and solvent extraction have been reported for the separation of bismuth from lead. However, these separations do not always give satisfaction for simple and rapid separation of small amounts of bismuth from large amounts of lead. Recently, Neirinckx[13] has reported the extraction separation of carrier-free bismuth from lead and zinc with di(2-ethylhexyl)phosphoric acid.

It has been shown that, by the present method using zinc-DBDTC, microgram amounts of bismuth can easily be separated from 1 g of lead.

EXPERIMENTAL

Reagents and apparatus

Standard lead solution (500 μg ml^{-1}). Dissolve 0.399 g of lead nitrate in water and dilute to 500 ml.

Standard antimony solution (500 μg ml^{-1}). Dissolve 0.685 g of potassium antimonyl tartarate (hemihydrate) in 2.5 *M* sulfuric acid with small amounts of tartaric acid, and then dilute with 2.5 *M* sulfuric acid to 500 ml.

Standard silver solution (500 μg ml^{-1}). Dissolve 0.394 g of silver nitrate in water and dilute to 500 ml.

Standard thallium solution (200 μg ml^{-1}). Dissolve 0.123 g of thallium sulfate (99.9%) in 5 ml of aqua regia with gentle heating and add 15 ml of 9 *M* sulfuric acid, followed by heating to slight white fumes, and dilute with 0.3 *M* sulfuric acid to 500 ml.

Standard bismuth solution (500 μg ml^{-1}). Prepare as previously described[11]

Prepare more dilute solutions from these stock solutions by dilution.

Zinc-DBDTC solution. Dissolve 0.30 g of zinc-DBDTC (B.D.H. Laboratory Chemicals) in 1 l of reagent-grade carbon tetrachloride. All reagents should be of the highest grade of purity obtainable.

Spectrophotometric measurements were made with a Hitachi Model EPU-2A spectrophotometer in 1-cm cells.

Atomic absorption spectrometric measurements were made with a Hitachi Model 208 atomic absorption spectrophotometer, fitted with a three-slot burner (air–acetylene). Hitachi hollow-cathode lamps were used.

An Iwaki KM-shaker was used.

General procedure

Transfer an aliquot of standard solution to a 100-ml separating funnel, and add enough acid and water to give the required acid concentration, the final total volume of aqueous phase being about 25 ml. Add 10.0 ml of zinc-DBDTC solution and shake for 60 s. After phase separation, drain the organic solution through a filter paper (No. 5B) into the optical cell. Measure the absorbance at a suitable wavelength for each metal against carbon tetrachloride or reagent blank.

Atomic absorption spectrometric measurements were made by the following procedure for determining the metals in the organic phase. After the extraction described above, drain the organic solution through the filter paper into the dry beaker. Transfer a 5-ml aliquot of this solution to a dry 50-ml beaker, and evaporate to dryness on a water bath. Digest the residues with nitric acid and evaporate to moist dryness without baking. Dissolve the residues in 1 *M* nitric acid and then determine the concentration of bismuth and lead by atomic absorption spectrometry at 283.3 nm for lead and at 306.8 nm for bismuth.

RESULTS AND DISCUSSION

Absorption spectra

Figure 1 shows the absorption spectra of metal–DBDTC chelates in carbon tetrachloride. The chelates of lead, silver and antimony have no absorption maximum in the visible region; only the thallium chelate has an absorption maximum at *ca.* 435 nm. Spectrophotometric measurements were made at 330 nm for lead, silver and antimony, and at 435 nm for thallium, since the reagent absorbed strongly at wavelengths below 325 nm. The molar absorptivities of the metal chelate extracted from 1 *M* nitric acid were about 6000, 4000, 10600 and 1200, for lead, silver, antimony and thallium, respectively. All the systems obeyed Beer's law up to 120 μg, except for thallium which was satisfactory to 450 μg. The same results were obtained for extractions from 1 *M* sulfuric acid or 1 *M* hydrochloric acid.

Extraction of metals from various acid solution

The extractions of lead, silver, antimony and thallium from sulfuric acid,

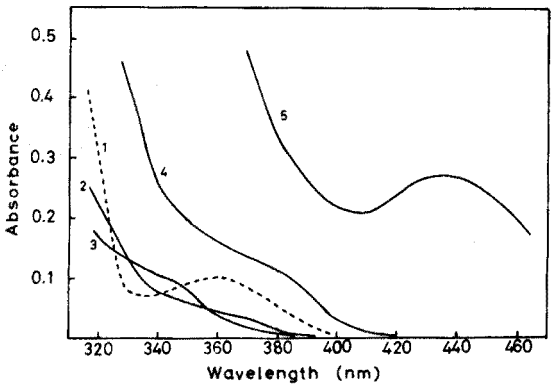

Fig. 1. Absorption spectra. (1) 0.03% Zn-DBDTC solution after shaking with 1 *M* sulfuric acid solution; reference: carbon tetrachloride. (2) Lead-DBDTC in carbon tetrachloride extracted from 1 *M* nitric acid solution containing 50 μg of lead; reference: reagent blank. (3) Silver-DBDTC in carbon tetrachloride extracted from 1 *M* sulfuric acid solution containing 35 μg of silver; reference: reagent blank. (4) Antimony(III)-DBDTC in carbon tetrachloride extracted from 1 *M* sulfuric acid solution containing 50 μg of antimony; reference: reagent blank. (5) Thallium(III)-DBDTC in carbon tetrachloride extracted from 1 *M* sulfuric acid solution containing 450 μg of thallium; reference: reagent blank.

hydrochloric acid and nitric acid at various acid concentrations were evaluated by the spectrophotometric technique. The extraction of bismuth from hydrochloric acid was also examined. Extractions were done for a period of 60 s, which was sufficient to obtain equilibrium in all cases.

On extraction from sulfuric acid a constant absorbance value was obtained over a wide range of acidity for each metal except for lead (Fig. 2). Figure 3 shows the extractions from hydrochloric acid. With all the metals the absorbance decreased sharply with increasing hydrochloric acid.

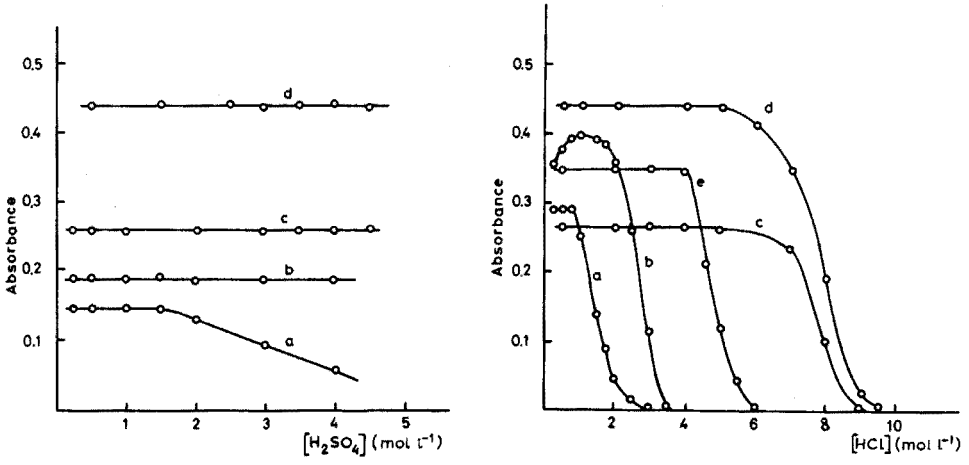

Fig. 2. Extraction of metals from various concentrations of sulfuric acid. (a) Lead, 50 μg; (b) silver, 50 μg; (c) thallium, 450 μg; (d) antimony, 50 μg.

Fig. 3. Extraction of metals from various concentrations of hydrochloric acid. (a) Lead, 100 μg; (b) silver, 100 μg; (c) thallium, 450 μg; (d) antimony, 50 μg; (c) bismuth, 60 μg.

This dependence of extraction efficiency of the metals on the concentration of hydrochloric acid was confirmed by atomic absorption spectrometry.

Although the absorption spectrum of the reagent used changed remarkably in 4 *M* nitric acid, no significant change of the absorption spectrum of the reagent was observed even in 9 *M* hydrochloric acid. Therefore, these extraction characteristics of the metals are probably due to the formation of chloro complexes of the metals in strong hydrochloric acid. There seems to be no chelate formation between the metal and DBDTC in strong hydrochloric acid. It is interesting to note that maximal extraction efficiency was observed for silver at about 1 *M* hydrochloric acid.

In the case of nitric acid, it was found that the acid concentrations for quantitative extraction had to be less than about 2 *M* for the metals examined.

Back-extraction study with hydrochloric acid

Back-extractions of the metals were studied with hydrochloric acid. After the metal had been extracted with zinc-DBDTC in carbon tetrachloride solution from 1 *M* nitric acid, the organic phase was shaken with various concentrations of hydrochloric acid for 60 s, and then the absorbance of the organic phase was measured in a similar way to that in the extraction study. In the studies of considerable amounts of lead and bismuth, *e.g.* 500 μg of lead and 400 μg of bismuth, atomic absorption spectrometry was also used to determine lead and bismuth in the organic phase after the back-extraction.

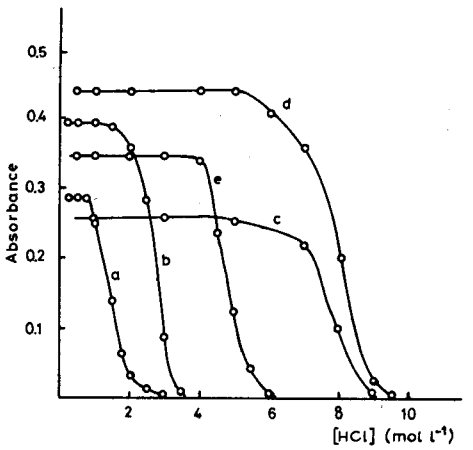

Fig. 4. Back-extraction of metals by washing with various concentrations of hydrochloric acid. (a) Lead, 100 μg; (b) silver, 100 μg; (c) thallium, 450 μg; (d) antimony, 50 μg; (e) bismuth, 60 μg. Back-extraction was made after the extraction of metals from 1 *M* nitric acid solution.

Figure 4 shows that the back-extraction characteristics are very similar to the corresponding extraction curves of each metal from hydrochloric acid, except for silver. Similar results were obtained for lead and bismuth by atomic absorption spectrometric measurements. At the lower acid concentrations, silver once extracted could not be back-extracted.

It was clear that all the metal ions examined could be back-extracted almost completely by washing with strong hydrochloric acid.

Separation of bismuth from lead

By means of this extraction system, it was possible to develop a procedure for separating bismuth from lead. In the presence of large amounts of lead, the extraction technique from hydrochloric acid cannot be applied directly because lead chloride precipitates.

Tests showed that bismuth was more readily extracted than lead even when large amounts of lead were present; lead was also extracted with unreacted DBDTC and the excess of lead remained in aqueous phase. It is suggested that the bismuth chelate with DBDTC is more stable than that of lead with DBDTC.

Therefore, the extraction of bismuth was made from 1 *M* nitric acid, and then the organic phase was washed with 3.5 *M* hydrochloric acid or washed twice with 3 *M* hydrochloric acid; under these conditions bismuth was not stripped whereas lead was. In this procedure the back-extraction should be made within a few minutes after the extraction, otherwise a turbidity occurs in the organic phase because of saturation by lead chelates.

The proposed separation procedure was applied to several synthetic samples. Known amounts of bismuth were added to lead nitrate solution (equivalent to 1 g of lead), previously freed from bismuth and copper by the extraction with zinc-DBDTC. Table I shows the results, for which the metals were determined by atomic absorption spectrometry. With a single washing with 3 *M* hydrochloric acid, a few micrograms of lead remained in the organic phase with bismuth. When the concentration of hydrochloric acid was increased to 3.5 *M*, the lead remaining was negligible. Almost all lead was back-extracted by washing twice with 3 *M* hydrochloric acid.

Table II shows the results by spectrophotometric measurements of bismuth at 370 nm. Good recoveries were obtained and Beer's law was obeyed by this

TABLE I

ANALYSIS OF SYNTHETIC SAMPLES

(Atomic absorption spectrometry of bismuth and lead in the organic phase after the separation. 1.0 g Pb was used in all cases)

Bi added (μg)	*Number of back-extraction*	*Acidity (M HCl)*	*Bi found (μg)*	*Pb found*[a] *(μg)*
0	1	3.5	0	n.d.
0	1	2.5	0	34
0	2	2.5	0	n.d.
100	1	3.5	98	n.d.
100	1	3.0	100	6
50	1	3.5	52	2
50	1	3.0	50	12
100	2	3.0	102	n.d.
100	2	3.0	98	n.d.
50	2	3.0	48	n.d.
100	1	3.5	100	n.d.
100	1	3.0	102	8

[a] n.d. = No detection of lead.

TABLE II

ANALYSIS OF SYNTHETIC SAMPLES

(Spectrophotometric determination of bismuth; acid concentration for back-extraction was 3.5 *M* hydrochloric acid)

Pd added (g)	*Bi added (μg)*	*Absorbance at 370 nm*	*Absorbance with no Pb present initially*
1.0	20	0.115	0.113
1.0	50	0.283	0.282
1.0	80	0.455	0.455

system. It was thus verified that bismuth is satisfactorily separated from large amounts of lead, and that no loss of bismuth was observed in this procedure.

It seems that this is generally sufficient for a pure bismuth fraction. Zinc in the reagent used was almost wholly transferred to the aqueous phase by shaking with acid solutions, and the zinc remaining in the organic phase was found to be insignificant (below 0.1 p.p.m.) by atomic absorption spectrometry.

If necessary, bismuth can be back-extracted by washing with 7 *M* hydrochloric acid.

SUMMARY

Solvent extraction of lead, silver, antimony and thallium from various acid solutions was investigated with zinc-DBDTC as chelating reagent. These metals were quantitatively extracted over a wide range of acidity with 0.03% zinc-DBDTC solution in carbon tetrachloride. A separation procedure for bismuth from large amounts of lead was developed; bismuth was extracted from 1 *M* nitric acid with zinc-DBDTC and was separated from lead by subsequently washing the organic phase once with 3.5 *M* hydrochloric acid or twice with 3 *M* hydrochloric acid. Satisfactory results were obtained in separating microgram amounts of bismuth from 1 g of lead.

RÉSUMÉ

L'extraction du plomb, de l'argent, de l'antimoine et du thallium dans un solvant est examinée, en utilisant le zinc dibenzyldithiocarbamate (DBDTC) comme réactif chélatant. Ces métaux ont été extraits quantitativement dans un large domaine d'acidité au moyen d'une solution à 0.03% de zinc-DBDTC dans le tétrachlorure de carbone. Une séparation du bismuth d'avec de grandes quantités de plomb est proposée; elle s'effectue en milieu acide nitrique *M*. Des résultats satisfaisants sont obtenus pour des quantités de bismuth de l'ordre du microgramme, en présence de 1 g de plomb.

ZUSAMMENFASSUNG

Die Extraktion von Blei, Silber, Antimon und Thallium mittels Zink-DBDTC

als Chelatisierungsreagenz aus verschiedenen sauren Lösungen wurde untersucht. Diese Metalle wurden in einem weiten Aciditätsbereich mit einer 0.03 %igen Zink-DBDTC-Lösung in Kohlenstofftetrachlorid quantitativ extrahiert. Es wurde ein Verfahren für die Abtrennung von Wismut von grossen Mengen Blei entwickelt; Wismut wurde aus 1 *M* Salpetersäure mit Zink-DBDTC extrahiert und vom Blei durch anschliessendes Waschen der organischen Phase entweder einmal mit 3.5 *M* Salzsäure oder zweimal mit 3 *M* Salzsäure abgetrennt. Zufriedenstellende Ergebnisse wurden bei der Abtrennung von Mikrogramm-Mengen Wismut von 1 g Blei erhalten.

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Analytica Chimica Acta, 69 (1974) 355–361

SOLVENT EXTRACTION SEPARATION OF RHODIUM FROM IRIDIUM WITH TRI-n-OCTYLAMINE AS A LIQUID ANION-EXCHANGER

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The separation of rhodium and iridium is one of the most difficult aspects of platinum metal analysis and few acceptable methods are available. Selective reduction by metals such as copper[1–3], antimony[4] or chromium[5] have been proposed, although a subsequent separation of the added metal was then required. Ion-exchange methods have been complicated by the fact that aged solutions of rhodium and iridium behave differently from fresh ones. A cation-exchange method[6] can be used for milligram amounts of the metals as their thiourea complexes if temperature is carefully controlled. Anion-exchange methods[7–9] have been used but require excess of salt for elution or Soxhlet-extraction for metal recovery.

Solvent extraction methods provide many useful separations but for rhodium and iridium there has been limited success. The use of tin(II) bromide complexes and isoamyl alcohol[10] provided quantitative results for microgram amounts of rhodium and iridium. Tetraphenylphosphonium complexes can be extracted[11] for 30–80 μg iridium from 1–2 mg rhodium. Tributyl phosphate has been used[12] to separate up to 200 μg of rhodium from up to 1 mg of iridium but the concentration of rhodium must be roughly known previously. Tri-n-octylamine (TOA) has been used to separate platinum and rhodium in 2 *M* hydrochloric acid[13]. The separation of rhodium from platinum and palladium with TOA in benzene to extract the rhodium–tin complex has been reported by Khattak and Magee[14].

It can be seen that there are few generally acceptable means of separating rhodium and iridium over a reasonably wide concentration range. A TOA solvent extraction method appeared to be most promising. TOA extractions for iridium(III), iridium(IV) and rhodium(III) singly as their chlorides from hydrochloric acid solutions have been studied with a continuous extraction system[15]. It was reported that the trivalent ions were only slightly extracted and, provided that chlorine was used to maintain the species, iridium(IV) was largely extracted. Calculations then indicated the possibility of a separation in 6 *M* hydrochloric acid although no experimental results were reported. The present work involves an extraction from 6 *M* hydrochloric acid with TOA solution to separate rhodium and iridium by conventional analytical techniques in a quantitative manner. In addition, the recovery of iridium from the organic phase with ammonia solution is reported.

EXPERIMENTAL

Apparatus

A Baird-Atomic Model 530 γ-spectrometer and Model 810C well counter

(Baird-Atomic, Bedford, Massachusetts), and a Perkin-Elmer Model 306 atomic absorption spectrophotometer with a Varian rhodium lamp, were used.

Reagents

An iridium solution (1 mg ml^{-1}) in 0.1 *M* hydrochloric acid was prepared from sodium hexachloroiridate hexahydrate, and a rhodium solution (1 mg ml^{-1}) in 1.2 *M* hydrochloric acid was prepared from sodium hexachlororhodate dodecahydrate; both salts were obtained from Johnson Matthey and Mallory Limited (Toronto).

Baker-analyzed reagent hydrochloric acid and ammonia solution, thiophene-free benzene (Fisher Scientific Company), and tri-n-octylamine (practical; Eastman Kodak) were used.

A 0.2 *M* solution of the amine (as received) in thiophene-free benzene was equilibrated on a Burrell "Wrist-Action" shaker for 1 h with four times its volume of 6 *M* hydrochloric acid before use.

Iridium-192 (Amersham-Searle Limited, Don Mills, Ontario) was diluted before use.

Procedures

The extractability of iridium in the presence of rhodium was studied with iridium-192 as tracer. The rhodium extractability was studied separately by atomic absorption spectrometry and the separation of rhodium from iridium was determined from the combination of the results.

Extraction of iridium

Samples containing the required amounts of inactive iridium and rhodium in a few milliliters of hydrochloric acid, and sufficient iridium-192 to give a counting rate of $13 \cdot 10^3$ counts min^{-1} were evaporated to dryness on a steam bath. The dry residues were treated with aqua regia and the solutions were evaporated three times to ensure that iridium was in the tetravalent state. Nitrous oxides were removed by adding 2 ml of concentrated hydrochloric acid to the samples and evaporating to dryness three times, the final time just before use of the individual sample. The dry residue was dissolved in 15 ml of 6 *M* hydrochloric acid and the solution was transferred to a 100-ml separatory funnel to which 15 ml of equilibrated 0.2 *M* TOA solution was then added. Chlorine was passed through the solution at 40 ml min^{-1} via a capillary tube until a slight haziness was apparent in the organic phase; this prevented the reduction of iridium by the amine. The separatory funnel was shaken by hand for 2 min and the phases were allowed to separate. The aqueous and organic phases were drained into test-tubes and their activities determined in the cavity of the well-counter.

The organic phase was then transferred to a clean separatory funnel and back-washed with 15 ml of 6 *M* hydrochloric acid, chlorine being passed as before. The activity of the aqueous phase indicated the loss of iridium on back-washing. The organic phase was then extracted with three 15-ml portions of 7 *M* ammonia solution to strip the iridium. The iridium activity in the strippings and in the residual organic phase were determined.

The radio-active procedure used was to count each sample five successive

times for a period of 100 s and to use the average activity after correcting for the background which was obtained from the average of fifteen successive 100-s measurements. The average deviation for measurements of the major iridium-containing phase was ±0.6%.

Extraction of rhodium

The samples for the extraction study were prepared identically to those described above. Reference samples containing only rhodium were used to determine the amount of rhodium initially present.

The extraction procedure was identical to that described above. The aqueous phase was separated for subsequent rhodium determination by atomic absorption. The organic phase was transferred to a clean separatory funnel and back-washed with acid as described previously. The acid back-wash was then analyzed for rhodium.

It was thought that there would be no interference from small amounts of iridium on the determination of rhodium by atomic absorption, and this was shown to be true for up to twice the concentration of iridium normally found in the aqueous phase after the extraction procedure. Standard rhodium solutions in the desired concentration range were prepared in 6 *M* hydrochloric acid. Sample solutions containing 1438 μg of rhodium were diluted to about 20 p.p.m. in 6 *M* hydrochloric acid while more dilute samples and back-washings were analyzed directly. The 343.5-nm line was used for analysis with the conditions basically as outlined in the Perkin-Elmer Handbook[16].

RESULTS AND DISCUSSION

The extent of iridium extraction in the presence of rhodium and the extent of iridium recovery from the organic phase are given in Table I. For the 100–1500

BLE I

TRACTION OF IRIDIUM IN THE PRESENCE OF RHODIUM

sent (μg)	*Rh present (μg)*	*% Ir extracted into organic phase after single extraction*	*% Ir in organic phase after back-wash*	*% Ir recovered from organic phase*
0	0	98.3[a] ± 0.3	97.9 ± 0.3	98.7 ± 0.1
0	93	98.1 ± 0.2	97.8 ± 0.2	98.7 ± 0.2
0	465	98.1 ± 0.2	97.6 ± 0.2	98.9 ± 0.2
0	1438	97.9 ± 0.2	97.4 ± 0.2	98.8 ± 0.4
0	0	99.2 ± 0.1	99.0 ± 0.1	99.0 ± 0.1
0	93	99.1 ± 0.1	98.9 ± 0.1	98.8 ± 0.4
0	465	98.8 ± 0.2	98.4 ± 0.1	98.3 ± 0.3
0	1438	98.5 ± 0.1	98.0 ± 0.2	98.0 ± 0.4
0	0	99.2 ± 0.1	99.0 ± 0.1	97.5 ± 0.4
0	93	99.2 ± 0.1	99.0 ± 0.1	98.0 ± 0.1
0	465	99.1 ± 0.1	98.9 ± 0.1	96.8 ± 0.4
0	1438	98.9 ± 0.1	98.7 ± 0.2	97.7 ± 0.1

ean value of at least three samples ± mean deviation.

μg range studied, 98–99% of the iridium was extracted into the organic phase with a single extraction. Other results showed that two additional extractions increased the extraction of iridium from the aqueous phase by 0.5–1.0% over the range of iridium and rhodium studied. The percentage of iridium extracted increased slightly (1%) with increasing iridium concentration. Within a series of samples with a fixed iridium concentration, the percentage of iridium extracted decreased slightly (0.7% or less) with increasing rhodium concentration. The back-washing of the organic phase resulted in an iridium loss of 0.5% or less to the aqueous phase.

The recovery of iridium from the organic phase by stripping with an ammonia solution varied from 97 to 99% depending on the iridium concentration. The lower recoveries were obtained for samples containing the greatest amount of iridium (1500 μg) and additional strippings of the organic phase failed to recover more iridium.

Some attempts were made to improve on the iridium extraction by evaporating the aqueous phase to dryness after the initial extraction and treating with aqua regia as done in the sample preparation. The residue was dissolved in 6 *M* hydrochloric acid and extracted with TOA as before. This procedure yielded slightly better results than a simple second extraction of the original sample, *i.e.*, 75% of the iridium remaining in the aqueous phase was extracted as compared with 50%. Thus the two extractions removed about 99.5% of the iridium but involved considerably more effort. It would appear that reduction of iridium(IV) to iridium(III) occurs during the extraction even in the presence of chlorine, so that completely quantitative recovery is prevented.

The extent of rhodium extraction in the presence of iridium is shown in Table II. These results show the necessity of back-washing the organic phase with acid as the single back-wash yielded about 2% of the rhodium present in the samples. For samples containing 93 or 465 μg of rhodium and 100 or 500 μg of iridium,

TABLE II

EXTRACTION OF RHODIUM IN THE PRESENCE OF IRIDIUM

Rh present (μg)	*Ir present (μg)*	*Rh found in aqueous phase after single extraction (μg)*	*Rh found in back-wash (μg)*	*Total Rh recovered (μg)*	*% Rh in aq. phase*
93	0	95[a] ± 2	2 ± 0.8	97 ± 2	104 ± 3
93	100	96 ± 2	2 ± 0.8	98 ± 1	105 ± 1
93	500	90 ± 1	2 ± 0.5	92 ± 2	100 ± 2
93	1500	79 ± 2	5 ± 1	84 ± 2	90 ± 2
465	0	451 ± 2	10 ± 0.5	461 ± 2	99.2 ± 0.4
465	100	452 ± 4	12 ± 2	464 ± 4	99.8 ± 0.9
465	500	449 ± 5	12 ± 0	461 ± 5	99.2 ± 0.9
465	1500	448 ± 3	12 ± 0.5	460 ± 3	99.0 ± 0.6
1438	0	1344 ± 13	34 ± 4	1378 ± 10	95.8 ± 0.7
1438	100	1350 ± 8	30 ± 0.5	1380 ± 8	96.0 ± 0.6
1438	500	1348 ± 10	31 ± 1	1379 ± 13	95.9 ± 0.7
1438	1500	1350 ± 8	31 ± 1	1381 ± 7	96.0 ± 0.5

[a] Mean value of four samples ± mean deviation.

little or no loss of rhodium from the aqueous phase was observed after the extraction and single back-wash procedure. Samples containing 93 μg of rhodium and 1500 μg of iridium exhibited a 10% loss of rhodium, presumably because of the high iridium concentration. With 1438 μg of rhodium, a rhodium loss of about 4% was incurred.

A cursory study of the effect of multiple extractions on the extractability of rhodium with 465-μg samples showed a slight increase in the loss of rhodium from the aqueous phase. Three extractions and a single 15-ml acid back-wash of the combined organic phases yielded a recovery in the aqueous phase of 98% rhodium as compared with 99% with a single extraction and back-wash.

TABLE III

SEPARATION OF RHODIUM FROM IRIDIUM AFTER SINGLE EXTRACTION AND BACK-WASH

Rh present (μg)	*Ir present (μg)*	*% Rh in aqueous phase*	*% Ir in organic phase*
93	100	105[a]	97.8
93	500	100	98.9
93	1500	90	99.0
465	100	98.8	97.6
465	500	99.2	98.4
465	1500	99.0	98.9
1438	100	96.0	97.4
1438	500	95.9	98.0
1438	1500	96.0	98.7

[a] Mean value of three or more trials.

The results shown in Table III were compiled from the results of the separate studies of rhodium and iridium extraction with a single extraction and back-wash. Thus for samples containing 93 and 465 μg of rhodium in the presence of 100–1500 μg of iridium, essentially rhodium-free iridium was obtained, except for the samples containing 93 μg rhodium and 1500 μg iridium. It would appear that an iridium concentration between 5 and 15 times the rhodium concentration is the maximum which can be tolerated without serious rhodium losses. With the 1438-μg rhodium samples, the rhodium loss to the organic phase becomes significant, of the order of 4%.

When other noble metals are present in the sample, a preliminary separation of these may be required. However, there are reports[14] that indicate that platinum and palladium would be extracted along with iridium by TOA from strongly acidic solutions.

The procedure outlined above provides a reasonably fast separation method for rhodium and iridium over a large concentration range with standard equipment. The method gives quantitative recovery of rhodium and 98–99% recovery of iridium for samples of suitable concentration.

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SUMMARY

Solvent extraction with tri-n-octylamine as a liquid anion-exchanger can be used to separate 93–465 μg of rhodium from 100–500 μg of iridium. With larger concentrations of rhodium and iridium, some rhodium is extracted along with the iridium. Single extractions and back-washing of the organic phase resulted in 97–99% of 100–1500-μg samples of iridium being extracted into the organic phase. More than 98% of the iridium extracted into the organic phase was recovered by stripping with ammonia solution.

RÉSUMÉ

L'extraction dans un solvent avec la tri-n-octylamine, comme échangeur anionique liquide, peut être utilisée pour séparer 93–465 μg de rhodium d'avec 100–500 μg d'iridium. Pour des concentrations plus fortes, il peut se produire un extrainement du rhodium lors de l'extraction de l'iridium. Pour des échantillons d'iridium de 100–1500 μg, 97 à 99% sont extraits dans la phase organique. Ensuite plus de 98% de l'iridium extrait est récupéré au moyen d'une solution ammoniacale.

ZUSAMMENFASSUNG

Durch Solventextraktion mit Tri-n-octylamin als flüssigem Anionenaustauscher können 93–465 μg Rhodium von 100–500 μg Iridium abgetrennt werden. Bei grösseren Konzentrationen von Rhodium und Iridium wird etwas Rhodium zusammen mit dem Iridium extrahiert. Durch einfache Extraktionen und durch Zurückwaschen der organischen Phase wurden 97–99% der Iridiumproben von 100–1500 μg erhalten, die in die organische Phase extrahiert worden waren. Mehr als 98% des in die organische Phase extrahierten Iridiums wurden durch Waschen mit Ammoniaklösung wiedergewonnen.

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ION-ASSOCIATION COMPOUNDS OF ANIONIC SURFACTANTS WITH IRON(II) CHELATES

PART I. EXTRACTION CONSTANTS

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Neutral aromatic chelating ligands such as 1,10-phenanthroline, 2,2′-bipyridine, and 2,2′,2″-tripyridine, are widely used for the colorimetric determination of iron(II). The complex cations which are formed can be extracted into organic solvents as ion-association compounds. Such extractions are used for the determination of iron or of the associated anion[1].

Taylor and Fryer[2] have used tris(1,10-phenanthroline)iron(II) (ferroin) and tris(2,2′-bipyridine)iron(II) cations for the colorimetric determination of anionic surfactants in sewage and sewage effluents. When the procedure is adapted to a radiometric one[3] with ferroin, labelled with iron-59, trace levels of surfactants can be determined in ground and potable waters. These methods are based on the formation of chloroform-soluble ion-association compounds[4] ($(\text{chelate})^{2+}(\text{surfactant})_2^-$). The chelate is added to an aqueous solution in formula excess of the surfactant present and the extent of extraction of the compound into chloroform is proportional to the amount of surfactant present.

Subsequent investigations[5] have shown that iron(II) chelates structurally similar to ferroin, can also form stable chloroform-soluble compounds with long alkyl-chain surfactants (sulphates and sulphonates). The chelates have high overall stabilities (log k_{form} about 20), high molar absorptivities (about 10^4), suitable organic solubilities, and are readily obtainable.

In this paper, results of the measurement of chloroform extractabilities of iron(II) chelate–surfactant compounds are reported and are correlated with alkyl chain length, with structure and polar group of the surfactant, and with size and structure of the chelate. The conditional extractability of these compounds is expressed as the extraction constant[6].

EXPERIMENTAL

Apparatus and reagents

The visible spectra of compounds were recorded with a Unicam SP800 spectrophotometer, and fixed wavelength measurements were made with a Unicam

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SP600 spectrophotometer.

Extraction device, counting equipment and counting vessel have already been described[2,3].

The ligands used were 1,10-phenanthroline (phen) and 2,2′-bipyridine (bipy; British Drug Houses Ltd, Poole), 2,2′,2″-tripyridine (tripy; Koch-Light Laboratories Ltd, Colnbrook), 5-methyl-1,10-phenanthroline (5-mephen), 5-nitro-1,10-phenanthroline(5-nitrophen), 5-bromo-1,10-phenanthroline (5-brphen), 5-chloro-1,10-phenanthroline (5-clphen), 5-phenyl-1,10-phenanthroline (5-phphen), 5,6-dimethyl-1,10-phenanthroline (5,6-dimephen), and 4,7-dimethyl-1,10-phenanthroline (4,7-dimephen) (all from G. Frederick Smith Chemical Co., Columbus, Ohio).

Stock iron(II) solution ($17.9 \cdot 10^{-3}$ *M*) was prepared from iron(II) ammonium sulphate and contained hydroxylammonium chloride (0.1%).

Inactive chelate solutions. ($8.95 \cdot 10^{-4}$ *M*). These were usually prepared by adding ligand ($5 \cdot 10^{-4}$ mol) dissolved in water (25 ml) or in the minimal amount of dilute hydrochloric acid, to stock iron(II) solution (5 ml) and then diluting to 100 ml. These solutions contain a 5- to 6-fold molar excess of ligand with respect to iron(II).

The bipy chelate reagent contained a 20-fold molar excess of bipy to prevent fading of the colour on extraction.

The tripy chelate reagent contained $4.3 \cdot 10^{-3}$ mol l^{-1} (0.1%) of tripy in hydrochloric acid (0.01 *M*). This solution was freshly prepared because the colour faded on standing.

Labelled iron(II) chelates ($8.95 \cdot 10^{-4}$ M). Iron(III)-59 supplied in 0.1 *M* hydrochloric acid (Radiochemical Centre, Amersham) had a specific activity of about 10 Ci g^{-1} (half-life 45.1 days, maximum β-energies 0.46 and 0.27 MeV, γ-energies 1.10 and 1.29 MeV). About 150 μCi of iron-59 was added to the inactive solutions above before dilution. When a slight excess of sodium lauryl sulphate was added to these reagents, virtually all the activity was extracted into chloroform, indicating that incorporation of the tracer into the chelates was complete.

Surfactants. Members of three homologous series of surfactants were used:

Sodium primary alkyl sulphates. Pentyl, hexyl, heptyl, octyl, nonyl and decyl sulphates were prepared from the corresponding alcohols by the following method. Chlorosulphonic acid (1.05 mol per mol of alcohol) was added dropwise to the pure alcohol in a flask fitted with a calcium chloride tube. The mixture was stirred during addition and for 10 min after, and the reaction temperature was maintained at 20–25° by an ice bath. The mixture was neutralized by pouring it into a calculated quantity of aqueous sodium hydroxide (5% excess). Ethanol–water (9+1) was added to give a solution containing 3–4% of sodium alkyl sulphate. Sodium chloride and sodium sulphate precipitated and were removed by filtering. Water was added to alter the concentration of ethanol to 50%, and the solution was then extracted with three equal portions of petroleum ether (40/60): the total volume of the ether was half the volume of the ethanolic solution. The ethanolic solution was evaporated to dryness to yield sodium alkyl sulphate, which was purified by recrystallizing from ethanol.

Sodium dodecyl sulphate (SDS, sodium lauryl sulphate; British Drug Houses Ltd) and sodium tetradecyl sulphate (Unilever Research Limited, Port Sunlight) were also used.

Sodium primary alkyl benzene sulphonates. Butyl-, hexyl-, octyl-, dodecyl- and tetradecyl-benzene sulphonates (Unilever) were used.

Sodium primary alkane sulphonates. Pentane-, octane-, decane-, dodecane- and hexadecanesulphonates (Unilever) and nonane-, undecane- and tridecane-sulphonates (R. N. Emanuel Ltd, Alperton) were used.

The purities of most of the surfactants were determined by the procedure described in Part II of this series[7], and were found to be better than 95%. Solutions ($2 \cdot 10^{-3}$ *M*) of the surfactants were prepared in water (usually) or in aqueous alcohol (C_{16} sulphonate which is poorly soluble in water alone).

Buffer solution (pH5). Sodium acetate (2 *M*) was adjusted to pH 5 with acetic acid (2 *M*).

The chloroform was of analytical-reagent grade.

Extractability, spectral characteristics, Beer's Law adherence and stoichiometry

Each chelate reagent was added to a reference solution of SDS or to a solution containing one of the other surfactants. The aqueous solution (5 ml) containing the chelate, the surfactant and buffer (0.5 ml) was extracted in a centrifuge tube (15 ml) with successive 2-ml portions of chloroform as already described[2]. The extracts were combined and made up to 5 or 10 ml in a graduated flask with the aid of the extraction device.

The spectral characteristics of the chelate–surfactant compounds were determined in both water and chloroform, and the formulae of the compounds in chloroform were confirmed by molar ratio and continuous variation techniques.

Beer's Law plots for chelate–surfactant systems were done by preparing a series of solutions containing SDS (0.1–0.25 ml) and the chelate in at least 5-fold molar excess. Each solution was extracted with chloroform and the absorbances of the extracts were measured (1-cm cell, chloroform reference) at the wavelength of maximum absorption for the chelate.

All the chelates were found to form chloroform-extractable stoichiometric compounds with surfactants. Fading of the chelate colour in chloroform occurred with the ligands 5-nitrophen and 5-phphen. The 5-phphen chelate is appreciably extracted and the dimephen chelates are slightly extracted in the absence of surfactant.

The stoichiometry of the compounds was found to be 1:2 for all the compounds which were extracted. The spectral characteristics of the chelates undergo little or no change on extraction (Table I). All the stable chelate-surfactant compounds obey Beer's Law over a wide range.

Relative extractabilities

Aqueous solutions (5 ml) each containing a chelate ($2 \cdot 10^{-4}$ mmol), an even-numbered alkyl-chain surfactant ($2 \cdot 10^{-5}$ mmol), and buffer (0.5 ml) were extracted with chloroform (3×2 ml). The first two extracts were combined and made up to 5 ml with the third. Absorbances of the extracts were then measured at the appropriate wavelengths.

In each surfactant series there is a member which forms a partially extractable compound with a particular chelate (Table II). This compound is defined as the "break-through" compound for the series. It is the compound which is *ca.* 50%

TABLE I

SPECTRAL DATA FOR IRON(II) CHELATES IN WATER AND IN CHLOROFORM

Ligand	*Water*		*Chloroform*	
	λ_{max} *(nm)*	*Molar absorptivity*	λ_{max} *(nm)*	*Molar absorptivity*
Phen	512	11,100	512	11,100
Bipy	522	8,650	522	8,650
Tripy	552	11,000	554	11,200
5-Clphen	512	11,700	514	11,770
5-Nitrophen	510	11,500	510	—
5-Mephen	516	11,500	516	11,550
5-Brphen	515	12,500	515	12,570
5-Phphen	522	12,700	520	—
4,7-Dimephen	512	14,000	514	13,940
5,6-Dimephen	520	12,600	520	13,000

TABLE II

BREAK-THROUGH COMPOUNDS OF IRON(II) CHELATE–SURFACTANT SERIES

Ligand	*Break-through alkyl-chain length*		
	Alkyl sulphate	*Alkylbenzene sulphonate*	*Alkane sulphonate*
Bipy	C_{10}	C_8	C_{12}
Tripy	C_{10}	C_8	C_{12}
Phen	C_8	C_6	C_{10}
5-Nitrophen	C_8	C_6	C_{12}
5-Clphen	C_6	C_4	C_8
5-Brphen	C_6	C_4	C_8
5-Mephen	C_6	C_4	C_8
Dimephen(4,7- and 5,6-)	C_5	below C_4	below C_8

extracted under the stated conditions. The higher adjacent even-numbered alkyl-chain homologue is almost completely extracted and other higher homologues are completely extracted. The lower adjacent homologue is hardly extracted at all, and other lower homologues are not extracted.

The break-through alkyl-chain length depends on the surfactant series and it also depends on the structure of the chelate (the number and type of substituents). The largest chelates usually form the most extractable compounds and the smallest, the least extractable ones with particular surfactants. Compounds containing 5-nitrophen are exceptions to this, probably owing to the hydrophilic nature of the nitro group.

The results indicate that homologous surfactants might be separated with the aid of appropriate chelates, and that short-chain surfactants might be determined using dimephen chelates (4,7- or 5,6-).

Extraction constants

MA_2 represents the chelate-surfactant compound which is partitioned between water (aq) and chloroform (org) and which is assumed to dissociate in water, ($MA_2 = M^{2+} + 2A^-$).

The extraction constant $\beta = Pk_{form} = [MA_2]_{org}/([M^{2+}][A^-]^2)$ where the partition coefficient $P = [MA_2]_{org}/[MA_2]_{aq}$ and the overall formation constant $k_{form} = [MA_2]_{aq}/([M^{2+}][A^-]^2)$. The distribution ratio, $D = [MA_2]_{org}/([MA_2]_{aq} + [M^{2+}])$. From these terms, expressions relating system parameters to the extraction constant may be derived.

If A is substoichiometric to M in the aqueous phase, then the solvent phase after extraction will contain only MA_2. If this solvent phase is equilibrated with pure water, then in the aqueous phase,

$$[A^-] = 2[M^{2+}], \quad \beta = [MA_2]_{org}/4[M^{2+}]^3, \quad \text{and} \quad [M^{2+}] = ([MA_2]_{org}/4\beta)^{\frac{1}{3}}$$

Further substitution and rearrangement gives

$$1/D = 1/(4\beta)^{\frac{1}{3}} \cdot 1/([MA_2]_{org})^{\frac{2}{3}} + 1/P$$

Hence if a chloroform solution of a chelate–surfactant compound is partitioned with water, and if the distribution ratio of the chelate between the two phases is measured, a plot of $1/D$ versus $(1/[MA_2]_{org})^{\frac{2}{3}}$ should yield a straight line with slope $1/(4\beta)^{\frac{1}{3}}$ and an intercept $1/P$. The expression is suitable for use with compounds that can be appreciably extracted into chloroform, and whose chloroform solutions can be back-extracted with water to give measurable concentrations of chelate in each phase.

If experimental conditions are arranged so that $[A^-]$ is much larger than $[M^{2+}]$, then values of $[A^-]_{aq}$ before and after partition are virtually the same. Substitution and rearrangement then gives

$$[M^{2+}] = [MA_2]_{org}/Pk_{form}[A^-]_{aq}^2$$

and finally

$$1/D = 1/P + 1/\beta[A^-]_{aq}^2$$

Hence if an aqueous solution containing chelate and a large known excess of surfactant is partitioned between chloroform and water, and if the distribution ratio of the chelate between the two phases is measured, a plot of $1/D$ versus $1/[A^-]_{aq}^2$ should yield a straight line with a slope of $1/\beta$ and with an intercept $1/P$. This expression is suitable for determining the extraction constants of compounds of low extractability, where significant changes in D can be obtained by varying $[A^-]_{aq}$.

In order to obtain comparable results, the partition conditions were standardized as far as possible. With few exceptions the measurements were made at minimal ionic strength and a working pH of about 5. Distribution ratios have been shown[5] to be independent of pH between 4 and 6.

For the back-extraction method. Aqueous solutions (5 ml) containing chelate (0.5–1.0 ml) and varying substoichiometric amounts of surfactant were prepared in glass-stoppered 15-ml centrifuge tubes. Each solution was shaken by hand for 1 min with 6 ml of chloroform. After centrifugation for 1 min, 5 ml of the chloroform

phase was transferred to another calibrated tube and back-extracted with 5 ml of distilled water by gentle inversions of the tube for 30 s. The tube was placed in a thermostatted water bath at 25° for 5 min, withdrawn, inverted several times for 10 s and replaced in the bath for 1 min. Withdrawal, inversion and replacement were repeated twice to ensure partition equilibrium at the bath temperature. Finally, the tube was removed from the bath, and the two phases were completely separated by centrifugation for 30 s. It was assumed that no further partition occurred across the small area of the liquid–liquid interface in the short period before the separate phases were analysed.

The concentration of chelate in each phase was measured colorimetrically or radiometrically, and the distribution ratio was thereby obtained. The extraction constant of the chelate–surfactant compound was obtained as described above with the aid of a computer.

In general, colorimetric techniques were used to determine the extraction constants of compounds of intermediate extractability (D between 0.1 and 20). The weight of surfactant added was adjusted so that the absorbances in both phases were never less than 0.05 (1-cm cells). Radiometric techniques were used to determine distribution ratios for compounds of higher chloroform extractability. In these determinations, the total activity of the phases was arranged to be about 70,000 counts min^{-1}.

For the forward-extraction method. Aqueous solutions (5 ml) containing chelate (0.25 ml) and varying measured excesses of surfactant were prepared. The amounts of added surfactants were selected to give a range of measurable distribution ratios. Each solution was extracted once with 5 ml of chloroform, the two phases were separated, and the concentration of chelate in each phase was measured, following a procedure similar to that used in the back-extraction method. The value of $[A^-]_{aq}$ was either calculated directly or was determined colorimetrically with 4,7-dimephen chelate. The extraction constant of the chelate–surfactant compound was computed as already described.

The extraction constants of a wide range of chelate–surfactant compounds were determined. Compounds containing the chelates of bipy, tripy, phen, 5-mephen, and 4,7-dimephen were investigated more thoroughly, particularly around the break-through regions.

The back-extraction method yielded extraction constant values greater than 10^7 mol^{-2}. Plots for all these compounds except the very highly extractable ones (β greater than 10^{11} mol^{-2}) gave good straight-line relationships. The standard errors of the values were obtained from residual errors[5].

Extraction constants of the very highly extractable compounds could not be determined precisely, because of adsorption and shorter alkyl-chain contamination. Only estimates of the constant for these compounds were made.

The radiometric and colorimetric techniques, when applied to the same compound, gave the same value for the extraction constant within the limits of the standard errors; *e.g.* for the bipy chelate–hexylbenzene sulphonate complex at 21°, $\beta = (4.05 \pm 0.18) \cdot 10^8$ and $(4.55 \pm 0.38) \cdot 10^8$ mol^{-2}, respectively. Most extraction constants were determined colorimetrically.

The extraction constants of all the break-through compounds were in the region 10^8–10^9 mol^{-2}.

The forward extraction method yielded values of 10^5–10^7 mol^{-2}. Plots for these compounds also gave good straight-line relationships in spite of the slight adsorption of the substoichiometric chelate. From these, extraction constants and standard errors were computed as before.

RESULTS AND DISCUSSION

All the determined extraction constants lie between 10^5 and 10^{15} mol^{-2} (Table III). There is continuity between the values obtained by the two methods of extraction, although no compound could be examined by both methods.

A number of factors affect the values obtained. Extraction constants are temperature-dependent: with the systems studied, values decrease with increasing temperature. However, the effect of temperature is small compared to the effect produced by altering the alkyl-chain length or the ligand.

The method used for equilibrating the compounds between chloroform and water affected the values obtained. Systems which were gently inverted so that each phase passed through the other, without the formation of an emulsion, gave good linear plots. Systems equilibrated with a fast electric stirrer, or by vigorous shaking, gave emulsions which led to inconsistent results.

Extraction constants were usually determined in the absence of buffer, in order to achieve minimal ionic strength and to prevent the formation of emulsions. Values determined in the presence of buffer were slightly lower than those determined in its absence. This effect is thought to be due to acetate ion competing with the surfactant for the chelate.

Extraction constants usually increase regularly with increasing chain length of homologous surfactants. The only exceptions are the bipyridine chelate compounds whose values remain almost constant above a certain chain length. Compounds with each of the other chelates have extraction constants whose logarithms are almost directly proportional to alkyl-chain length in each series. Extraction constants of adjacent homologous compounds differ from each other by a factor of about 10.

Extraction constants and break-through regions are dependent on the associated ligand. For a surfactant of particular chain-length, the largest chelates, those of 4,7- or 5,6-dimethylphenanthroline form the compounds with the highest constants, whilst the smallest chelate, that of bipyridine, forms the compound with the lowest one. Each may be placed in order of its ability to form extractable compounds. The following order of ligands is consistent for all surfactant series studied.

4,7-dimephen = 5,6-dimephen > 5-mephen = 5-brphen > 5-clphen > phen > tripy > bipy

Extraction constant measurements make it possible to distinguish between the extractabilities of chelates whose surfactant break-through regions are similar. For example, the tripy chelate forms rather more readily extractable compounds with a particular surfactant than does the bipy chelate; similarly the 5-mephen chelate is a rather better extracting cation than the 5-clphen chelate.

The extraction constant of a chelate-C_n alkyl sulphate is similar to those

TABLE III

EXTRACTION CONSTANTS OF CHELATE–SURFACTANT COMPOUNDS

(Values given as log β in mol^{-2}. Numbers in parentheses are relative errors (%). Superscripts indicate the techniques used: (colorimetric (c), radiometric (r), forward extraction (f), back extraction (b), and the temperatures (°C))

Chain length	*Bipy*	*Tripy*	*Phen*	*5-Mephen*	*4,7-Dimephen*
Alkyl sulphates					
C_5				6.87(5)$^{cb\,25}$	8.64(5)$^{cb\,25}$
C_6			5.46(2)$^{rf\,25a}$	8.47(2)$^{cb\,23}$	9.63(7)$^{cb\,25b}$
			5.92(1)$^{cf\,25}$		
C_7			7.50(6)$^{cb\,25}$	9.39(4)$^{cb\,23}$	10.24(8)$^{cb\,25}$
C_8	5.85(5)$^{rf\,25a}$	6.26(2)$^{rf\,25a}$	8.82(10)$^{cb\,23}$	10.67(16)$^{cb\,23}$	10.92(55)$^{cb\,25}$
	5.95(2)$^{cf\,25}$		8.61(10)$^{cb\,25}$		
C_9			8.95(18)$^{cb\,23}$	10.52(3)$^{cb\,23}$	
C_{10}	8.64(8)$^{cb\,25}$	8.99(4)$^{cb\,25}$	10.87(9)$^{cb\,23}$		
C_{12}	9.67(4)$^{cb\,23}$	10.88(9)$^{rb\,25}$	12.53(35)$^{rb\,23}$		
	9.55(8)$^{cb\,25}$				
C_{14}	9.59(6)$^{cb\,25}$	12.33(2)$^{rb\,23}$	14.5(76)$^{rb\,22}$		
Alkane sulphonates					
C_8			5.16(3)$^{rf\,25a}$	8.00(4)$^{cb\,25}$	9.42(3)$^{cb\,25}$
			5.46(3)$^{cf\,25}$		
C_9			7.37(9)$^{cb\,25}$	9.16(6)$^{cb\,25}$	10.28(7)$^{cb\,25}$
C_{10}	5.59(3)$^{rf\,25a}$	5.98(2)$^{rf\,25a}$	7.97(5)$^{cb\,25}$	10.12(8)$^{cb\,25}$	
	5.85(3)$^{cf\,25}$	6.28(2)$^{cf\,25}$	8.45(8)$^{cb\,22}$		
C_{11}	7.02(12)$^{cb\,25}$	7.63(6)$^{cb\,25}$	9.02(11)$^{cb\,25}$		
C_{12}	7.98(5)$^{cb\,25}$		10.51(21)$^{cb\,23}$		
	8.33(37)$^{cb\,24}$				
C_{13}	8.24(2)$^{cb\,25}$	10.05(4)$^{cb\,25}$	10.47(6)$^{cb\,25}$		
C_{16}			13.7(80)$^{rb\,23}$		
Alkyl-benzene sulphonates					
C_4			5.60(3)$^{rf\,25a}$	8.49(10)$^{cb\,22}$	9.79(11)$^{cb\,25}$
			5.97(1)$^{cf\,25}$		
C_6	6.16(2)$^{cf\,25}$		8.58(2)$^{cb\,25}$	10.59(19)$^{cb\,23}$	
			8.61(4)$^{rb\,21}$		
			8.66(8)$^{cb\,21}$		
C_8	7.96(25)$^{cb\,25}$	9.08(13)$^{cb\,25}$	10.31(7)$^{cb\,25}$		
C_{12}	9.44(26)$^{cb\,25}$	11.06(5)$^{cb\,22}$	12.8(52)$^{rb\,21}$		
C_{14}	9.09(20)$^{cb\,25}$	12.98(37)$^{rb\,22}$			

[a] Determinations were made in the presence of buffer solution (0.5 ml).

[b] The values obtained for this C_6 surfactant were 8.18(4)$^{cb\,25}$ for 5-Clphen, 8.74(12)$^{cb\,25}$ for 5-Brphen, and 9.78(11)$^{cb\,25}$ for 5,6-Dimephen.

of compounds of the same chelate with C_{n+2} alkane sulphonate, or C_{n-2} alkyl benzene sulphonate. The superior extractability of alkylbenzene sulphonates is due to the greater bulk and covalency of the compounds that they form: both of these features aid chloroform extractability. Alkyl sulphates are extracted more readily than corresponding alkane sulphonates probably because of the greater effective chain-length of alkyl sulphates.

SUMMARY

The chloroform extractabilities of iron(II) chelate–anionic surfactant compounds are measured. Extraction constants of these compounds are determined by absorptiometric or radiometric techniques. Values of the constants depend on the structure of the chelate and on the structure and the alkyl-chain length of the surfactant.

RÉSUMÉ

Des mesures d'extractibilité dans le chloroforme sont faites pour des chélates fer(II)–surfactants anioniques. Les constantes d'extraction de ces composés sont déterminées a l'aide de techniques absorptiométriques ou radiométriques. La valeur de ces constantes dépend de la structure du chélate, ainsi que la structure et de la longueur de la chaîne alcoylée du surfactant.

ZUSAMMENFASSUNG

Die Extrahierbarkeit von Verbindungen zwischen Eisen(II)-Chelat und anionischen Tensiden mittels Chloroform wurde untersucht. Die Extraktionskonstanten dieser Verbindungen wurden durch absorptiometrische oder radiometrische Verfahren bestimmt. Die Werte der Konstanten hängen von der Struktur des Chelates und von der Struktur und der Alkylkettenlänge des Tensids ab.

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ION-ASSOCIATION COMPOUNDS OF ANIONIC SURFACTANTS WITH IRON(II) CHELATES

PART II. SELECTIVE DETERMINATION OF SURFACTANTS

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In Part I of this series[1], the extraction constants of a number of iron(II) chelate–surfactant compounds in chloroform–water systems were reported. The values usually increased with increasing alkyl-chain length and with increasing size of the chelating ligand. Differences between the extraction constants of compounds of adjacent homologous surfactants with a particular chelate were mostly quite marked. These differences indicated that longer-chain surfactants might be separated and distinguished from shorter-chain ones by use of the appropriate chelate, and that short-chain surfactants which are undetectable by other methods might now be determined. In this paper, factors are examined which affect the extraction and separation of chelate–surfactant compounds, and a method is proposed for the selective determination of homologous surfactants.

Mixed compound formation

The cations which were examined were all divalent; when one was added to a mixture of surfactants, compounds could be formed which contained two different anions. The extraction constants of such compounds are likely to lie between those for compounds of each of the corresponding anions alone. If mixed compounds are formed, separations of homologous surfactant mixtures will be less effective than predicted from the extraction constants of binary compounds.

The formation of mixed compounds was demonstrated for ferroin–alkyl sulphate and ferroin–alkyl sulphonate systems. Solutions containing either sodium heptyl sulphate and sodium octyl sulphate, or sodium nonane sulphonate and sodium decane sulphonate, were treated with ferroin and extracted with chloroform in the usual way. The absorbances of the extracts (5 ml or made up to 10 ml) were compared with those of the extracts from solutions containing the same quantities of ferroin with one surfactant only. Each pair of surfactants differed from each other by only one carbon atom, and were in the break-through region[1] for the

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series with ferroin. This choice was made in order to produce conditions for maximum mixed compound formation in the separation region.

Results in Table I show that the amount of surfactant extracted from a mixture was always greater than the combined amounts extracted from corresponding separate surfactant solutions. The synergic effect was due to mixed surfactant compounds being formed with the chelate cation, in addition to compounds containing a single surfactant. The formation of such compounds caused the separation to be poorer than that predicted from extraction constant data.

TABLE I

EXTRACTION OF SOLUTIONS CONTAINING ONE OR TWO SURFACTANTS AND EXCESS O FERROIN

Surfactant added		*One partition (5 ml)*[a]		*Three partitions (2 ml each)*[a]		*Five partitions (2 ml each)*[a]	
Chain length	*Amount ($mmol \cdot 10^4$)*	*Surfactant found ($mmol \cdot 10^4$)*	*SF*	*Surfactant found ($mmol \cdot 10^4$)*	*SF*	*Surfactant found ($mmol \cdot 10^4$)*	*SF*
Ferroin–heptyl sulphate (C_7)–octyl sulphate (C_8)							
C_7	1.93	0.10		0.14		0.25	
C_8	1.77	0.67		0.88		1.19	
C_7+C_8	3.70	1.07	3.58	1.39	3.62	1.80	3.48
C_7	4.83	0.48		0.78			
C_8	4.43	2.31		2.89			
C_7+C_8	9.26	3.39	3.65	4.00	3.53		
Ferroin–nonane sulphonate (C_9)–decane sulphonate (C_{10})							
C_9	1.83	0.08		0.19			
C_{10}	2.00	0.61		0.90			
C_9+C_{10}	3.83	0.89	3.61	1.24	3.42		
C_9	4.58	0.49		0.57			
C_{10}	5.00	2.09		2.39			
C_9+C_{10}	9.58	2.97	3.07	3.49	3.10		

[a] Aqueous solutions contained $9 \cdot 10^{-4}$ mmol of chelate, and sodium acetate buffer (pH 5, 0.2 *M*)

Separation factor (*SF*) is defined in the usual way as

$$\frac{\text{Ratio of quantities in the extract } (R)}{\text{Ratio of original quantities in the aqueous solution}}.$$

R was calculated from experimental data being equal to

$$\left[B + \frac{C-(A+B)}{2}\right] \Big/ \left[A + \frac{C-(A+B)}{2}\right]$$

where A and B are the quantities extracted from the separate surfactant solutions, and C is the quantity extracted from the mixture. This expression is derived using the simplification that the difference between C and $(A+B)$ is accounted for by mixed compounds.

The results indicated that the separation factor for a pair of surfactants was not significantly affected by an increase in the number of partitions, but that it decreased somewhat with increasing surfactant concentration for the alkane sulphonate pairs.

SEPARATION CONDITIONS

Either a single chelate or a succession of chelates was added to aqueous solutions each containing two or more surfactants. The solutions were then extracted with chloroform and the absorbances of standard extract volumes were measured. The number of longer-chain surfactants which was extracted depended principally on the chelate used, as predicted from extraction constant data[1]. The quantity of surfactants extracted in the break-through region depended also on the number of surfactants present and on their relative concentrations, on the ionic strength, pH, and volume of the aqueous solution, on the quantity of chelate, and on the quantity, type and number of portions of solvent. These variables were studied in turn, in an attempt to optimize conditions for the separation of adjacent homologous surfactants.

Three-component mixtures

Solutions containing either sodium alkyl-benzene sulphonates (C_4, C_6 and C_8) or sodium alkane sulphonates (C_8, C_{10} and C_{12}) were treated with the chelate of 2,2′-bipyridine (bipy) for alkyl benzene sulphonates, or 2,2′,2″-tripyridine (tripy) for alkane sulphonates or 1,10-phenanthroline (phen) or 4,7-dimethyl-1,10-phenanthroline (4,7-dimephen) and extracted with chloroform. In a second set of experiments the three most appropriate chelates were added consecutively to each solution in the order given above; extractions with chloroform were made after each addition. Additions and extractions may be made in this way because the stability constants of iron(II) chelates reflect their ability to form extractable compounds with surfactants.

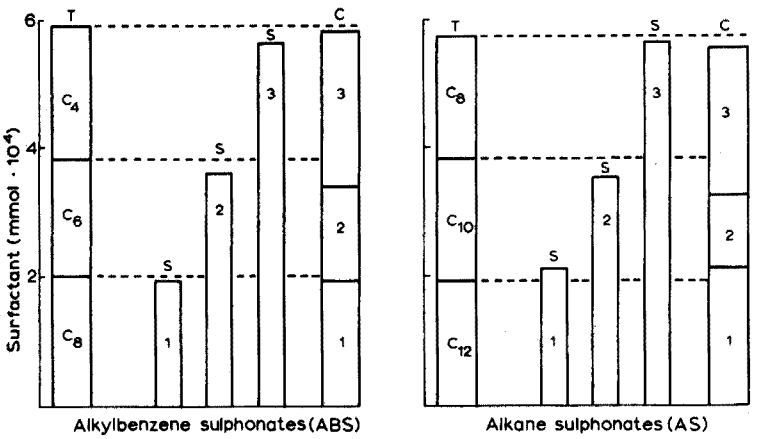

Fig. 1. Fractionation of simple surfactant mixtures. (T) total surfactant taken; (S) separate addition of chelate; (C) consecutive additions; (1) bipy (ABS) or tripy (AS); (2) phen; (3) 4,7-dimephen. 5 × 2-ml chloroform extracts were used. Conditions otherwise similar to those described for Table I.

Results are shown in Fig. 1. Both procedures gave high total recoveries of both types of surfactant. However, the recovery of the surfactants of intermediate chain length was worse when consecutive additions were made. All subsequent work was carried out with separate additions of iron(II) chelate solutions.

More complex mixtures

Aqueous solutions containing surfactant homologues were each treated with a chelate and extracted with chloroform. The quantities of surfactants in the extracts were measured as described above. Results are shown in Fig. 2 and in Table II.

Figure 2a indicates that the surfactants in the mixtures are almost totally extracted as their compounds with the 4,7-dimephen chelate. Even pentyl sulphate (C_5) is largely extracted, whereas when present alone, it is only slightly extracted. Each chelate is suitable for separating and determining surfactants with chains greater than a particular length. The two series were fractionated, but clean separations

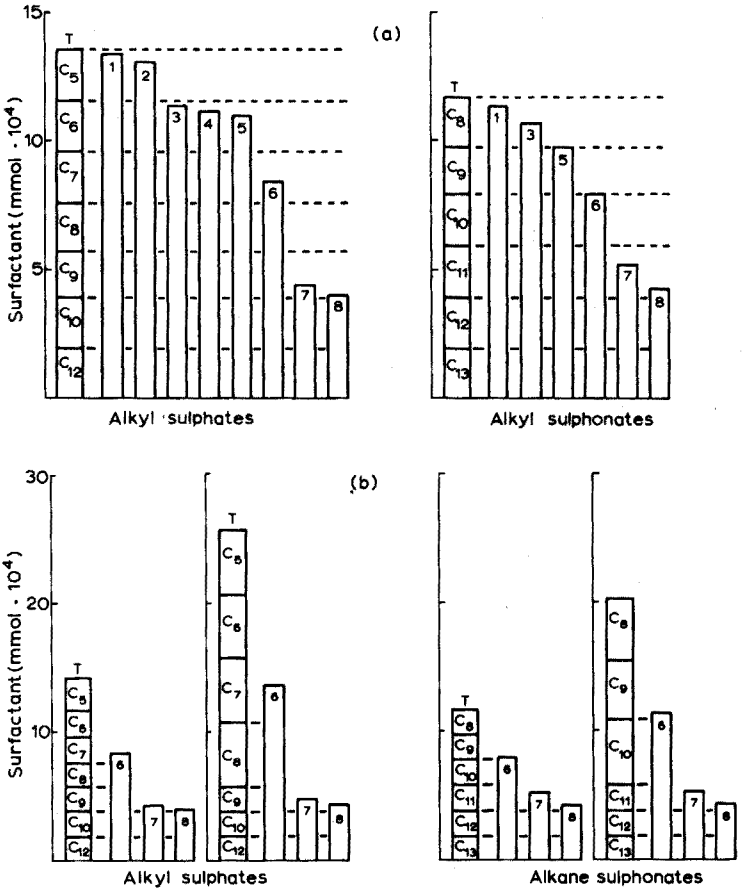

Fig. 2. (a) Mixtures containing similar molar quantities of each surfactant. (b) Effect of larger quantities of short-chain surfactants. (1) 4,7-dimephen; (2) 5,6-dimephen; (3) 5-mephen; (4) 5-brphen; (5) 5-clphen (6) phen; (7) tripy; (8) bipy. Aqueous solutions contained $2 \cdot 10^{-3}$ mmol of chelate. Conditions and symbols otherwise similar to those described for Fig. 1 and Table I.

between adjacent homologues were not achieved. This was due firstly to insufficient differences between extraction constants, and secondly to the synergic effect produced by mixed compound formation.

Figure 2b indicates that addition of larger amounts of short alkyl-chain surfactants to equimolar mixtures results in increased overall extraction. The effect is quite small when the bipy and tripy chelates are used, because the chain lengths of the added surfactants are appreciably shorter than the break-through chain lengths for these chelates.

TABLE II

EFFECTS OF LARGE AMOUNTS OF SHORT ALKYL-CHAIN SURFACTANTS ON THE EXTRACTION OF LONGER-CHAIN HOMOLOGUE MIXTURES

(Conditions were the same as those for Fig. 2)

Ligand	*Short-chain surfactant added (mmol·10⁴)*	*Surfactant found (mmol·10⁴)*
Alkyl sulphates: $5.74 \cdot 10^{-4}$ mmol (1.95, 1.96 and 1.84 mmol·10⁴ of C_{12}, C_{10} and C_9, respectively)		
Phen	None	5.57
	C_5 (20.0)	5.96
	C_6 (20.0)	8.14
	C_7 (20.0)	14.88
Bipy	C_7 (20.0)	4.21
	C_8 (18.6)	6.17
	C_9 (18.4)	6.03
Alkane sulphonates: $5.87 \cdot 10^{-4}$ mmol (1.95, 1.97 and 2.00 mmol·10⁴ of C_{13}, C_{12} and C_{11}, respectively)		
Phen	None	5.87
	C_8 (19.4)	7.03
	C_9 (19.3)	12.45
Bipy	C_9(19.3)	4.34
	C_{10} (20.0)	6.77
	C_{11} (20.0)	13.25

Table II indicates that the presence of a short-chain surfactant in relatively large quantity has little effect on the total surfactant extracted when its chain length is more than two carbon atoms short of the break-through region for the chelate-surfactant system.

Ionic strength

Some typical aqueous ferroin–surfactant systems, like those used to study mixed compound formation, were extracted without further additions. Similar systems were also extracted after the addition of buffer solution, buffer solution plus sodium chloride, or in one instance, sodium chloride alone. Extractions were either with 5 ml of chloroform in a closed system or with three 2-ml portions, making up to 5 ml with the third portion. The absorptiometrically measured quantities of extracted surfactants are listed in Table III together with the separation factors achieved for mixtures.

TABLE III

EFFECT OF IONIC STRENGTH ON EXTRACTABILITY OF SOME FERROIN–ALKYL SULPHATES AND –ALKANE SULPHONATES

(Conditions are similar to those described for Table I. Sodium chloride (2 ml) was added as a 20% solution)

Surfactant added		*Partitions*	*No additions*			*Buffer*			*Buffer and NaCl*			*NaCl*	
Chain length	*Amount (mmol·10⁴)*		*Surfactant found (mmol·10⁴)*	*% Extracted*	*SF*	*Surfactant found (mmol·10⁴)*	*% Extracted*	*SF*	*Surfactant found (mmol·10⁴)*	*% Extracted*	*SF*	*Surfactant found (mmol·10⁴)*	*% Extracted*
Ferroin–heptyl sulphate (C_7)–octyl sulphate (C_8)													
C_7	4.83	1	1.01	21		0.48	10		1.14	24			
C_8	4.43		2.94	66		2.31	52		3.18	71			
C_7+C_8	9.26		4.84	52	2.54	3.39	37	3.65	4.68	51	2.78		
Ferroin–nonane sulphonate (C_9)–decane sulphonate (C_{10})													
C_9	1.83	1	0.12	6		0.08	4		0.32	17			
C_{10}	2.00		0.78	39		0.61	31		1.11	56			
C_9+C_{10}	3.83		1.22	31	3.08	0.89	23	3.61	1.62	41	2.68		
C_9	1.83	3	0.49	25		0.19	10		0.45	23			
C_{10}	2.00		1.31	66		0.90	45		1.50	75			
C_9+C_{10}	3.83		1.74	44	2.36	1.24	32	3.42	2.12	54	2.73		
C_9+C_{10}	3.83	1										1.35	34

The percentages of surfactant extracted from solutions containing buffer plus sodium chloride were usually greater than those from the other corresponding solutions. The lowest percentages were always from solutions containing buffer alone, but these solutions led to the highest separation factors. Increase in the number of partitions led to a significant decrease in separation factor when buffer and sodium chloride were both absent. The percentage extraction of surfactant was greater in the presence of sodium chloride than in its absence.

pH and buffer concentration

Previous work showed that extraction constants of chelate–surfactant compounds are lowered in the presence of buffer[1]. This was confirmed by a series of experiments with ferroin–octyl sulphate, –nonane sulphonate and –decane sulphonate systems, which showed that the extractabilities of these compounds decreased with increasing concentration of sodium acetate buffer, but that extractabilities were unaffected by pH changes between 4 and 6, the range maintained throughout this work. Acetate ion appears to compete with anionic surfactant for the chelate.

Reagent excess

Aqueous solutions (5 ml) containing sodium nonane sulphonate and sodium decane sulphonate (0.2 μmol of each), sodium acetate buffer (pH 5, 0.2 *M*) and either 0.5 or 1.0 μmol of ferroin were extracted with chloroform (5 ml). The absorbances of the extracts indicated that 23 and 31%, respectively, of the total surfactant had been extracted, and that the reagent excess affected the extent of extraction, particularly for compounds in the break-through region.

Solution volume

Aqueous solutions (20 ml or 10 ml) each containing a series of sodium alkane sulphonates (C_8–C_{13}, 0.2 μmol of each), buffer as above, and a chelate (2 μmol) were extracted with chloroform (10 ml). The percentage extractions calculated from the measured absorbances are shown in Table IV. Change in aqueous volume led to a significant change in the amount of surfactant extracted. The results showed

TABLE IV

EFFECT OF THE VOLUME OF AQUEOUS PHASE ON THE EXTRACTABILITY OF SURFACTANTS FROM A SODIUM ALKANE SULPHONATE MIXTURE (C_8–C_{13})

Ligand	*% Extraction*	
	Aqueous volume	
	20 ml	*10 ml*
4,7-Dimephen	90.0	96.3
5-Mephen	74.0	82.9
5-Clphen	64.6	75.7
Phen	44.2	52.5
Tripy	19.5	24.0
Bipy	14.0	18.1

that consistent extractions can only be obtained when the volumes of the aqueous and chloroform phases are standardized.

Solvent

A number of solvents which had been shown to extract chelate–surfactant compounds from aqueous solution[2] were examined as extractants suitable for differentiating surfactant homologues.

Replacement of chloroform by nitrobenzene caused a shift in the break-through region of a chelate–surfactant series of about two carbon atoms towards shorter chain lengths, *e.g.* from about C_{12} to C_{10} for the bipy chelate–alkyl sulphate system. Large blanks were obtained, however, and this, together with the toxicity of the solvent, rendered it unsuitable for general use.

Extraction constants for two compounds with dichloromethane were measured[1]. Results in Table V indicate that dichloromethane is a rather better extractant than chloroform. Break-through regions were shifted by about one carbon atom when chloroform was replaced by dichloromethane, but there were no significant advantages for separation purposes.

TABLE V

EXTRACTION CONSTANTS OF TWO CHELATE–SURFACTANT COMPOUNDS

(Results given as log β. Numbers in parentheses are % relative errors. Temperature, $24 \pm 1°$)

	Log β	
	Chloroform–water	*Dichloromethane–water*
Ferroin–lauryl sulphate	12.53(34)	13.6[a]
Ferroin–hexylbenzene sulphonate	8.58(24)	9.63(18)

[a] Estimated value.

Trichloroethylene, chlorobenzene, 1,2-dichloroethane and 1,1,1-trichloroethane proved to be poor extractants, and were also unsuitable because of their tendency to form emulsions and cloudy extracts.

Chloroform–carbon tetrachloride mixtures have been previously used[2] to extract ferroin–lauryl sulphate. A mixture containing 15% chloroform was found to extract about 50% of the compound in a single extraction from an equal volume of aqueous solution. In the present work, break-through regions for some chelate–surfactant series with $CHCl_3/CCl_4$ (15% and 40% $CHCl_3$) were established and are listed in Table VI together with comparable break-through regions with chloroform alone. Figure 3 shows the variation in extractability of two homologous ferroin–surfactant compounds with the chloroform content of the $CHCl_3/CCl_4$ extractant. Substitution of $CHCl_3/CCl_4$ mixtures for chloroform alone can produce shifts in break-through regions of up to four carbon atoms towards longer chain lengths. Dilute chloroform solutions (6–8%) produce even larger shifts, but solvent mixtures containing less than 10% of chloroform cause some precipitation of chelate–surfactant compounds. Mixtures of chloroform (15%, w/v) with trichloroethane,

TABLE VI

SOME BREAK-THROUGH REGIONS WITH CHLOROFORM AND CHLOROFORM–CARBON TETRACHLORIDE MIXTURES

Ligand	*Break-through region (alkyl-chain length)*								
	Alkane sulphonate			*Alkyl sulphate*			*Alkyl benzene sulphonate*		
	1[a]	*2*[b]	*3*[c]	*1*	*2*	*3*	*1*	*2*	*3*
5-Mephen	8	10	12	6	8	10	4	6	8
Phen	10	12	14	8	10	12	6	8	10
Bipy	12	14	—	10	12	14	8	10	12

[a] Chloroform.
[b] 40% (w/v) chloroform in carbon tetrachloride.
[c] 15% (w/v) chloroform in carbon tetrachloride.

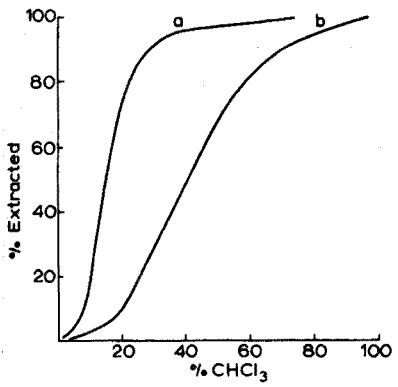

Fig. 3. Effect of chloroform content on the extraction of two ferroin compounds by chloroform–carbon tetrachloride mixtures. (a) ferroin–lauryl sulphate; (b) ferroin–decyl sulphate. Aqueous solutions (5 ml) of ferroin (1 μmol), surfactant (0.2 μmol) and sodium acetate buffer (pH 5, 0.2 *M*). Partitions with 3×2 ml of solvent. Extracts made up to 5 ml for absorbance measurements.

chlorobenzene, or trichloroethylene were also tried as extractants. Break-through regions of ferroin–alkyl sulphates with these mixtures were around C_{11}, C_{10} and C_{11}, respectively. No significant advantages over $CHCl_3/CCl_4$ were offered by these mixtures, and none of them caused a marked change in separation factors from those obtained with chloroform alone.

Selection of reagents and conditions

Each of the chelates examined in this work forms solvent-extractable compounds with members of homologous surfactant series whose carbon chains exceed a specific length. The length depends on the chelate and on the solvent used, and covers a range of seven carbon atoms in each series. Table VII lists the chelate systems which were selected for use in a method to determine surfactants with

TABLE VII

BREAK-THROUGH REGIONS WITH SELECTED CHELATES

Ligand	*Solvent*	*Alkyl-chain length*			*Spectral data for reagent*	
		Sulphate	*Sulphonate*	*Benzene sulphonate*	λ_{max} *(nm)*	ε_{max}
Phen	15% $CHCl_3$ in CCl_4	12	14[a]	10[a]	512	11,100
Bipy[b]	$CHCl_3$	10	12	8	522	8,650
Tripy[b]	$CHCl_3$	10	12	8	554	11,200
Phen	$CHCl_3$	8	10	6	512	11,100
4,7-Dimephen	$CHCl_3$	5	7	3[a]	514	14,000

[a] Estimated.
[b] Bipy is the more readily available reagent, but tripy is the more sensitive one.

chain lengths greater than specified values. The choice is based on the regular spacings of the break-through regions and on the availability of the ligands.

On the basis of the present work, the following conditions are proposed to optimize the extraction of surfactants with chain lengths greater than the appropriate break-through region, and the separation of adjacent surfactants in the break-through region:

(i) an aqueous solution volume of 5 ml buffered to pH 5;

(ii) a quantity of reagent equivalent to about twice the total surfactant present;

(iii) three solvent partitions for each determination (4 ml, 4 ml and 2.5 ml) and a bulked extract volume of 10 ml for absorbance measurement.

EXPERIMENTAL

Apparatus

Spectrophotometer and extraction device as previously described[1].

Reagents

Ammonium iron(II) sulphate. A 0.02 *M* solution containing 0.1% (w/v) hydroxylammonium chloride was prepared.

Iron(II) chelates (0.001 *M*). These were prepared from the iron(II) solution and the appropriate ligand. 4,7-Dimephen chelate and ferroin should contain a ligand–iron(II) molar ratio of 6:1. Bipy chelate should contain a ligand–iron(II) ratio of 40:1. A solution of 2,2′,2″-tripyridine in 0.01 *M* hydrochloric acid was used.

The solvents used were of analytical-reagent grade. The mixed solvent contained 15% (w/v) of chloroform in carbon tetrachloride.

Recommended Procedure

All apparatus should be washed with nitric acid (30%) and with distilled water. In a conical centrifuge tube (15 ml) place the sample (about 2.5 ml, containing not more than 2 μmol of total surfactant), acetate buffer, pH 5 (0.5 ml)[1], either chelate (2 ml), or iron(II) solution (2.0 ml) and tripy solution (0.5 ml), and water

to give a total volume of 5 ml. The chelate chosen will depend on the minimum chain length to be determined (Table VII). Extract with two successive 4-ml portions of solvent (Table VII), by stirring the contents of the tube vigorously for 30 s and centrifuging for 1 min. Combine the extracts in a 10-ml graduated flask. Wash the aqueous solution with solvent (2.5 ml) and dilute the extracts to the mark with the washings. Measure the absorbance (A) at the appropriate wavelength against a solvent blank in 1-cm cells. Perform a reagent blank (B) following the above procedure without the addition of sample.

Calculate the amount of surfactant (S) extracted from the sample by the expression

$$S = \frac{2 \times (A - B) \times 10{,}000}{\varepsilon} \mu\text{mol}$$

where ε is the molar absorptivity of the chelate used (Table VII).

APPLICATIONS

Synthetic mixtures

Mixtures of homologous even-numbered alkyl-chain surfactants were analysed. All determinations were performed in duplicate. Results (Table VIII) show that all the surfactants are quantitatively extracted as their compounds with the 4,7-dimephen chelate. For each surfactant series, the break-through region of this chelate is less than the carbon-chain length of the shortest surfactants. When other chelates are used to separate the longer from the shorter surfactants in a series, the quantity of surfactants which is extracted is generally different from, but close to, the quantity predicted from the appropriate break-through region. The greatest differences are when a major component of the series has a carbon-chain length either just less than that of the break-through region (positive) or in the break-through region (negative).

Biodegradation studies

Synthetic sewage liquors. Mixtures of sodium alkylbenzene sulphonates (25 p.p.m. total) containing from six to eight alkyl carbons, with the benzene rings randomly distributed along the alkyl chains, were degraded by digestion with activated sewage sludge[3]. Degradation was followed firstly by means of a methylene blue method[4], but this cannot measure total surfactant because some of the branched-chain surfactants are not extracted. Measurements were subsequently made by means of the present method with the 4,7-dimephen chelate. It was shown that all the surfactants were completely extracted and that the total degradation can be followed.

River water. A solution of sodium dodecane sulphonate ($2 \cdot 10^{-4}$ M) in river water (500 ml) was allowed to stand for about one month. At regular intervals, the surfactant concentration of an aliquot (1 ml) was determined with either the ferroin or 4,7-dimephen chelate. The solution was stood in a beaker covered with paper tissue to prevent contamination by airborne detergents. At appropriate times, distilled water was added to compensate for evaporation and sampling. The results, summarized in Fig. 4, indicated that during the degradation shorter-chain

TABLE VIII

DETERMINATION OF SURFACTANTS IN SYNTHETIC MIXTURES

Ligand	*Alkyl-chain length*							*Extracted surfactant (mmol·10⁴)*		
	Break-through region	*Nominal composition of mixture (mmol·10⁴)*						*Predicted*[a]	*Found*[b]	%
		4	*6*	*8*	*10*	*12*	*14*			
a. Alkyl Sulphates										
4,7-Dimephen	5		2.0	2.0	2.0	2.0	2.0	10.0	10.2	102
			8.0	2.0	2.0	2.0	2.0	16.2	16.4	101
			2.0	8.0	2.0	2.0	2.0	15.8	15.8	100
			2.0	2.0	8.0	2.0	2.0	16.1	15.9	99
			2.0	2.0	2.0	8.0	2.0	16.1	16.1	100
Phen	8	As Above						8.0	8.1	101
								8.0	8.9	111
								13.6	13.0	96
								13.9	13.7	99
								13.9	13.9	100
Bipy	10	As Above						6.0	5.8	97
								6.0	5.9	98
								6.0	6.8	113
								11.9	10.7	90
								11.9	11.5	97
Phen with $CHCl_3/CCl_4$	12	As Above						4.0	4.5	113
								4.0	4.5	113
								4.0	5.1	128
								4.0	5.7	143
								9.9	10.3	104
b. Alkane Sulphonates										
4,7-Dimephen	7			2.0	2.0	2.0		6.0	6.2	103
				8.0	2.0	2.0		12.0	12.1	101
				2.0	8.0	2.0		11.8	12.2	103
				2.0	2.0	8.0		12.1	12.3	102
Phen	10	As Above						4.0	3.6	90
								4.0	4.1	103
								10.0	8.3	83
								9.9	9.5	96
Tripy	12	As Above						2.0	2.1	105
								2.0	2.0	100
								2.0	3.1	155
								7.9	7.2	91
c. Alkyl Benzene Sulphonates										
4,7-Dimephen	3	3.0	1.8	1.9		0.9		7.6	7.7	101
		8.6	1.8	1.9		0.9		13.3	13.2	99
		3.0	7.1	1.9		0.9		13.0	13.0	100
		3.0	1.8	7.7		0.9		13.5	13.4	99
		3.0	1.8	1.9		3.8		10.4	10.5	101

(Continued

TABLE VIII (*contd.*)

Ligand	Alkyl-chain length							Extracted surfactant ($mmol \cdot 10^4$)		
	Break-through region	Nominal composition of mixture ($mmol \cdot 10^4$)						Predicted[a]	Found[b]	%
		4	6	8	10	12	14			
Phen	6	As Above						4.6	5.0	109
								4.6	5.8	126
								9.9	9.6	97
								10.4	11.1	107
								6.5	7.6	117
Tripy	8	As Above						2.8	3.0	107
								2.8	3.1	111
								2.8	3.9	139
								8.6	8.7	101
								5.7	6.0	105

[a] Including any surfactant in the break-through region.
[b] Mean of two values which always agreed within 2%.

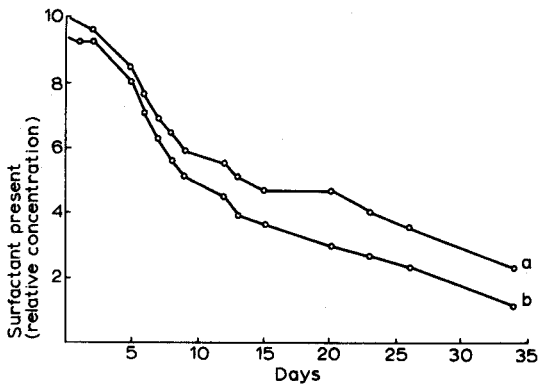

Fig. 4. Degradation of dodecane sulphonate in River Dee water, measured with iron(II) chelates. (a) 4,7-dimephen chelate; (b) phen chelate. The surfactant present includes extractable anionic material present in the river water itself.

surfactants were possibly formed. These were extractable with 4,7-dimephen chelate, but not with ferroin. A similar solution in distilled water showed little degradation over a period of several months. Sodium lauryl sulphate ($2 \cdot 10^{-4}$ *M*) in river water or distilled water was degraded more rapidly, but no evidence for the formation of shorter-chain surfactants was found, probably because degradation was by hydrolysis.

Interferences

Shorter-chain surfactants. The extent to which lower surfactants interfere positively with the extraction and measurement of higher ones is shown in Fig. 5. There is a linear relationship between the quantity of lower surfactant present and the total surfactant extracted. Sodium decyl sulphate is the only one of the surfactants

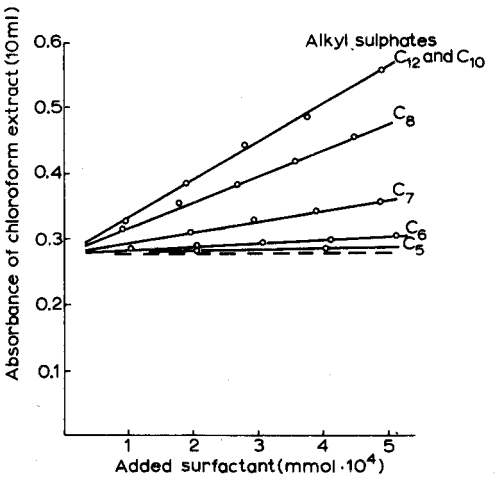

Fig. 5. Effect of shorter alkyl-chain surfactants upon the extraction of ferroin–lauryl sulphate. The dashed line indicates the absorbance of standard ferroin–lauryl sulphate.

TABLE IX

PERMISSIBLE CONCENTRATIONS OF FOREIGN COMPOUNDS

Compound	*Concentration (p.p.m.)*[a]
Cobalt nitrate	5
Chromium nitrate	5[b]
Copper sulphate	5
Nickel nitrate	5
Potassium thiocyanate	10
Potassium dichromate	20[b]
Iron(III) ammonium sulphate	20
Iron(II) ammonium sulphate	20
Sodium fluoride	30
Lead acetate	50[c]
Sodium tetraborate	50[c]
Disodium hydrogen phosphate	100
Potassium aluminium sulphate	100[c]
Zinc sulphate	100[c]
Sodium nitrate	200
Sodium chloride	250

[a] These concentrations produce errors not exceeding $\pm 2\%$ in the absorbance of an extract from aqueous solution (5 ml) containing the 4,7-dimephen chelate (2 μ-mol) and sodium lauryl sulphate (0.5 μmol).
[b] Above this concentration, high absorbances are obtained which decrease on standing.
[c] Not examined above this concentration.

examined whose sensitivity is the same as that of sodium lauryl sulphate itself. Ferroin–octyl sulphate, the compound in the break-through region, is 71% extracted, and the lower compounds (C_7, C_6, C_5) are 29, 9 and 3% extracted respectively. The results are consistent with those obtained from the analysis of synthetic mixtures. They show that, because of mixed compound formation, serious errors can occur

in the determination of higher surfactants when similar quantities of lower surfactants are present.

Foreign ions. The effect of foreign matter on the absorbance of extracts from aqueous solutions containing the 4,7-dimephen chelate was studied (Table IX). This chelate gave the highest blanks of those which were examined, and formed slightly chloroform-soluble ion-association compounds with a number of anions. The serious interferences from transition metal ions are similar to those observed in earlier work with ferroin[5], and are due to competition with iron(II) for the ligand. Interferences from anions are more marked with the 4,7-dimephen chelate than with ferroin. Salt concentrations should therefore be kept low, or maintained constant, for reproducible results. The effect of relatively high salt concentrations (sodium acetate and sodium chloride) was reported earlier in this paper.

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SUMMARY

Homologous anionic surfactants may be separated from each other by solvent extraction of their ion-association compounds with iron(II) chelates. Separations of components from a number of surfactant mixtures were investigated, and the effect of solution variables on extraction and separation was studied. A method is proposed for the determination of surfactants of various chain lengths. Applications of this method to synthetic mixtures and to biodegradation experiments are described.

RÉSUMÉ

Les surfactants anioniques homologues peuvent être séparés les uns des autres par extraction dans un solvant de leurs chélates fer(II). Une méthode est proposée pour le dosage de surfactants à chaîne de longueurs diverses. Des applications de cette méthode sont décrites pour des mélanges synthétiques et des expériences de biodégradation.

ZUSAMMENFASSUNG

Homologe anionische Tenside können voneinander durch Extraktion ihrer Ionenassoziationsverbindungen mit Eisen(II)-Chelaten getrennt werden. Trennungen von Komponenten einer Anzahl von Tensidgemischen sowie der Einfluss von Lösungsvariablen auf die Extraktion und die Trennung wurden untersucht. Es wird eine Methode für die Bestimmung von Tensiden unterschiedlicher Kettenlänge vorgeschlagen. Anwendungen dieser Methode auf synthetische Gemische und bei Untersuchungen des biologischen Abbaus werden beschrieben.

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Analytica Chimica Acta, 69 (1974) 389–407

QUANTITATIVE MEASUREMENTS OF INORGANIC MERCURY AND ORGANOMERCURIALS IN WATER AND BIOLOGICAL MEDIA BY GAS–LIQUID CHROMATROGRAPHY

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At present, few analytical methods exist which permit quantitative and unambiguous evaluation of both the inorganic and organic mercury content of a sample with essentially identical techniques of sample handling and instrumentation.

In the case of mercury, problems arise when attempts are made to quantify the various chemical forms of the element, as may be necessary in environmental health studies, via widely disparate techniques; the shortcomings of this practice have been discussed in some detail[1].

In the determination of the mercury content of various foodstuffs, for example, methylmercury and other organomercurials are evaluated by gas–liquid chromatography[2] with total levels of mercury in the same sample measured by either activation analysis or flameless atomic absorption spectrometry. Inorganic mercury is then taken as the difference between levels of total and organic mercury.

In a recent report by Magos[3], further refined by Magos and Clarkson[4], methods are described for determination of inorganic and organic mercury by flameless a.a.s. techniques. In this case, organic mercury is not determined as a specific compound, and in an analytical application any organomercurial detected is assumed to be methylmercury.

As part of a broad program dealing with the biochemical pathology of heavy metals, we have explored the use of gas–liquid chromatography in assessing all of the forms of mercury(II) present in various media.

A recent report[5] described a gas-chromatographic procedure for inorganic and organic mercury based on Peter's reaction[6] involving the generation of an aryl mercurial for g.c. analysis from an inorganic species via a reaction in the sampling media with selected arenesulfinates.

In this paper, the determination of inorganic mercury is incorporated into the g.c. scheme for different mercury species in a range of media. The chemical basis of the method lies in the ability of various organometallic agents to undergo electrophilic reaction with inorganic mercury under a variety of conditions to yield alkyl and aryl mercurials:

Reaction			Ref.
$[Co(III)(CN)_5–R]^{3-}$	$+Hg^{2+} \rightarrow R–Hg^+$ R = methyl, ethyl, benzyl...	(1)	7
$R_4–Sn(IV)$	$+Hg^{2+} \rightarrow R–Hg^+$ R = methyl, ethyl, propyl	(2)	8
$[Cl–CH_2–Cr(III)(H_2O)_5]^{2+}$	$+Hg^{2+} \rightarrow Cl–CH_2–Hg^+$	(3)	9
$R_2Tl(III)(O_2C–R')$	$+Hg^{2+} \rightarrow R–Hg^+$ R = methyl, ethyl... $R' = CH_3, C_3H_7$	(4)	10
$[(Ar)_4B]^-$	$+Hg^{2+} \rightarrow Ar–Hg^+$ Ar = phenyl...	(5)	11

Samples containing the organomercurial generated from inorganic mercury can then be manipulated by g.c. techniques for organomercurial analysis[2].

In the course of these investigations, existing g.c. procedures for organomercurial analysis have been modified by changes in sample size and column packing employed. Media studied with satisfactory results include water, urine, whole blood, serum and soft tissue homogenates.

EXPERIMENTAL

Apparatus

A Glowall Model 320 dual-oven gas–liquid chromatograph, equipped with an electron-capture detector (^3H foil) and 18-in. glass coil columns, was employed. Column packing consisted of Durapak Carbowax 400 (low K′) on Porasil F (Waters Associates, Framingham, Mass.) and 10% diethyleneglycol succinate (DEGS) on Anakrom SD, 70/80 mesh (Analabs, Inc., North Haven, Conn.). Preparation of the latter packing involved use of a fluidizing apparatus (Applied Science Laboratories, State College, Pa.). The carrier gas used was a 95%+5% argon–methane mixture and isothermal mode temperature parameters included: (a) Durapak C.W. 400 (low K′): column, 140° or 170°, injector port, 210° and detector, 190°; (b) 10% DEGS: column, 180°, injector port, 210–225° and detector, 210°.

Reaction vessels for conversion of inorganic mercury in various media to an organomercurial were 1-dram and 2-dram glass screw cap vials (Fisher Scientific Co). Preparation consisted of a soap and water wash followed by 24-h immersion in 50% analytical-grade nitric acid and finally six rinsings with deionized water. The screw caps were prepared for first-time use by manually removing cap liner and adhesive followed by immersion in xylene for 24 h to remove any adhering residue. Subsequent and routine cleaning of caps involved a soap and water rinse, a 24-h immersion in saturated EDTA salt solution and finally deionized water rinses. Nitric acid is corrosive to the cap material and should be avoided. All other glassware, including pipettes and reagent bottles, were vigorously cleaned with nitric acid before use.

Reagents

Baker and Adamson reagent-grade nitric, sulfuric, perchloric and hydrochloric acids are satisfactory for use without further purification. Reagent-grade sodium dichromate, cadmium chloride, sodium hydroxide and potassium permanganate (J. T. Baker Chemical Co.) were utilized directly. Reagent-grade anhydrous magnesium sulfate (Baker and Adamson) and L-cysteine (free base; Sigma Chemicals) were used without purification. Pesticide-grade benzene (Fisher Scientific) served as extracting solvent for organomercurials. Methanol was of analytical grade (Mallinckrodt Chemical Works).

Methyl-, ethyl- and phenylmercury(II) chloride were commercial samples (Eastman Chemicals) while chloromethylmercuric chloride was synthesized by the procedure of Dodd and Johnson[9]. In the synthesis of pentacyanoalkylcobaltate(III) reagents, cobalt(II) chloride, potassium cyanide, acetone and ethyl ether were used as obtained (Baker and Adamson), and alkyl(ethyl and methyl)iodide reactants (Eastman Chemicals) were reacted directly.

Tetraethyl- and tetramethyltin were obtained from Alfa Inorganics (Ventron). Dimethylthallium(III) acetate was synthesized by treatment of the corresponding carbonate with dilute acetic acid, the carbonate being obtained by treatment of dimethylthallium(III) chloride (Alfa Inorganics) with sodium carbonate. Tetraphenylborate (sodium salt) was employed as obtained (Fisher).

Pentacyanoalkylcobaltate(III) reagents, as the potassium salts, were prepared by the method of Kwiatek and Seyler[12]. The mixture of pentacyanoiodocobaltate-(III) and pentacyanoalkylcobaltate(III) was characterized by n.m.r. and i.r. spectral data, the methylcobalt compound showing a singlet at τ 8.90 (D_2O) and a pair of CN-stretching frequencies in the i.r. at 2089 and 2120 cm^{-1} (lit. i.r.[12], 2087 and 2120 cm^{-1}). During synthesis, the reaction flask was covered with aluminum foil to avoid light and the alkylcobalt reagent was stored in an amber bottle.

Procedures for analysis of inorganic and organic(alkyl, aryl)mercury in various media

Water and urine (acidic medium) with pentacyanomethylcobaltate(III). Pipette a sample of water or urine (1.0 ml) into a 1-dram acid-washed vial, the neck of which is wrapped with several turns of 0.5-in. wide teflon tape to prevent leakage on sealing. In like manner, prepare a blank and samples containing added inorganic mercury incubated for 10 min. Add concentrated analytical-grade sulfuric acid (0.1 ml) and 0.2 ml of 0.5 *M* sodium dichromate solution, and treat the solution or mixture (urine) with a freshly prepared aqueous solution (0.3 ml, 0.5 *M*) of the pentacyanomethylcobaltate(III) reagent for 30 min. Add 0.2 ml of saturated sodium chloride solution followed by 2.0 ml of pesticide-grade benzene, seal the vials, and extract the aqueous phase by vigorous agitation in a Vortex-Genic apparatus operated at maximum speed for 1 min. Centrifuge the vials at *ca.* 3000 rev. min^{-1} for several minutes, open and remove an aliquot (1.0 ml) of the benzene layer by pipette to a second 1-dram vial containing 1.0 ml of a freshly prepared aqueous 2% cysteine solution. Re-extract the organomercurial(s) into the aqueous phase, by agitation as before, for a period of 30 s. Centrifuge the vials again at 3000 rev min^{-1} for several min, and then carefully remove the benzene layer. Treat the retained aqueous layer with concentrated hydrochloric acid (0.1 ml) and add 1.0 ml of pesticide-grade benzene. Remove the liberated organomercurial(s) into the organic layer by agita-

tion for 30 s. After centrifugation, transfer the benzene layer (*ca.* 0.5 ml) to a third vial containing 50–100 mg of anhydrous magnesium sulfate. Then inject an aliquot of the dried benzene layer into the gas chromatograph.

For the determination of inorganic mercury in the presence of methylmercury, carry out sequential analysis in aliquot pairs, treating one with methylcobalt reagent and the other with a like volume of deionized water. Determine the total methylmercury content of the sample treated with the methylcobalt reagent by g.c.; this represents inorganic mercury methylated by the reagent and any methylmercury already present. The sample from which the methylating reagent is omitted yields only methylmercury already in the sample. The difference in peak mass or area is due to inorganic mercury, and can be calculated from standards.

Analysis of urine and water samples which may contain inorganic mercury and organomercurials other than methylmercury, involves simultaneous analysis of the mercurials on the Durapak Carbowax 400 column (see Apparatus) if ethylmercury is present; if phenylmercury is of interest, a second analysis at a higher column temperature (170°) is necessary.

Water and urine (alkaline medium with cadmium ion). Pipette water or urine samples (1.0 ml) into 1.0-dram vials along with inorganic mercury standard solutions (0.1–1.0 p.p.m.) contained in either water or urine. Also prepare a reagent blank. Make alkaline by addition of 0.1 ml of 0.5 *M* sodium hydroxide solution. Then add aqueous cadmium chloride solution (0.2 ml, 0.44 *M*) and set aside for 30 min. Introduce freshly prepared pentacyanomethylcobaltate(III) solution (0.3 ml, 0.5 *M*) and leave the methylation to proceed for 30 min. Further manipulation of the samples for g.c. analysis is identical to that described for mercury analysis in acidic media (*vide supra*). Samples taken for both inorganic and organic mercury analysis are treated in the same fashion as described for acidic media.

Whole blood or serum. Introduce whole blood or serum (0.5 ml) into acid-washed 2-dram vials along with volumes of whole blood or serum containing inorganic mercury (0.1–1.0 p.p.m.) which have been prepared at least 10 min before the addition of any reagents to permit binding of added mercury to groups in the blood sample. Also prepare a serum or whole blood blank containing no, or a known measurable amount of, mercury. Add a solution of sodium dichromate (0.5 ml, 0.5 *M*) in perchloric acid (2 *M*) to each vial and set aside for 1 h. Then add the methylcobalt reagent (0.5 ml, 0.3 *M*) and allow the alkylation of inorganic mercury to proceed for 30 min. Further sample work-up is identical to that carried out with water and urine. Where analysis of inorganic and methylmercury is necessary, a serum or whole blood sample containing a known amount of methylmercury is carried through the analysis as already described.

Soft tissue homogenates. Add freshly prepared soft tissue homogenates (5% w/v) in deionized water to 2-dram vials, along with control homogenates containing added inorganic mercury (0.05–0.2 p.p.m.); the controls must be left after addition of inorganic mercury for at least 10 min to allow binding of mercury by homogenate components. Similarly, prepare a homogenate blank containing no, or a known measurable, amount of background mercury. Treat the samples with sodium dichromate solution (0.5 *M*) and concentrated sulfuric acid (0.1 ml). Wrap the vial necks with teflon tape, seal and mix for 15 s with the Vortex-Genic agitator at maximum speed. Then set aside for 5 h at room temperature. Introduce the methylcobalt

reagent (0.3 ml, 0.5 *M*) and methylate for 30 min at room temperature. The balance of the assay sequence is that for other media described above. Determination of methylmercury in the presence of inorganic mercury is carried out in sequential fashion as already indicated.

Measurements of inorganic mercury and organomercurials in water and urine with tetraethyltin. Place samples of water or urine (0.5 ml) in 1-dram acid-washed vials. Similarly, prepare samples of water or urine containing known added amounts of inorganic mercury (0.1–1.0 ppm), as well as a sample blank of either medium. After 30 min of treatment with sodium dichromate (0.5 ml, 0.2 *M*) in perchloric acid (2 *M*), add an equal volume of freshly prepared tetraethyltin (0.2 *M*) solution in reagent-grade methanol (1.0 ml), and shake the teflon tape-sealed vials on an Eberbach shaker equipped with a multi-hole aluminum block[13] at room temperature for 45 min. Then, as for the procedures with methylcobalt reagent, add saturated sodium chloride, extract ethylmercury and other mercurials with benzene and treat the benzene layer as described above.

For the g.c. analysis, use the 10% DEGS–Anakrom SD packing. Determine inorganic mercury as ethylmercury(II) chloride. The presence of methylmercury is noted at the same column conditions, while phenylmercury, if present, is best determined with the Durapak Carbowax 400 packing at 170°. Samples containing, or suspected of containing, ethylmercury are analyzed in sequential fashion as in the case of methylmercury analysis using the methylcobalt reagent. In this case, ethylmercury in known amount is added to a urine or water sample which is carried through the analysis. Evaluation of levels of inorganic mercury in the samples is directly related to the corresponding peak area or mass obtained by analysis of the spiked matrix standards. Quantitative measurement of other organomercurials similarly involves proportionality between peak area (mass) of the organomercurial as related to a blank sample of matrix containing a known amount of the mercurial(s).

Measurements of inorganic mercury and organomercurials in water and biological media with sodium tetraphenylborate(III). Treat samples of urine or serum (1.0 ml) with 0.1 ml each of 1.0 *M* sodium hydroxide and 1.0 *M* cadmium(II) ion and leave for 15 min in the case of urine and 30 min in the case of serum. To the samples add 0.2 ml of freshly prepared 0.2 *M* sodium tetraphenylborate in deionized water and leave for 15 min at room temperature. For urine samples, treat with 0.2 ml of concentrated hydrochloric acid and extract for 1 min in a Genie-Vortex shaker with 2.0 ml of chromatographic-grade benzene. For serum samples proceed similarly but use 3.0 ml of extracting solvent. Then dry the benzene layers over magnesium sulfate (anhydrous) and take aliquots for g.c. analysis, using a column temperature of 170° and Durapak Carbowax 400 (low K′) as packing.

For samples of soft tissue (aqueous 5% homogenate, 1.0 ml), mix with 0.2 ml of the cadmium and alkaline solutions as above and vortex. After addition of sodium tetraphenylborate solution (0.2 ml, 0.2 *M*), heat the sample vessels in a sand bath at 50° for 90 min. Proceed further as described above for other media.

For co-determination of phenylmercury and inorganic mercury, the sequential procedure is identical to that employing the other reagents, with inorganic mercury determination being made by difference. Evaluation of methyl- and/or ethylmercury is carried out by analysis of the latter organomercurials at a column temperature of 140°.

RESULTS AND DISCUSSION

Measurements of inorganic mercury in various media with pentacyanoalkylcobaltate-(III) reagents

Pentacyanoalkylcobaltate(III) compounds were extensively investigated as reagents for the determination of inorganic mercury involving organomercurial formation and subsequent measurement of the newly formed mercurial by gas–liquid chromatography.

As prepared by the method of Kwiatek and Seyler[12] and with methyl or ethyl iodide, pentacyanomethyl- and -ethylcobaltate(III) are isolated as 1 : 1 mixtures with pentacyanoiodocobaltate(III).

$$2\,[Co(II)(CN)_5]^{3-} + R\text{–}I \rightarrow [Co(III)(CN)_5\text{–}R]^{3-} + [Co(III)(CN)_5I]^{3-}$$

There was no apparent effect on the analytical utility of the alkylcobalt compounds owing to the presence of the halogenated cobalt product, and all data presented in this report dealing with cobalt reagents involve use of the 1 : 1 mixtures.

Compared to pentacyanomethylcobaltate(III), the ethyl analog is synthesized in lower yield and appears to have a lower degree of stability both in the solid form and in solution[14]. Therefore, the analytical application of these reagents was centered on the methyl derivative.

With pentacyanomethylcobaltate(III), alkylation of inorganic mercury under the conditions used proceeds only to the stage of monomethylation; the further use of neutral buffered solutions of inorganic mercury and the reagent in variable amounts furnished no evidence for the formation of dimethylmercury[15]. In acidic media, of course, any dimethylmercury formation would be followed by cleavage to the monomethylmercury.

Analytical application of these materials for mercury involves the use of highly acidic as well as oxidizing media, hence the mercury-alkylating capacity of these reagents in such media for trace levels of mercury was examined.

In an acidic medium up to 3 *M* in sulfuric or 6 *M* in perchloric acid, the methyl- and ethylcobalt reagents convert inorganic mercury to the corresponding organomercurial in varying yield, the best yield (*ca.* 80%) being seen with the methyl analog. In a medium consisting of acidic (3 *M*) dichromate (0.5 *M*) solution, methylation of inorganic mercury by pentacyanomethylcobaltate(III) is not materially altered, although a slight increase in mercury recovery compared to the acidic medium without oxidant was noted. Acidic solutions of permanganate also permit methylation to occur at time intervals of 30 min or less. Longer reaction times lead to decreasing recovery of methylmercury. It is likely that permanganate decomposes some of the generated methylmercury; in fact, when methylmercury was exposed to acidic permanganate medium for increasing periods of time, increased losses occurred.

Volatile products of partial acid decomposition of the methylcobalt agent, acetamide and acetonitrile[16], do not pose problems in an analytical scheme involving gas–liquid chromatography, as these materials separate cleanly with the packings employed here (see Experimental). In dilute alkaline media, the pentacyanoalkylcobaltate(III) compounds appear to be stable for a period of days in solutions protected from light.

Evaluation of the inorganic mercury content in various samples with the alkylcobalt reagents involves gas–liquid chromatographic techniques first developed by Westöö[2]. In the present work, various aspects of the procedure were refined, including column packings and altered manipulations of the samples of various media. The overall procedure was scaled down to sample sizes of 1.0 ml or less of liquid media including water, urine, whole blood, serum and tissue homogenates. The relatively dilute tissue homogenates found to be satisfactory (5%), furthermore, require less than 0.1 g of original soft tissue. Satisfactory sampling vessels were found to be 1- or 2-dram vials which had been freed of chromatographic interferences. Vessels were tightly sealed with teflon tape, although adequate sealing was also achieved with teflon cap liners.

A variety of column packings for separation of methylmercury and other organomercurials was evaluated, including those enjoying wide use for organic mercury, must be faced for media only partially modified in terms of organic with the organocobalt reagent for inorganic mercury, were obtained with a relatively new packing in which the stationary phase is chemically bonded to the support, Durapak Carbowax 400 (low K′) on Porasil F, 80/100 or 100/120 mesh. Use of this packing avoids detector contamination and sensitivity loss owing to column bleed, permits elution of the organomercurials at moderate temperatures with minimal sample retention and does not require the periodic dressing necessary with certain packings[17]. Column life over the temperature ranges employed and with the instrument continuously operational was greater than six months.

The Westöö procedures for isolation of organomercurials, as well as the present methods, do not involve mineralization of the samples, whereby all organic matter is destroyed. Consequently, the problem of recovery of elements, including mercury, must be faced for media only partially modified in terms of organic matter which may bind the metal and interfere with the analysis. Attempts were made to minimize such interferences in several ways. The use of a strongly acidic media containing oxidants which would chemically modify those groups most strongly binding mercury, specifically sulfhydryl groups, appears to be reasonably successful. One may also employ an excess of some ion which competes with mercury for the same binding sites in organic matter, with or without the simultaneous use of binding group-modifying oxidants. Westöö[2] employed inorganic mercury to assist in liberation of methylmercury, which is clearly of no value if inorganic mercury levels are also sought. The use of copper(II) ion has been reported[17]. The use of both copper(II) and cadmium(II) ion was examined in optimizing liberation of both inorganic mercury and organomercurials in various media; in concert with an acidic medium containing dichromate, neither metal ion materially influenced recoveries of inorganic or organic mercury. Cadmium(II) ion, used in excess, was found to be valuable in analyses for inorganic mercury in water or urine, with an alkaline medium in the absence of oxidant.

A related problem is the manner of spiking of samples with mercury or any metallic element whereby recovery data is obtained. Before manipulation of any samples, methylmercury was added to the sample followed by addition of saturated sodium chloride solution and extraction with benzene. In these samples, little or no liberation of bound methylmercury by halide occurred. It was found that no recovery of added methylmercury occurred after *ca.* 1 min of incubation of the

mercurial with the medium. Since inorganic mercury potentially possesses more coordinating sites than the methyl analog, binding time was arbitrarily expanded to 10 min. Such a procedure is, admittedly, a crude simulation of the binding of mercury as it occurs *in vivo* but ensures that, qualitatively, mercurials added to the samples are not circulating unbound in the medium thereby introducing large errors in relating recovery data for the element with the actual extent of metal isolation in routine sample analysis.

In the analysis of various media for inorganic mercury and organomercurials, samples were acidified in varying degree with sulfuric acid or perchloric acid followed by addition of dichromate as oxidant, and samples were set aside for varying periods of time to liberate the mercurials. An alternative method in the analysis of water or urine was the addition of excess of cadmium ion followed by dilute sodium hydroxide solution. The use of an oxidant in the case of acidified media, and excess cadmium ion in the case of alkaline water or urine, was necessary to effect satisfactory recovery of added inorganic mercury and organomercurials. As pointed out earlier, permanganate causes the decomposition of organomercurials over extended periods of time; no such effect was seen with dichromate.

As noted before, the methylation of inorganic mercury by pentacyanomethylcobaltate(III) proceeded in acidified media containing dichromate, or alkaline media containing cadmium ion, and was optimal at 30 min, although shorter periods (10–15 min) may be employed in water analysis. Isolation of the organomercurials as the chloride derivative was accomplished by addition of sodium chloride and extraction with benzene.

Partitioning of organomercurials into benzene from a variety of appropriately treated samples occurs in high yield and with satisfactory reproducibility under the conditions described. Emulsion formation was not encountered when phase separation was carried out by centrifugation at 3000 rev min^{-1}.

In water or urine analysis, acidification of the sample to 1.5 *M* in acid (sulfuric) is apparently sufficient, with dichromate as oxidant, to preclude loss by binding of mercury to vessel walls or components present in the medium. Furthermore, no standing time is necessary before addition of the methylating reagent. With serum and whole blood, perchloric acid was better than other acids, yielding minimal difficulty with emulsion formation. Samples of whole blood or serum required a treatment interval with dichromate in perchloric acid of 1 h before addition of the methylating agent. In the case of tissue homogenates, samples were set aside for a minimum of 5 h.

Determination of any organomercurials already present in a given sample in addition to inorganic mercury involves the use of sequential or simultaneous measurement depending on the nature of the organomercurial(s).

Should the determination of methylmercury in the presence of inorganic mercury in a specific sample be desired, a sequential analysis is carried out: aliquots of the sample are treated identically save for the exclusion of the methylating reagent from one of the samples, an equivalent volume of deionized water being used instead. Methylmercury determined in the sample with no methylating reagent yields methylmercury already present in the sample and is quantified by comparison to a sample containing a known amount of methylmercury. The sample treated with methylating reagent furnishes methylmercury arising from inorganic mercury and

methylmercury already present. Differences in the g.c. peak areas for the two aliquots are ascribed to methylated inorganic mercury, and are evaluated by comparison with the amount of inorganic mercury recovered from samples to which a known amount had previously been added. Such a sequential analysis may be carried out with all of the media described in this report and actual chromatographic data for sequential analysis using tissue homogenate are presented later (Fig. 4).

Inorganic mercury and ethylmercury can be evaluated by simultaneous analysis. Phenylmercury is measured on Durapak Carbowax 400 (low K′) at a higher temperature (170°) than that used for methyl- and ethylmercury. Alternatively, temperature-programmed g.c. separations of organomercurials can be done on an instrument with dual-column capability.

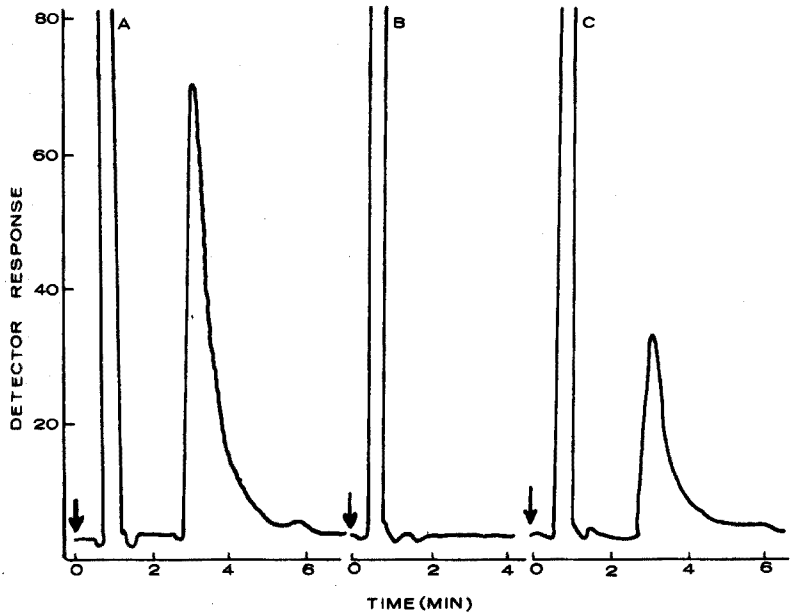

Fig. 1. Chromatograms for inorganic mercury(II) in urine with pentacyanomethylcobaltate(III). (A) Standard solution of methylmercury(II) chloride in benzene; (B) blank urine sample treated with alkylating agent; (C) urine sample incubated (10 min) with added inorganic mercury(II). Packing: Durapak Carbowax 400 (low K′) on Porasil F, column, 140°; injector, 210°; detector, 190°.

Representative chromatograms obtained in the determination of inorganic mercury at trace levels in urine, via formation of methylmercury, are shown in Fig. 1. Under a variety of conditions of column packing, temperature and flow rate, the peak arising from treatment of inorganic mercury in all media tested with the methylcobalt reagent was identical to that obtained with an authentic sample of methylmercury (chloride) in benzene. Furthermore, the material giving rise to the peak was removed from benzene extracts of alkylating media on treatment with thiol compounds such as cysteine, and was not influenced by similar treatment with aqueous media not containing these agents. Working calibration curves obtained by the addition of varying amounts of inorganic mercury to water or urine followed

by sample work-up and g.c. analysis of the resulting methylmercury (chloride) were linear over the range employed (200–1000 ng Hg ml^{-1} of sample). While it was invariably necessary to clean up the initial benzene extract obtained in the analysis of biological media, the purification step involving back-extraction into an aqueous cysteine solution followed by mercurial liberation with acid into a second benzene volume would not always be necessary with water, depending on the amount and nature of any organic matter present.

Recovery data (10 samples in replicate) were obtained for inorganic mercury added to water or urine followed by a 10-min binding period and analytical work-up, for both acidic dichromate media and alkaline media containing cadmium ion. Results for the former conditions are included in Table I. No particular advantage seems to be offered by one method over the other for water or urine. Recovery from both urine and water was achieved with reasonable precision, being better for water than for urine. The analytically useful detection limit appears to be 30 ng Hg ml^{-1} of sample (15 ng Hg ml^{-1} of benzene layer), while the lower detection limit is 10 ng Hg ml^{-1} of sample.

Since the quantities of mercury in unpolluted natural waters are invariably much less than may be directly detected by the proposed method[18], studies on the use of resins for collection of very small amounts of the element in whatever form from large volumes of water are in progress.

Measurement of inorganic (and organic) mercury in serum and whole blood

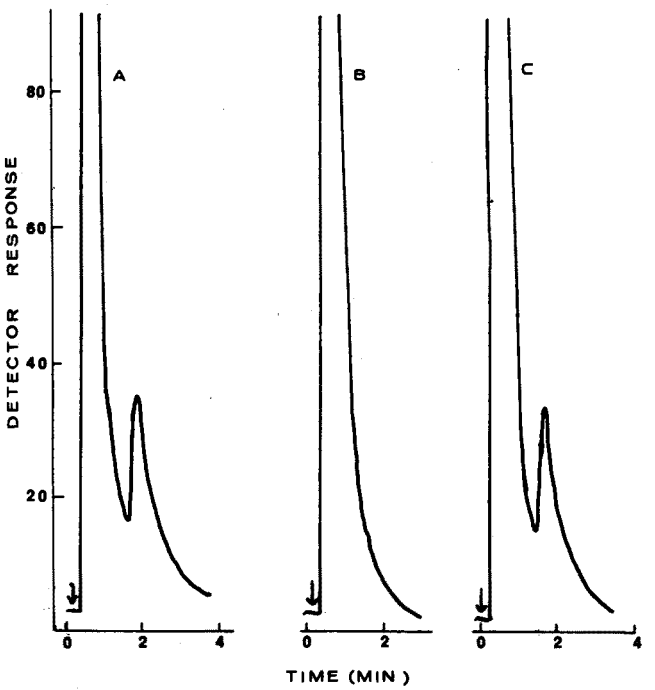

Fig. 2. Chromatograms for inorganic mercury(II) in whole blood with the methylcobalt reagent. (A) Standard solution of methylmercury chloride in benzene; (B) blank whole blood sample; (C) whole blood sample containing added inorganic mercury incubated for 10 min. Conditions as in Fig. 1.

involves some modification of the procedure employed with water and urine. Volumes of whole blood or serum taken for analysis are reduced to 0.5 ml and treated with an oxidant for 1 h before introduction of the methylating reagent. Work-up of the samples is essentially that described for water and urine analysis, including the use of cysteine solution for purification of the original extract.

Figure 2 shows representative chromatograms for inorganic mercury in whole blood. Corresponding chromatographic data for serum were not markedly different. Linear calibration plots over the added inorganic mercury range used were obtained with whole blood and serum. Recovery data for addition of inorganic mercury to whole blood serum (five samples in replicate) are included in Table I; higher recovery was noted with whole blood but better precision was evident for serum.

TABLE I

RECOVERY DATA FOR INORGANIC MERCURY ADDED TO VARIOUS MEDIA

Medium	*Mercury added* ($\mu g\ Hg\ ml^{-1}$)	*% Recovery* (*s*)
A Methyl(pentacyano)cobalt(III)		
Water	1.0	80.5 (4.3)
Urine	1.0	68.8 (5.2)
Serum	1.0	69.8 (4.5)
Whole blood	3.2	68.4 (11.2)
Homogenate (kidney)	2.6	77.4 (17.0)
B Tetraethyltin		
Water	0.4	42.1 (6.2)
Urine	0.4	63.0 (6.4)
C Sodium tetraphenylborate(III)		
Urine	3.6	105.6 (8.9)
Serum	3.6	80.4 (9.7)
Homogenate (liver)	3.6	93.4 (8.3)

Measurements of organomercurials in whole blood and serum samples which also contain inorganic mercury involved essentially those procedures described above for water and urine.

Evaluation of inorganic and organic mercury in soft tissue homogenates, owing to the nature of the matrix, required modification of the procedure employed with other media, chiefly the use of an extended treatment time with acidic solutions of dichromate. While the optimal liberation time of mercurials in soft tissue was considerably greater than with other media studied, the methylating efficiency for inorganic mercury was the same as in other cases, 30 min sufficing. Dilute homogenate mixtures appeared to give best results, a 5% (w/v) mixture in deionized water furnishing maximum recovery data. In Fig. 3 representative chromatograms for inorganic mercury in 5% rat kidney homogenate are shown, while recovery data (five samples) are included in Table I.

Sequential analysis of tissue homogenate for inorganic mercury and methylmercury content may be carried out readily. In Fig. 4 are shown a set of chroma-

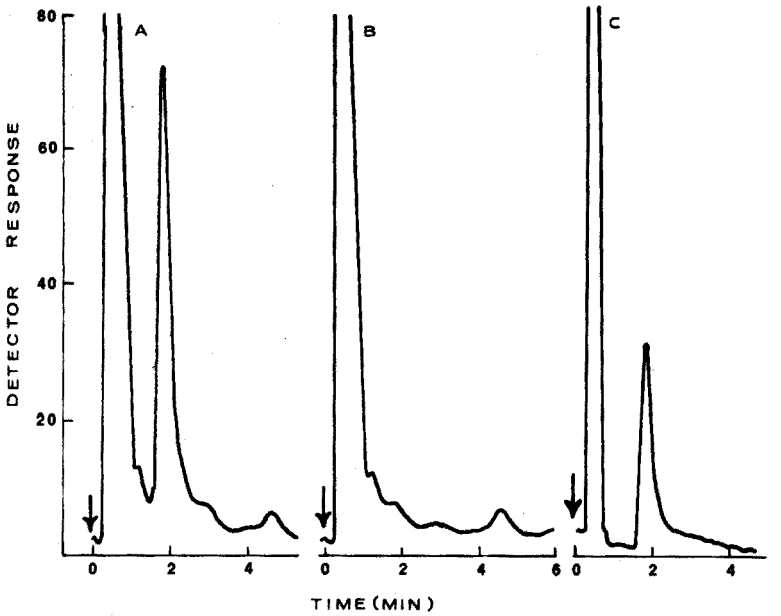

Fig. 3. Chromatograms from measurement of inorganic mercury(II) in rat kidney homogenate (5%) with the methylcobalt reagent. (A) Standard solution of methyl mercury chloride in benzene; (B) blank homogenate sample; (C) sample of homogenate incubated with added inorganic mercury (10 min). Conditions as in Fig. 1.

tograms obtained for an aliquot pair of kidney homogenate (5%) samples containing both inorganic mercury and methylmercury. Total methylmercury content (A) consists of methylmercury originally present as well as methylmercury arising from methylation, while methylmercury alone is depicted as a smaller peak (C). Evaluation data of inorganic mercury and ethylmercury are shown in Fig. 5, with serum and kidney homogenate as matrix.

Measurement of inorganic mercury in various media with tetraalkyltin reagents

Recently, several reports have appeared regarding the ability of peralkyltin compounds to alkylate inorganic mercury to the monoalkylmercurial stage[8]. Yields of methyl-, ethyl- and propylmercury are satisfactory when reaction is carried out in mixtures of water and water-miscible organic solvents such as methanol.

The alkylating behavior of these tin compounds towards mercury was examined under a variety of experimental conditions of time, temperature, stirring and mixtures of organic solvents with water.

Combining methanolic solutions of methyl- and ethyltin with an equivalent volume of acidified water containing added inorganic mercury provided varying yields of methyl- and ethylmercury depending on the time and method of shaking. Best results were achieved if the mixtures, in tightly sealed 2-dram vials, were shaken in an Eberbach reciprocating shaker equipped with a multi-hole block and cover which permitted horizontal shaking[13]. Optimal shaking time was 45 min at room temperature.

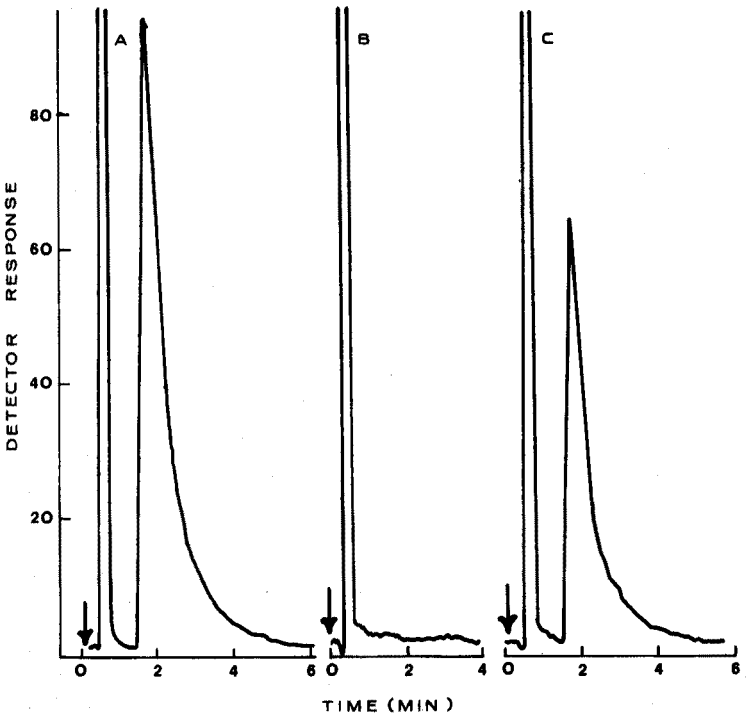

Fig. 4. Sequential analysis chromatograms from analysis for inorganic and methylmercury in rat kidney homogenate (5%) with the methylcobalt reagent. (A) Total methylmercury (methylmercury present and methylated inorganic mercury); (B) blank homogenate sample containing no mercurials; (C) sample of homogenate containing only methylmercury. Conditions as in Fig. 1.

In an acidified sample containing an oxidant such as dichromate, tetramethyltin reacts with inorganic mercury to give poor yields of methylmercury, relative to acidic media with oxidant omitted, probably because of destruction of the methyltin reagent. Much higher recovery of mercury, as ethylmercury, was observed under like conditions with tetraethyltin as alkylating agent.

Methanolic solutions of tetraalkyltin compounds are unstable on standing, and solutions of these reagents should be prepared just before use. The commercial reagents are satisfactory.

Early investigations of these tin reagents showed that the Durapak Carbowax 400 (low K′) columns, which were satisfactory for the analysis based on pentacyanoalkylcobaltate(III), were not useful in the case of the tin compounds; a large interfering peak, probably arising from decomposition of the tin reagent, overlapped the elution region of the alkylmercury (chloride).

Separation and reduced detection of this interfering material was achieved with 10% DEGS on Anakrom SD, 70/80 mesh. This packing was prepared with a commercially available "fluidizer" and was found to require minimal periodic treatment with mercurials to optimize elution.

To date, the use of tetraethyltin in organic mercury analysis has been restricted to water and urine. Unsatisfactory recoveries of added mercury from whole blood, serum or tissue homogenate were encountered.

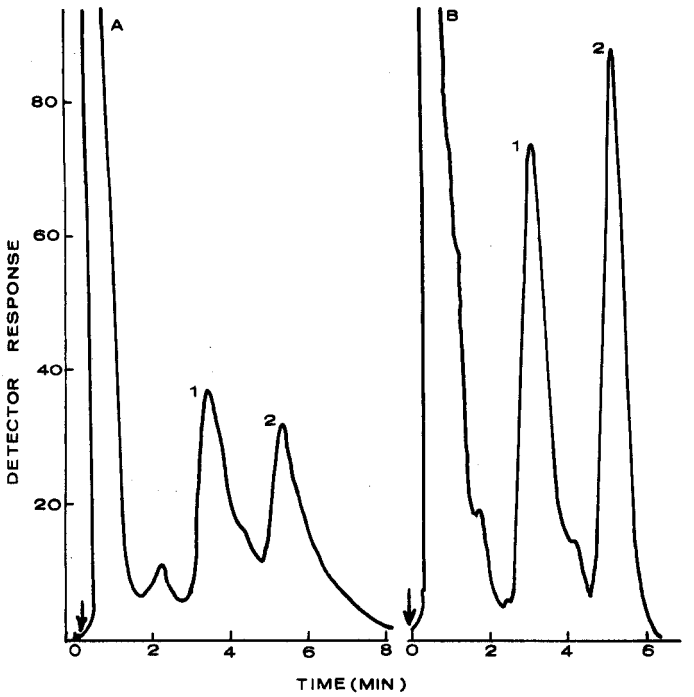

Fig. 5. Simultaneous analysis chromatograms from measurement of ethyl- and inorganic mercury(II) in (A) serum and (B) rat liver homogenate. A-1 and B-1 inorganic (methyl) mercury; A-2 and B-2, ethylmercury. Conditions as in Fig. 1.

Volumes of water and urine taken for analysis with the tin reagent were half that (0.5 ml) employed with the methylcobalt reagent, while the acid used was dilute perchloric instead of sulfuric. The samples were treated with dichromate in perchloric acid for 30 min, and then with a freshly prepared solution of tetraethyltin in methanol.

Inorganic mercury is measured as ethylmercury(II) chloride, the levels of any methyl- or phenylmercury present being determined distinct from the ethyl analog. Methylmercury and inorganic (as ethyl-) mercury were determined at the same column temperature while phenylmercury was best detected by use of the Carbowax 400 (low K′) packing at 170°. Mixtures of inorganic and ethylmercury in this case require sequential analysis analogous to the methods for methylmercury with pentacyanomethylcobaltate(III).

Chromatographic data obtained for inorganic mercury in water and urine with tetraethyltin as alkylating agent are presented in Fig. 6.

Calibration curves were linear over the range of added mercury investigated (300–1000 ng Hg ml^{-1} of sample). Recovery data for added mercury from urine and water are given in Table I. It can be seen that recovery was lower for water than for urine, but in both cases was lower than that obtained with the methylcobalt reagent. Simultaneous measurement of inorganic mercury and methylmercury in water and urine is shown in Fig. 7.

In alkaline media, with or without added cadmium ion, alkylation with tetra-

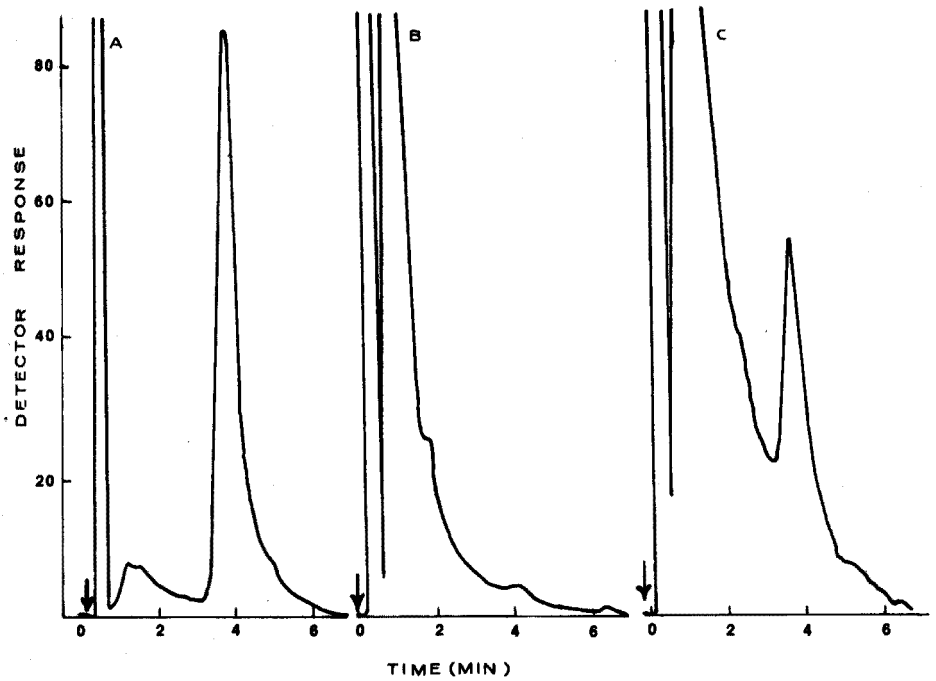

Fig. 6. Chromatograms from measurement of inorganic mercury(II) in urine with tetraethyltin. (A) Standard sample of ethylmercury chloride in benzene; (B) blank urine sample; (C) urine sample incubated (10 min) with added inorganic mercury. Packing: 10% DEGS on Anakrom SD, 70/80 mesh, column, 180°; injector, 210°; detector, 190°.

alkyltin was only about 10–20%, in contrast to satisfactory data obtained with pentacyanomethylcobaltate(III).

Measurement of inorganic mercury in various media with tetraphenylboron and other reagents

Inorganic mercury in water, blood, urine and tissue homogenates was also determined with sodium tetraphenylborate, an attractive reagent owing to its ready availability at moderate cost.

Unlike the other organometallic agents studied, arylboron compounds appeared to be too unstable under acidic conditions and required modification of the assay. In a dilute alkaline medium also containing excess of cadmium reagent, arylation of inorganic mercury to form phenylmercury proceeds satisfactorily as determined by recovery studies with various biological media. Poorest results were obtained with whole blood.

Table I shows recovery data for urine, serum and tissue homogenates with sodium tetraphenylborate. Mercury recovery from urine is quantitative, with good recoveries being noted with tissue (93.4%) and serum (80.4%).

Representative chromatograms for inorganic mercury in tissue homogenates are given in Fig. 8. Sequential analysis chromatographic data (not shown) were obtained for various mixtures of inorganic mercury as well as the methyl and ethyl analogs, in identical manner to procedures noted with the other reagents.

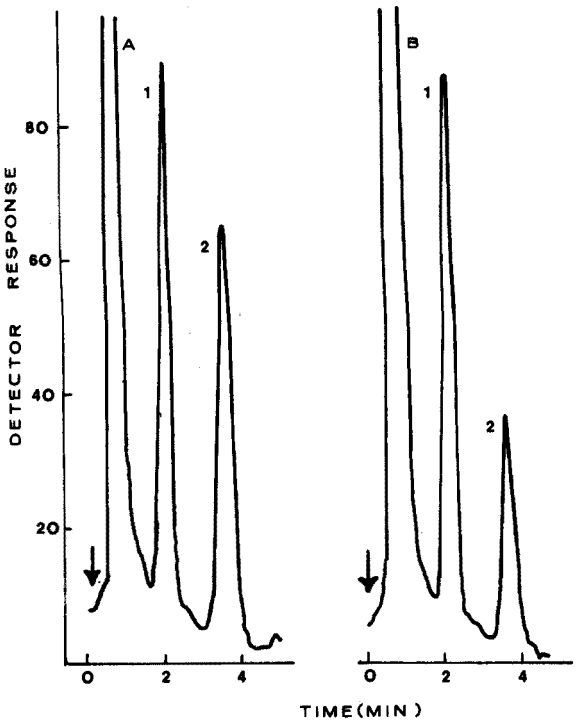

Fig. 7. Simultaneous analysis chromatograms for methyl- and inorganic mercury in (A) urine and (B water. A-1 and B-1, methylmercury; A-2 and B-2, inorganic mercury. Conditions as in Fig. 6.

Assessment of inorganic mercury along with methyl- and/or ethylmercury require the use of two column temperatures, phenylmercury being obtained by differentia assay.

Problems arose in attempting to utilize chloromethyl(penta-aquo)chromium (III) in mercury determinations, owing to the unsatisfactory gas-chromatographi properties of chloromethylmercury(chloride), the organomercurial which woul arise on reaction of the chromium reagent with inorganic mercury. Preliminar studies indicated that chloromethylmercury undergoes decomposition in the chroma tograph yielding relative peak sizes which are but a fraction of those for methyl mercury in solutions possessing identical metal content. Furthermore, the peal that was observed under a variety of conditions of flow and temperature wa indistinguishable from that of methylmercury.

It has been reported that dialkylthallium(III)carboxylates in aqueous solu tion alkylate inorganic mercury in high yield under relatively mild conditions c temperature and reaction time[10]. To date, efforts to exploit this route as an analytica method with acetic acid solutions of water and urine have been unsuccessful; alkyla tion of mercury via this route probably requires that the carboxylate be totall unprotonated, which precludes the use of acidic media.

Intercomparison studies involving the use of the g.c. techniques describe here, flameless atomic-absorption spectrometry and neutron activation analysi applied to samples obtained from methylmercury-treated laboratory animals ar

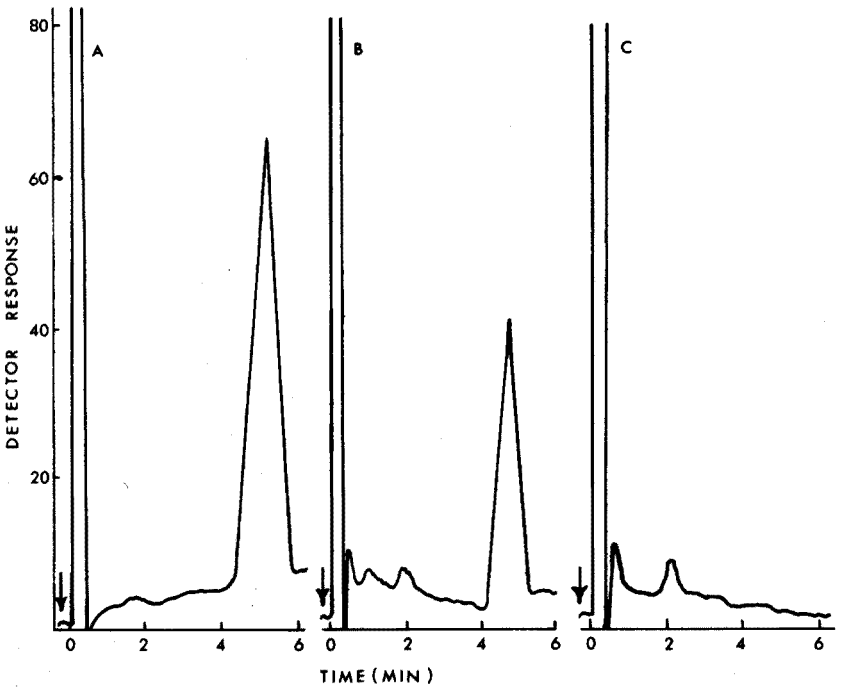

Fig. 8. Chromatograms from measurement of inorganic mercury with sodium tetraphenylborate in kidney homogenate. (A) Standard solution of phenylmercury chloride in benzene; (B) kidney homogenate sample with added inorganic mercury(II); (C) kidney homogenate sample without added mercury.

TABLE II

INTERCOMPARISON STUDY OF TOTAL MERCURY LEVELS IN WHOLE BLOOD SAMPLES OF RATS

(All results are given as $\mu g\ ml^{-1}$)

Sample	*G.l.c.*	*N.a.a.*	*F.a.a.s.*
C_1	0.00	1.65	
C_2	0.00	1.05	
201	32.10	8.80	
202	21.54	5.25	
203	31.62	12.10	
204	34.30	11.00	
205	41.63	13.40	
206	28.60	7.95	
	Range 21.54–41.63	Range 5.25–13.40	$r=0.30$
C_1	0.00	—	1.00
C_2	0.00	—	0.00
101	109.12	—	107.0
102	120.76	—	139.0
103	147.76	—	155.5
104	206.08	—	123.0
105	183.60	—	168.0
	Range 109.12–206.08		Range 107.0–168.0
			$r=0.91$

presently in progress. Some preliminary data were obtained from a comparative study of whole blood from methylmercury-poisoned rats.

In the first part of Table II results are presented for mercury in whole blood by activation analysis and gas chromatography, while the lower part of the table shows g.l.c. *versus* f.a.a.s. levels of mercury. Total mercury by g.l.c. is the sum of methylmercury and inorganic mercury. Sample volumes available did not permit three-way analysis of the same samples, so that these preliminary results consist of two sets of results involving two methods per set. It is of interest to note that data obtained by g.l.c. and f.a.a.s. are generally in good agreement, while the blood set analyzed by g.l.c. and n.a.a. furnished much higher levels by g.l.c. The correlation coefficient (r) for g.l.c. and f.a.a.s. is 0.91 while that for g.l.c. and n.a.a. is 0.30.

More extensive cooperative studies are necessary and are presently being organized.

We thank Dr. J. Weaver, North Carolina State University, and Mr. D. Fox, National Institute of Environmental Health Sciences, for intercomparison of methodology studies. Support of this investigation by the National Institutes of Health Grant ES-00481 is gratefully acknowledged. Assistance in initial studies was by the Triangle Universities Consortium on Air Pollution funded by the Office of Manpower Development, U.S. Environmental Protection Agency.

SUMMARY

Gas–liquid chromatography is used to determine inorganic mercury in the presence and absence of organomercurials in water and biological media after alkylation or arylation. Best results for inorganic mercury were realized with pentacyanomethylcobaltate(III) and tetraphenylborate(III), via the generated methyl and phenyl mercurial. Tetraethyltin, forming ethylmercury, was less satisfactory. Lower detection limits with these reagents were in the range 10–30 ng Hg ml^{-1} of medium. Co-determination of inorganic mercury and various organomercurials was carried out by sequential or simultaneous procedures with several column temperatures and packings. Optimal chromatographic results were achieved with Durapak Carbowax 400 (low K′) on Porasil F and 10% DEGS on Anakrom SD.

RÉSUMÉ

La chromatographie gaz–liquide est utilisée pour le dosage du mercure inorganique, après alkylation ou arylation, en présence et en l'absence d'organomercuriels, dans l'eau et dans des milieux biologiques. Les résultats les meilleurs ont été obtenus avec le pentacyanométhylcobaltate(III) et le tétraphénylborate(III), via méthyl- et phénylmercure. L'étain tètraéthyle, formant de l'ethylmercure a donné les résultats les moins satisfaisants. Les limites de détection les plus basses sont de l'ordre de 10–30 ng Hg ml^{-1}. Les résultats chromatographiques optima ont ont été obtenus avec le pentacyanométhylcobaltate(III) et le tétraphénylborate(III), Anakrom SD.

ZUSAMMENFASSUNG

Anorganisches Quecksilber wird in Gegenwart und in Abwesenheit von Organoquecksilber-Verbindungen in Wasser und in biologischen Stoffen nach Alkylierung oder Arylierung mittels Gas–Flüssig-Chromatographie bestimmt. Die besten Ergebnisse für anorganisches Quecksilber wurden über die mit Pentacyanomethlcobaltat(III) und Tetraphenylborat(III) erhaltenen Methyl- und Phenylquecksilber-Verbindungen erzielt. Tetraäthylzinn, das Äthylquecksilber ergibt, war weniger zufriedenstellend. Die unteren Nachweisgrenzen lagen bei diesen Reagenzien im Bereich 10–30 ng Hg ml^{-1} des Mediums. Die gemeinsame Bestimmung von anorganischem Quecksilber und von verschiedenen Organoquecksilber-Verbindungen erfolgte durch aufeinanderfolgende oder simultane Verfahren bei verschiedenen Säulentemperaturen und -füllungen. Die besten chromatographischen Ergebnisse wurden mit Durapak Carbowax 400 (niedriges K′) auf Porasil F und 10% DEGS auf Anakrom SD erhalten.

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DETERMINATION OF NITRATE IN WATER WITH A NEW CONSTRUCTION OF ION-SELECTIVE ELECTRODE

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The determination of the nitrate content of water may give valuable information about pollution by decomposing organic material or by nitrate fertilizers. The standard methods of nitrate determination are based on spectrophotometric procedures in which organic compounds are oxidized or nitrated to a coloured species, or nitrate is reduced to nitrite or ammonia for the subsequent determination. The disadvantages of these methods are too well established to require further emphasis.

Several attempts to apply ion-selective electrodes for these purposes have been described. Keeney *et al.*[1] and Milham *et al.*[2] have used the commercial Orion 92-07 electrode for the determination of nitrate in water. The latter authors eliminated the interferences of chloride, nitrite and organic anions by addition of a masking solution containing aluminium sulphate, silver sulphate, boric acid and aminosulphonic acid. In this paper, the results of the determination of nitrate ions with a new construction of liquid-state ion-selective electrode are described; the concentrations that can be determined are considerably smaller than those discussed previously[1, 2].

EXPERIMENTAL

Reagents and apparatus

A 0.01 *M* standard solution of potassium nitrate was prepared.

Silver sulphate (0.44%) solution was obtained by dissolving 1.10 g of silver sulphate in water and diluting to 250 cm^3; 1 cm^3 of this solution corresponds to 1 mg of precipitated chlorides.

A phosphate buffer (0.5 *M*, pH 2.2) was prepared by diluting 29 g of 85% phosphoric acid with water, adding 7 g of potassium hydroxide and diluting up to 250 cm^3 with water.

Potentiometers, Elpo N-512 (Poland) and Radelkis OP-205 (Hungary), were used for potential measurements with the ion-selective electrode; a saturated calomel electrode served as reference. A Radelkis OP-711-1/A glass electrode was used for pH measurements.

Construction of the nitrate ion-selective electrode

A new simplified design of the liquid-state ion-selective electrode was developed. This consists of a wick made of natural or synthetic porous polymer

which is saturated with the liquid ion-exchanger. This wick is in contact with the external solution containing the nitrate activity to be measured. The other "membrane" contact is made directly by a platinum wire fixed in the wick. To increase the liquid-exchanger capacity, the internal part of the wick is in contact with a chemically neutral porous foam (polyurethane foam) soaked with the exchanger (Fig. 1).

The liquid ion-exchanger used in the electrode was of the tris(4,7-diphenyl-1,10-phenanthroline)nickel(II) type in *p*-nitrocymene (Orion 92-07). With the given construction, the electrode can be used in a horizontal position, and is free of any disturbance caused by the presence of small air bubbles under the electrode membrane. Its impedance is in the range $1–1.2 \cdot 10^6$ ohms, which is similar to that of the crystalline solid-state electrodes. The working life-time for electrodes of this type was found to be at least 6 weeks. Reconditioning of the electrode was very simple, involving only soaking of the wick with a few drops of new exchanger, and draining any small excess of the exchanger solution by leaving open to the air for a short time.

Procedure for natural waters

To 100 cm^3 of the sample water, add 2 cm^3 of the phosphate buffer and 10 cm^3 of silver sulphate solution. Immerse the nitrate ion-selective electrode and saturated calomel reference electrode in the solution and measure the potential after 1–2 min of stirring with a magnetic stirrer and read the nitrate content from a calibration curve.

The standards for the calibration curve were prepared in the same way as the sample, but from double-distilled water with the addition of the standard nitrate solution.

When the standard addition procedure was used, the potential was measured twice. For the first measurement, sample was prepared as described above (E_1); the second measurement was made after addition of 1 cm^3 of 0.01 *M* potassium nitrate (E_2). The nitrate concentration in the sample (c_x) was calculated from:

$$c_x = \frac{c_{st} \cdot V_{st} \cdot \alpha}{V(10^{(E_1 - E_2)/S} - 1)}$$

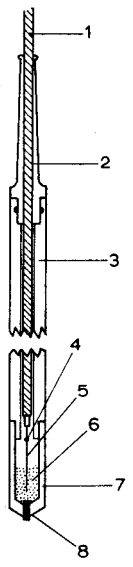
where c_{st} and V_{st} are the concentration and volume of the standard, respectively, V is the initial volume of the sample, S is the slope of the electrode characteristics, and α is the dilution factor (in this case, 1.12).

RESULTS AND DISCUSSION

Electrode characteristics

The electrode characteristics are mainly determined by the ion-exchanger used for a particular ion. Therefore, the response of the electrode with the proposed construction based on the Orion 92-07 exchanger is similar to that of the commercial Orion electrode. The linear response range was found to be $10^{-1}–6 \cdot 10^{-5}$ *M*, with a slope close to Nernstian response. However, on the basis of a calibration curve, sufficiently accurate results may be obtained down to 10^{-5} *M*.

The electrode response is not pH-sensitive in the range 2–12 for 10^{-1} *M*



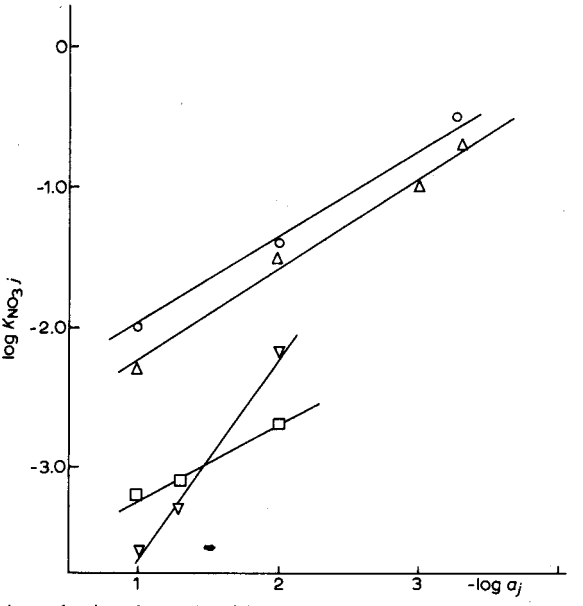


Fig. 1. Cross section of the liquid-state ion-selective electrode with wick. 1, cable; 2, plastic body, upper part; 3, plastic body, main part; 4, cable junction; 5, platinum wire; 6, porous sponge reservoir; 7, teflon cap; 8, porous wick.

Fig. 2. The logarithmic values of selectivity coefficients for the nitrate electrode as a function of concentration of the interfering ion. (○), HCO_3^-; (△), Cl^-; (▽), $H_2PO_4^-$; (□), SO_4^{2-}.

nitrate ion concentrations. The alkaline limit shifts to lower pH values as the nitrate concentration decreases, indicating the competition of hydroxyl ions.

The selectivity coefficients for interfering ions, calculated from the Eisenman equation[3]

$$E_1 = E_2 = S \log \frac{a_{(2)NO_3} + K_{NO_3 j} + a_j^{1/z}}{a_{(1)NO_3}}$$

at the *ca.* 0.1 *M* concentration level were close to those reported by Orion[4]. However, the values were not constant and tended to increase for more dilute solutions (Fig. 2). Such behaviour is similar to that observed for other types of electrodes[5]. In these calculations, because of the necessity of adding rather high concentrations of sulphate or dihydrogenphosphate ions, the activity coefficients for each case were calculated on the basis of the equation

$$\log f_i = -\frac{0.5 z^2 \sqrt{I}}{1 + \sqrt{I}}.$$

The reproducibility of the electrode response was investigated over short as well as long periods of time. Within a series of measurements, when the response was recorded 1–2 min after changing the solution, a reproducibility within ± 0.5 mV was observed. The potential reproducibility between measurements on successive days usually lay within a few mV, although it was sometimes greater in the first period after preparation of a new electrode. However, the changes in absolute potential value were parallel at different concentrations and consequently did not affect the linearity of the calibration curve (Fig. 3).

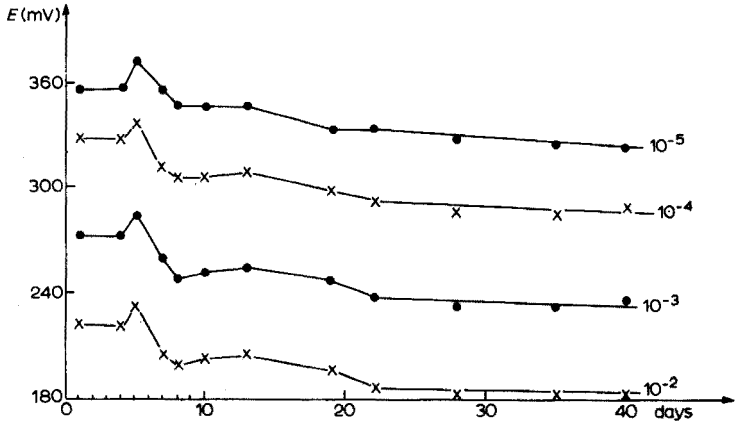


Fig. 3. Changes of absolute potential values with time for the wick electrode, at various levels of concentration.

Determination of nitrate in tap water

The described electrode was used for the determination of nitrate concentrations in tap water. As a reference procedure, the colorimetric method based on oxidation of brucine[6] was adopted.

The determination with the ion-selective electrode may be done directly from the calibration curve or by the standard addition procedure. Both procedures applied to water samples without any preliminary treatment gave results of 16.6 mg dm^{-3} compared to 13.0 mg dm^{-3} determined colorimetrically. This corresponds to a +27% error; the precisions for a series of 5 determinations were 0.62 and 1.51 mg dm^{-3}, respectively, for the calibration curve and standard addition procedures. The results for synthetic mixtures were also unsatisfactory, indicating significant influence of chloride and hydrogencarbonate ions. To eliminate these effects, the interfering ions were masked by addition of silver sulphate (chloride) and acidic phosphate buffer (hydrogencarbonate). After the addition of these substances, the measurements could be carried out without any separation. For synthetic mixtures (Table I), the results in the presence of a normal content of

TABLE I

DETERMINATION OF NITRATE IN SYNTHETIC MIXTURES

(55 mg Cl^- dm^{-3} and 300 mg HCO_3^- dm^{-3} with various amounts of nitrate)

Nitrate content mg dm^{-3}	*Nitrate found mg dm^{-3}*	*Error (%)*
3.2	3.7	+17
6.3	6.5	+3.2
7.6	7.5	−1.3
9.5	9.3	−2.1
12.6	12.3	−2.4
15.8	15.1	−4.4

chloride and hydrogencarbonate were promising, indicating an accuracy better than ±5%, except for the smallest content, which corresponds to a point of significant curvature in the potential-response diagram.

The direct determination in tap water was equally accurate (Table II). For a series of 5 independent measurements, the standard deviation was less than 0.25 mg dm^{-3}, being slightly better for the calibration curve procedure than for the standard-addition method.

TABLE II

DETERMINATION OF NITRATE IN TAP WATER

Nitrate content[a] *mg* dm^{-3}	*Nitrate found* *mg* dm^{-3}	*Error* (%)	*S* (*mg* dm^{-3})	S_r (%)
Calibration curve method				
8.9	8.8	−1.1	0.15	1.7
9.3	8.7	−6.5	0.13	1.4
10.0	9.5	−5.0	0.18	1.8
Standard addition method				
8.9	8.3	−6.7	0.21	2.4
9.3	8.3	−10.8	0.24	2.6
10.0	9.3	−7.0	0.19	1.9

[a] Brucine method; average of 5 determinations.

CONCLUSIONS

The new construction of the nitrate ion-selective electrode enables successful determination of the nitrate content of water. This design gives reproducible results and can be operated during relatively long periods of time. Its regeneration or rather reconditioning is simple, and needs only a small amount of ion-exchanger. The results for nitrate determination are of very reasonable precision, although for individual samples some systematic errors of a few per cent (up to 10.8%) appear; such errors may, at least partially, be also attributed to the errors of the reference brucine method. Similar procedures described in the literature[1,2] show precisions of the same order, but in the present case the nitrate content is at least two times lower. The proposed method needs no separation of interfering ions and therefore is competitive with the classical procedures, being quicker, simpler and less subject to accidental errors. Therefore it may be used successfully for rapid control of the nitrate content in tap water.

SUMMARY

A new design of the liquid-state electrode for nitrate ions is proposed. It contains a porous wick soaked with the liquid ion-exchanger, and has no internal reference solution. This electrode was used for nitrate determination of tap water at the level 8–10 p.p.m. The effects of chloride and hydrogencarbonate are

eliminated by the addition of silver sulphate and a phosphate buffer, which also maintains constant ionic strength. Precision of a series of measurements is better than 2%, but the results show differences up to 10% compared to the colorimetric brucine procedure.

RÉSUMÉ

Une construction nouvelle d'électrode ionique sélective est proposée pour le dosage des nitrates dans l'eau. Elle est constituée d'une mèche poreuse imbibée du liquide échangeur d'ions et ne contient pas de solution de référence interne. Cette électrode est utilisée pour le dosage des nitrates dans l'eau du robinet, à des concentrations de 8 à 10 p.p.m. L'influence des chlorures et du bicarbonate est éliminée par addition de sulfate d'argent et d'un tampon au phosphate, permettant également de maintenir constante la force ionique. La précision est meilleure que 2%; cependant les résultats donnent une différence allant jusqu'à 10%, comparés à ceux obtenus par la méthode colorimétrique à la brucine.

ZUSAMMENFASSUNG

Es wird eine neue Art der Flüssig-Elektrode für Nitrationen vorgeschlagen. Sie enthält einen porösen Docht, der mit dem flüssigen Ionenaustauscher getränkt ist, und hat keine innere Vergleichslösung. Diese Elektrode wurde für die Nitratbestimmung in Oberflächenwasser im Bereich 8–10 p.p.m. angewendet. Der Einfluss von Chlorid und Hydrogencarbonat wird durch die Zugabe von Silbersulfat und eines Phosphatpuffers eliminiert, der ebenfalls die Ionenstärke konstant hält. Die Reproduzierbarkeit bei einer Serie von Messungen ist besser als 2%, jedoch zeigen die Ergebnisse Abweichungen von bis zu 10% gegenüber dem kolorimetrischen Brucin-Verfahren.

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Analytica Chimica Acta, 69 (1974) 415–424

SINGLE-POINT TITRATIONS

PART I. THE DETERMINATION OF BASES

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There are two approaches to the determination of bases, namely titration with a strong acid and direct potentiometry. The first is capable of giving very high accuracy whereas direct potentiometry, even in the most favourable cases, yields only moderate accuracy. If direct potentiometry is used, weak bases can be determined only if their pK values are known, and mixtures of bases cannot be determined at all. In practice, therefore, titrations are normally the only technique considered, although they are time-consuming and rather cumbersome, especially for automatic process analysis.

An intermediate technique could be very useful for a number of routine applications, namely partial reaction of the bases with a mixture of weak acids, the acids being in excess. Equal volumes of sample and acid mixture could be taken. If the concentrations and pK values of the weak acids in the mixture were known, a single potentiometric measurement of pH would yield the same information as a titration. It would not be necessary to know the pK values of the bases in the sample and the technique would be as useful for a single base as for a mixture of bases. It is possible to prepare a mixture of weak acids which yields an almost linear relation between the amount of base in the sample and the pH. It would also be easy to construct a meter or a digital instrument to read directly in milliequivalents of base in the sample. This technique seems to have received very little attention. Leithe[1] described the technique and its advantages. He worked out a number of examples including acid mixtures for the determination of bases as well as base mixtures for the determination of acids. He used the term "Einpunkt-Titration", a name which might be reserved for this special technique. Oehme and Dolezalova[2] used a similar technique for the analysis of sulphuric acid in plating baths. Similar approaches have also been used in other cases, *e.g.* the preparation of buffers with special properties. However, there seems to be no quantitative treatment of the technique. Leithe's mixtures appear to have been prepared empirically and large deviations from linearity were accepted.

This paper describes a method for the calculation of a reagent composition and an evaluation of the prepared mixtures. Further experimental evaluation of the method is in progress as well as extension to systems other than acid–base titrations.

THEORY

A mixture of weak acids must be made so that when it is titrated, part of the titration curve will be linear as shown in Fig. 1. The abscissa represents the amount of base added from the burette during the titration. When such a calibration curve has been made the amount of base in a sample can be determined graphically. The experimental procedure consists of adding the sample to the mixture of acids and reading the pH. For example for Fig. 1, if the pH is 5.0, the amount of base in the sample corresponds to 4.0 ml of 0.1 *M* sodium hydroxide, *i.e.* 0.4 mmol.

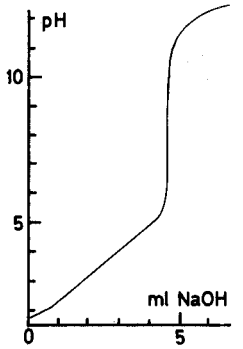

Fig. 1. Titration of a mixture of several weak acids with 0.1 *M* sodium hydroxide.

If the calibration curve had been a straight line, evaluation of the result could have been made simpler. The conditions for obtaining a linear curve will now be derived. The first step is to derive a relationship between the concentration of base, b, and the pH, or rather the hydrogen ion concentration, in the mixed solution. It is the reverse of the calculation of pH in a mixture of weak acids and a strong base. For a dibasic acid, H_2A, the following conditions can be written:

$$b+[H^+]=[OH^-]+[HA^-]+2[A^{2-}] \quad (1)$$

$$A_{tot}=[H_2A]+[HA^-]+[A^{2-}] \quad (2)$$

$$[HA^-]=K_1[H_2A]/[H^+] \quad (3)$$

$$[A^{2-}]=K_1K_2[H_2A]/[H^+]^2 \quad (4)$$

$$[OH^-]=K_w/[H^+] \quad (5)$$

Equations (3) and (4) are valid only if the ionic strength is kept constant. The constants K_1 and K_2 will then be conditional constants valid for this special ionic strength. Further, it must be assumed that the base is so strong that the number of undissociated molecules can be neglected. If K_2 is set to zero, the equations will describe a monobasic acid. If n acids are present, the relationship sought will be

$$b=\frac{K_w}{[H^+]}-[H^+]+\sum_{i=1}^{n}\frac{A_{itot}(2K_{i1}K_{i2}+[H^+]K_{i1})}{([H^+]^2+K_{i1}[H^+]+K_{i1}K_{i2})} \quad (6)$$

This equation can be used to calculate the amount of base, if A_{itot}, K_{i1} and K_{i2} are known and $[H^+]$ is measured. The highest accuracy should be obtained when

the available pH span is evenly covered with weak acids so that the relationship between pH and b is a straight line. The next step in the derivation is therefore to substitute pH into eqn. (6). In a constant ionic strength medium, the glass electrode responds according to Nernst's equation. A special pH scale can therefore be defined so that:

$$\mathrm{pH} = -\log[\mathrm{H}^+], \quad \mathrm{H}^+ = 10^{-\mathrm{pH}} \tag{7}$$

The pH scale used is obtained by operational comparison with an NBS buffer at a single point named the calibration point and applying eqn. (7) over the rest of the range. There will be only small differences between this scale and a scale defined by calibration against several NBS buffers along the operating range. On the other hand, eqn. (7) will not give a correct value of the hydrogen ion concentration, but if the error is small it can be cancelled by a corresponding adjustment of the pK values. A procedure for empirical adjustments of the pK values will be given later. The electrode response must be Nernstian and should be checked in separate experiments. By substituting eqn. (7) into eqn. (6), we can write:

$$b = K_w \cdot 10^{\mathrm{pH}} - 10^{-\mathrm{pH}} + \sum_{i=1}^{n} A_{i\,\mathrm{tot}} E_i \tag{8}$$

where

$$E_i = \frac{2K_{i2} + 10^{-\mathrm{pH}}}{\left(\frac{10^{-2\mathrm{pH}}}{K_{i1}} + 10^{-\mathrm{pH}} + K_{i2}\right)} \tag{9}$$

Equation (8) is the titration curve of the acid mixture.

The final step in the derivation is to find a relationship which can be used for adjusting the concentrations $A_{i\,\mathrm{tot}}$, eqn. (8), until a linear relation between pH and the concentration of base is obtained. The desired line is given by

$$\mathrm{pH} = kh + l \tag{10}$$

where h is a base variable for a mathematically linear case, and k is the desired proportionality constant; the constant l is discussed below. b is the experimentally obtainable variable expressed by eqn. (8). The relation between h and b is shown in Fig. 2.

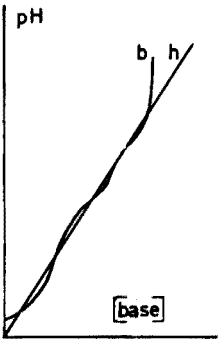

Fig. 2. The relation between a mathematically linear base variable, h, and a physically obtainable variable, b.

A function G is then formed:

$$G = \int (h-b)^2 \mathrm{dpH} \tag{11}$$

The concentrations $A_{i\mathrm{tot}}$, which over a certain pH range give a minimal value for G, should be found. If G is differentiated with respect to $A_{i\mathrm{tot}}$, a linear equation system will result if

$$\frac{\partial G}{\partial A_{i\,\mathrm{tot}}} = 0 \tag{12}$$

The equation will be

$$\begin{pmatrix} \int E_1 E_1 & \int E_1 E_2 \ldots \int E_1 E_n \\ \int E_2 E_1 & \int E_2 E_2 \ldots \int E_2 E_n \\ \int E_n E_1 & \int E_n E_2 \ldots \int E_n E_n \end{pmatrix} \cdot \begin{pmatrix} A_{1\,\mathrm{tot}} \\ A_{2\,\mathrm{tot}} \\ A_{n\,\mathrm{tot}} \end{pmatrix} = \begin{pmatrix} \int cE_1 \\ \int cE_2 \\ \int cE_n \end{pmatrix} \tag{13}$$

where $c = -[K_w \cdot 10^{\mathrm{pH}} - 10^{-\mathrm{pH}} - (\mathrm{pH}-l)/k]$. The integration of the elements in the matrix, eqn. (13), is performed over the pH range where a linearization is desired. The solutions to eqn. (13) are the concentrations which produce a buffer with the desired properties. The value of k can be selected so that a suitable operating range is obtained, say, 1 meq/pH unit for equal amounts of acid mixture and sample. A strong acid is obtained by putting $K_2 = 0$ and taking a large number for K_1, *e.g.* 10^{10} A monobasic acid is obtained by putting $K_2 = 0$.

Equation (13) was solved in a calculator as described in a later section. It turned out that negative values of $A_{i\mathrm{tot}}$ were sometimes obtained. A negative value of the concentration of a strong acid is equal to the corresponding concentration of a strong base. Negative values of the weak acid concentrations cannot be realized, and these solutions to eqn. (13) must be discarded. There is an increased probability of negative results if the calculations are made with too low precision. It is advantageous to select acids with pK values spaced as evenly as possible along the range and under these conditions the calculations usually resulted in positive answers. The results also depend on the selection of the integration interval. The best result was obtained with a lower pH limit selected so that the $10^{-\mathrm{pH}}$ term in eqn. (8) does not dominate the sum. The highest pH limit should be set close to the pK_i value of the weakest acid.

The value of l in eqn. (10) must be selected manually. The selection is arbitrary and a value of 2.80 pH units was used throughout. The l value corresponds to the pH in an acid mixture before any base addition and it can be used as a calibration point. It is convenient to take l equal to a value slightly higher than the lower pH limit of the linear range. If another value than $l = 2.80$ is desired, a new value l^* can be obtained by adjustment of the amount of strong acid

$$A^*_{1\,\mathrm{tot}} = A_{1\,\mathrm{tot}} + (l - l^*)/k \tag{14}$$

where $A_{1\,\mathrm{tot}}$ is the concentration of strong acid required for $l = 2.80$, and $A^*_{1\,\mathrm{tot}}$ the corresponding concentration for another starting point, l^*.

CALCULATIONS

A programmable calculator HP 9810 was used. Altogether four programs were written. In the first, the elements of the coefficient matrix in eqn. (13) were calculated with Simpson's formula for evaluating the integrals. The results were calculated for 2000 and 4000 intervals and the correction formula

$$S = S(h) + [S(h) - S(2h)]/15 \tag{15}$$

was used where $S(h)$ is the approximation obtained with the interval length h. The time for the calculator to perform this step was a few hours. The right-hand side of eqn. (13) was calculated with a second program in the same way. The results were then entered into the machine and the solution to eqn. (13) was obtained by a program given in the HP manual for solving an equation system. The concentrations $A_{i\mathrm{tot}}$ were obtained.

A rounding error results in an error in the solution. Depending on the nature of the coefficient matrix in eqn. (13), it might increase when the number of acids is increased. Four significant figures in the concentration will thus require a calculation of the integrals with six significant figures if four acids are used. If more acids are present, still more significant figures are necessary for the stated accuracy of the result.

The final program used calculated a theoretical titration curve from eqn. (8), employing the obtained $A_{i\mathrm{tot}}$, as well as the mean error, and the size and location of the maximal error.

THEORETICAL RESULTS

It was then possible to calculate an acid mixture for general laboratory use. The following aspects were taken into account in the selection of constituents. It was assumed above that the ionic strength should be kept constant. The reagent should therefore always contain a high concentration of inert electrolyte. The pK values of tribasic acids are more sensitive to changes in ionic strength than mono- and dibasic acids, hence no tribasic acids were used. Another aspect is that an acid mixture for general use should be subjected to as few side-reactions as possible. Such side-reactions can be complex formation, precipitation or redox reactions with components of the sample. The following selections were therefore restricted to carboxylic acids, but for other purposes mixtures containing other types of acids might be more suitable.

All bases of the sample should be protonated completely. If a precision of 1% is desired for the method, at least 99% of the bases of the sample must be protonated when it is mixed with the reagent. The available range of Figs. 1 and 2 should therefore extend up to a pH which is at most 2 units less than the pK_a value of the weakest base in the sample. A suitable approximate range will therefore be between pH 3 and pH 6.

If the pH of the acid mixture was 3.0 before addition of the sample and 6.0 after, the electrical range would be 3 pH units. It will then be necessary to make the pH measurement with an accuracy of ± 0.015 pH unit in order to know the amount of base in the sample with an accuracy of 1%. This example shows

that the demand on linearity must be high. A titration curve of the acid mixture should not show deviations from the ideal line, see Fig. 2, larger than about ± 0.02 pH units.

Several examples were calculated (Table I). The first solution contains one strong acid and three weak acids, and it is interesting to note that this is a sufficient number to give a quite low mean error. The stated limits for the deviations, ± 0.02 pH units, are found at the lower and upper end of the range, respectively. In between the limits, the linearity is much better. The negative value of the hydrochloric acid concentration indicates that the acid mixture should be partially titrated with sodium hydroxide. A calculated titration curve is shown in Fig. 3, and it is also extended to negative values of the base variable to show the shape at the end of the interval. Negative values of the base variable correspond to a sample containing excess of strong acid. A portion of the curve has been enlarged to show the excellent linearity. The enlarged part was selected so that it contained the largest error except for the interval ends.

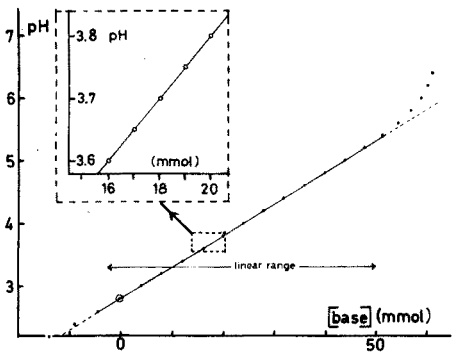

Fig. 3. The calculated titration curve for solution 1 in Table I. The enlarged inset contains the largest deviation but for the interval ends. ⊙ indicates the calibration point at pH = 2.800. The abscissa gives the base consumption per liter of solution.

Solution 2 in Table I is an example of a mixture containing five weak acids. As expected, the mean deviation is lower in this solution than in solution 1 because there are more adjustable parameters. The interval is again chosen so that a maximum deviation of ± 0.02 pH units is allowed. Solution 3 shows that when β-alanine is exchanged for glycollic acid, a slight improvement occurs (compare solutions 1 and 3 in Table I); the pK_a values are then more symmetrically spaced. Solution 4 is an example of the possibility of extending the range by including an acid with a higher pK_a value.

Solutions 1–4 were calculated for a proportionality constant of 20 mmol l^{-1} (pH unit)$^{-1}$, l/k, in eqn. (10); 10 ml of acid mixture would then react with up to $(5.35–2.75) \cdot 20 \cdot 10/1000 = 0.52$ mmol of base, *i.e.* it will have a range equal to a 0.05 *M* acid in a 10-ml burette in an ordinary titration. For many routine applications, stronger samples are encountered; solution 5 (Table I) is an example of a solution suitable when the amount of base is five times higher. In other respects, it is equal to solution 1. As expected, the linear pH range is slightly extended to lower values for the stronger solution. It can also be seen that the concentrations in solution 5

TABLE I

CALCULATED ACID MIXTURES FOR SINGLE-POINT TITRATION OF BASES

	pK_1	pK_2	*Solution 1*	*Solution 2*	*Solution 3*	*Solution 4*	*Solution 5*
Proportionality constant $1/k$ (mmol l^{-1})			20	20	20	20	100
Acids[a] (mol l^{-1}):							
HCl	−10	—	−0.01358	−0.01538	−0.01567	−0.03052	−0.07462
Malonic acid	2.66	5.32	0.02063	0.02471	0.02566	0.01870	0.10730
Malonic acid monobenzyl ester	3.35	—	—	0.00126	—	—	—
β-Alanine	3.60	—	0.01851	0.00119	—	—	0.09705
Glycollic acid	3.83	—	—	0.01754	0.02008	0.02063	—
Acetic acid	4.55	—	0.01712	0.01084	0.00951	0.01091	0.08134
Maleinic acid	1.92	6.22	—	—	—	0.02122	—
Range for less than 0.02 pH unit deviation:							
Lower pH			2.75	2.85	2.85	2.85	2.70
Upper pH			5.35	5.45	5.50	6.15	5.35
Mean deviation (pH units)			0.0038	0.0022	0.0024	0.0031	0.0027

[a] Negative sign for HCl means addition of NaOH instead. Only the acid group of β-alanine was taken into account in the calculations as the amine group is fully protonated within this pH range. The compound is supplied as an internal salt and the corresponding amount of strong acid should therefore be added. In example 1 the solution should thus be $-0.01358+0.01851=0.00493$ M with respect to HCl.

cannot simply be obtained by multiplying those of solution 1 by five. A separate calculation must be made for each proportionality constant.

In practice, a stock solution should be made with twice the tabulated concentrations plus a high concentration of inert salt. If equal volumes of this stock solution and sample solution are taken, the final concentrations will be those used in the calculations. As already pointed out[1,2], it is possible to displace the range by addition of an accurately known amount of strong acid. If all samples are known to contain between 2.0 and 2.5 mmol of base, the concentration of strong acid can be increased by 0.2000 *M* to 0.1864 *M* hydrochloric acid in solution 1. A reading of pH = 2.800 then equals 2.000 mmol of base, pH = 3.800 equals 2.200 mmol, etc., if 10 ml of acid mixture is taken. This procedure will, of course, improve the accuracy of the method.

Determination of conditional pK_a values

As every practising chemist knows, the tabulated constants seldom describe his chemical system correctly. A means for simple adjustment of the constants is therefore essential for the single-point titration method. The adjustment should involve finding a conditional constant including corrections for errors caused by the pH definition adopted. By comparing the calculated curve and an experimental curve, a correction can be derived. A function g can be calculated from eqn. (8) with some reasonably accurate values of the pK_i's and with the A_i values obtained from eqn. (13). Such a function g is shown in Fig. 3 for solution 1. Function f is a similar curve obtained experimentally by titration of a portion of the acid mixture with strong base under such conditions that volume changes can be neglected.

Now, suppose that the difference between f and g can be ascribed solely to errors in the pK_i values. If δ_i denotes the difference in the ith pK value, it is possible to write the functions

$$g = \sum_i \frac{A_i}{10^{pK_i - pH} + 1}, \qquad f = \sum_i \frac{A_i}{10^{pK_i + \delta_i - pH} + 1} \tag{16}$$

if only monobasic acids are included. If dibasic acids are present they can be treated as two independent monobasic acids with pK values of pK_1 and pK_2. For a given pH, the difference can be taken as:

$$\Delta(pH) = g(pH) - f(pH) = \sum A_i \left[\frac{1}{10^{pK_i - pH} + 1} - \frac{1}{10^{pK_i + \delta_i - pH} + 1}\right] = \sum \Delta_i(pH) \tag{17}$$

$$\Delta_i(pH) = A_i \left[\frac{10^{\delta_i} - 1}{(10^{pK_i - pH} + 1)(10^{\delta_i} + 10^{pH - pK_i})}\right] \tag{18}$$

If all δ_i values are small, all Δ_i values can be expanded in a Taylor series around $\delta_i = 0$:

$$\Delta_i(pH) = A_i \left[\frac{-1 + 10^0 + \delta_i \ln 10 + \delta_i^2 \ln^2 10/2! + \ldots}{(10^{pK_i - pH} + 1)(10^{pH - pK_i} + 10^0 + \delta_i \ln 10 + \delta_i^2 \ln^2 10/2! + \ldots)}\right] \tag{19}$$

If the δ_i values are small, eqn. (19) can be simplified further so that

$$\Delta_i(pH) \approx A_i \delta_i h(pH, pK_i) \tag{20}$$

where

$$h(\mathrm{pH}, \mathrm{p}K_i) = \frac{\ln 10}{(10^{\mathrm{p}K_i - \mathrm{pH}} + 1)(10^{\mathrm{pH} - \mathrm{p}K_i} + 1)} \tag{21}$$

For a given value of pH equal to pH_K:

$$\Delta(\mathrm{pH}_K) = \Sigma\, \delta_i A_i h(\mathrm{pH}_K, \mathrm{p}K_i) \tag{22}$$

If a pH_K value corresponding to each pK value is selected, a linear equation system will be obtained from eqn. (22)

$$\begin{aligned} &\delta_1 A_1 h(\mathrm{pH}_1, \mathrm{p}K_1) + \delta_2 A_2 h(\mathrm{pH}_1, \mathrm{p}K_2) + \ldots + \delta_n A_n h(\mathrm{pH}_1, \mathrm{p}K_n) = \Delta(\mathrm{pH}_1) \\ &\delta_1 A_1 h(\mathrm{pH}_n, \mathrm{p}K_1) + \delta_2 A_2 h(\mathrm{pH}_n, \mathrm{p}K_2) + \ldots \delta_n A_n h(\mathrm{p}K_n, \mathrm{p}K_n) = \Delta(\mathrm{pH}_n) \end{aligned} \tag{23}$$

δ_i can be obtained as a solution to eqn. (23) and the corrected values, $\mathrm{p}K_i^*$ values can be obtained from

$$\mathrm{p}K_i^* = \mathrm{p}K_i + \delta_i \tag{24}$$

TABLE II

EFFICIENCY OF THE ADJUSTMENT PROCEDURE FOR THE pK VALUES TO FIT AN EXPERIMENTAL CURVE

	Malonic acid		*β-alanine*	*Acetic acid*
	pK_1	pK_2	pK_1	pK_1
Supposed "true" constants	2.61	5.41	3.70	4.63
Tabulated constants, *cf.* Table I	2.66	5.32	3.60	4.55
Obtained δ_i, eqn. (23)	−0.053	0.094	0.099	0.079
"Corrected" constants	2.607	5.414	3.699	4.629

Suppose that a solution is made up with the concentrations given in Table I, solution 1. Its calculated titration curve, *g*, is shown in Fig. 3. Suppose also that the "true" conditional constants are those given in the first row of Table II. By using eqn. (8) it is then possible to generate a curve, *f*, expressing the "true" constants. It represents an "experimental" titration curve with no errors in concentrations or pH measurements. By using eqn. (23), corrections for the "wrong" pK values in Table I can be obtained. The corrected pK values are also shown in Table II, and it is seen that the procedure results in values which come very close to the assumed "true" values. If there are large corrections an iterative procedure is necessary. It might then be necessary to calculate another set of concentrations for a new solution 1 based on the corrected constants in order to obtain a sufficiently linear relationship.

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SUMMARY

Equations are derived for the calculation of acid mixtures, which upon titration with base show a linear relation between pH and the amount of base. Three to five weak acids were used and a linearity of better than ± 0.02 pH units was obtained. The use of such mixtures for analysis of the base content of samples by means of a single pH measurement is described. A procedure for obtaining conditional pK_a values of the components of the acid mixture is also described. The single-point titration method is advocated for use when better accuracy than that of direct potentiometry is desired but less than that of an ordinary titration can be accepted. It is not necessary to know the pK_b or the number of weak bases.

RÉSUMÉ

Des équations sont établies pour le calcul de mélanges d'acides, la relation pH/quantité de base lors du titrage etant linéaire. Des mélanges de trois à cinq acides faibles ont été utilisés; une linéarité de ± 0.02 unité de pH (ou mieux encore) a été obtenue. La détermination de la teneur en base peut ainsi être faite par simple mesure de pH. Une méthode est également décrite permettant d'obtenir des valeurs pK_a conditionnelles des constituants d'un mélange. Cette méthode est plus exacte que la potentiométrie directe; il n'est pas nécessaire de connaître le pK_b ou la quantité de bases faibles.

ZUSAMMENFASSUNG

Es werden Gleichungen für die Berechnung von Säure-Gemischen abgeleitet, die nach Titration mit Base eine lineare Beziehung zwischen dem pH-Wert und der Menge der Base aufweisen. Drei bis fünf schwache Säuren wurden verwendet, und es wurde eine Linearität von besser als ± 0.02 pH-Einheiten erzielt. Die Anwendung solcher Gemische auf die Bestimmung des Basengehaltes von Proben mittels einer einzigen pH-Messung wird beschrieben. Ausserdem wird ein Verfahren zur Ermittlung der konditionellen pK_a-Werte der Komponenten des Säure-Gemisches beschrieben. Die Methode der Einpunkt-Titration wird empfohlen, wenn eine bessere Genauigkeit als die bei der direkten Potentiometrie gefordert wird, wenn aber eine geringere Genauigkeit als die bei einer gewöhnlichen Titration hingenommen werden kann. Es ist nicht notwendig, die pK_b-Werte oder die Anzahl der schwachen Basen zu kennen.

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COULOMETRIC DETERMINATION OF SERUM IRON*

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Serum iron has been determined primarily by spectrophotometric and atomic absorption methods. Coulometric determination has not been possible because the complex serum matrix contains too many substances which could interfere with a titration. In addition, none of the existing titrations for iron are compatible with the usual methods of iron separation. A new coulometric determination for iron[1] which involves titration of iron bound to 1,10-phenanthroline, facilitates coordination of the separation and titration steps. Iron–1,10-phenanthroline, known as ferroin, can be quantitatively formed in the serum matrix. Since the complex is very stable and resistant to oxidation, it proved to be a good choice for the separation of iron from serum for titration.

In the present paper, coulometric titrations are applied to the determination of serum iron following separation as the ferroin complex. This investigation is part of a continuing study on the application of coulometric titrations as reference methods in clinical chemistry[2,3].

EXPERIMENTAL

Apparatus

The titration cell was equipped with two side arms which were isolated from the body of the cell by fritted-glass discs. The central compartment was made from a weighing bottle of *ca.* 12-ml volume. All compartments were filled with generating solution. A platinum wire was used in the central compartment for the generating anode. The auxiliary generating electrode was a platinum foil electrode which was placed in a side-arm. A constant current of 9.65 μA was provided by a ChrisFeld Microcoulometric Quantalizer, Model B.

A Sargent Model XV Polarograph with Microrange Extender was used to maintain a constant voltage of 1.025 V across the indicating electrodes and to monitor the current passing between them. A saturated calomel electrode in the remaining side-arm and a platinum electrode in the central compartment were used for the indicating system.

Reagents

The generating solution was prepared by saturating a 3 *M* sulfuric acid solution with cerium(III) sulfate.

* Taken in part from the Ph.D. Dissertation of Sharon W. McClean, University of Maryland, 1972.

The acetate buffer of pH 4.6 was prepared by combining 52 ml of 0.2 *M* acetic acid and 48 ml of 0.2 *M* sodium acetate.

An iron solution of 0.02301 g l^{-1} was made by dissolving 0.02301 g of electrolytic grade iron powder in 2 ml of concentrated hydrochloric acid, and then diluting to 1 l with deionized water.

Procedure

Place 0.5 ml of serum in a 10-ml cylindrical centrifuge tube with tapered bottom. Add 1 ml of 20% trichloroacetic acid and 1 ml of deionized water by means of an Automatic Dilutor (Lab Industries, Berkeley, California). Cover the tube with Parafilm and heat for 10–15 min in a water bath maintained at 90–95°. After cooling, place a second Parafilm cap over the first, and centrifuge the tube for a few seconds to dislodge droplets of condensed water. Add 1 ml of chloroform and replace the Parafilm cap with a ground-glass stopper. Then shake the tube vigorously for 30–60 s. Allow built-up pressure to escape by loosening the lid. Place a Parafilm cap over the ground-glass stopper and extend down the tube for 1 in. Centrifuge at 1470 g for 10 min. After centrifugation, decant the supernatant liquid into a Buchner funnel with a sintered-glass disc of coarse porosity. Rinse the tube three times with 2 ml of deionized water delivered from a 10-ml pipet. To avoid dislodging the protein plug, allow the rinse water to flow down the sides of the tube. Use the remainder of the 10 ml to rinse the sides of the Buchner funnel, draining into a separatory funnel.

To the separatory funnel, add 2 ml of 20% ammonium acetate, 2 ml of 0.25% hydroxylammonium chloride, 1 ml of 0.003 *M* 1,10-phenanthroline, and 2 ml of 1 *M* sodium perchlorate, using 0–10 ml Measureomatic Dispensers (Sargent-Welch Co., Chicago, Illinois). Extract the contents with three 1-ml portions of chloroform. To the combined chloroform extracts in a 25-ml Erlenmeyer flask, add 1 ml of acetate buffer, and heat the flask in a 90–95° water bath for about 2 min. After evaporation of chloroform, place the contents of the flask in the titration cell, and rinse the flask with 1 ml of 4 *M* sulfuric acid followed by 2 ml of generating solution. Insert the electrodes and titrate at a generating current of 9.65 μA with 1.025 V impressed across the amperometric indicating electrodes.

To calculate the weight of iron in a given sample, Faraday's Law is applied. The constant generation current is multiplied by the electrolysis time, *t*. This product is divided by Faraday's constant to yield the number of equivalents of iron titrated. When this number is multiplied by the equivalent weight of iron, the result is the amount of iron present. Thus, for a current of 9.65 μA, the weight of iron (g) $= (5.58 \cdot 10^{-9})t$.

RESULTS AND DISCUSSION

Before serum was used, a suitable extraction of ferroin from aqueous solution had to be developed. Various quantitative solvent extraction procedures for ferroin–X_2 were considered; X is an anion called a "promoter" which makes possible or promotes the extraction by making the ferroin complex electrically neutral. Several promoters[4, 5] and the solvents[4, 6] used with them were considered and rejected either because of their interference with the coulometric titration or

the difficulty of back-extracting the complex from the organic solvent.

Since perchloric acid is frequently employed in lieu of sulfuric acid for cerium titrations, it could be assumed that perchlorate ion as the promoter would not interfere with the titration. Chloroform required several extractions to remove ferroin perchlorate quantitatively from water, and upon standing for long periods, ferroin perchlorate precipitates from chloroform[6]. Despite these disadvantages, chloroform seemed the best solvent, since it would not affect the coulometric titration, did not form long-lasting emulsions with water, and had a relatively low boiling point (60°).

Hydroxylamine has been used to reduce iron in serum [7,8]; it will not reduce copper, a possible interference in serum[6] at pH values lower than 5. Ferroin is stable over the pH range 2–9. It was found that increasing the acidity of the aqueous solution decreases the extraction of ferroin perchlorate into chloroform. To decrease copper interference, to improve the extraction of ferroin perchlorate by chloroform, and to take advantage of the middle portion of the ferroin stability range, a pH of 4.0–4.5 was maintained for the extraction. This choice of pH is in keeping with the pH usually chosen for ferroin solvent extraction, regardless of the solvent or promoter employed. Acetate buffer is most commonly used for ferroin formation[9] and was the choice here.

Others have shown for iodide[4] and for dodecylsulfate[5] that the anion:ferroin ratio should be at least 1000:1 for quantitative extraction. That ratio was used in this work. The phenanthroline:iron ratio was always greater than 6:1, to assure an adequate supply against possible interference. The phenanthroline:iron ratio is not critical to complex formation since this rate is reported to be extremely rapid[10].

Preliminary work indicated that ferroin perchlorate returned unoxidized from chloroform to an acetate buffer of pH 4–5, if the chloroform was evaporated by heating. Before the back-extract produced by evaporating the chloroform could be titrated quantitatively, it was necessary to determine the effect on titration times of adding acetate buffer to the titration cell. The effect was tested by performing a pretitration, and then adding various amounts of acetate buffer along with 5 μg of iron bound to 1,10-phenanthroline. This sample was titrated, then another 5 μg of iron was added and titrated. From these tests it appeared that the two major factors influencing titration time are the changes in hydrogen-ion concentration and in solution volume. The former causes a decrease in titration time, the latter an increase.

Although the two effects might to a large extent be cancelled by the use of a blank, it seemed wise to make an acetate buffer solution of ferroin acidic enough before its introduction into the titration cell, to produce a normal time without reliance on a blank. Therefore, two different procedures for handling the sample were used. The alternative procedure involved adding 1 ml of 4 *M* sulfuric acid to the 1 ml of acetate buffer containing ferroin extracted from chloroform; 1 ml of this 2-ml mixture was introduced into the titration cell.

The recommended procedure consists of emptying the acetate buffer into the cell, then rinsing the buffer container first with 1 ml of 4 *M* sulfuric acid, and finally with 2 ml of generating solution. All of the original sample is titrated in this procedure.

The first procedure titrates only one-half of the original sample, and relies on the assumption that the volume of the acetate buffer is not diminished by evaporation of the chloroform. The second procedure titrates the entire sample but suffers from several defects. The increase in solution volume is 50%, four times that of the first procedure. This increase in solution volume and reagents used raises the blank titration time about 25 s to 150 s for a 10-μA generating current. If one were titrating amounts of iron larger than 5 μg, a larger cell could be employed and the problem of volume change lessened, if not eliminated.

TABLE I

RECOVERY OF IRON FROM WATER

Fe taken (μg)	*Fe found (μg)*	*Recovery (%)*	*Procedure*	*s[a] (μg)*
A. With acetate buffer				
1.003	0.961	96.3	Alternative	0.067
1.150	1.135	98.7	Alternative	0.020
B. With protein-free filtration reagents				
1.180	1.135	96.2	Alternative	0.090
1.180	1.192	100.8	Recommended	0.075

[a] Standard deviations were calculated on the basis of 3–7 trials. In most instances, more than 5 trials were used.

The recoveries for iron added to deionized water by the alternative procedure are shown in Table I (Part A). The recommended procedure was not employed until some reagent changes had been made to accommodate the special requirements of serum. Iron was added either as ferroin or iron(III) standard. Hydroxylamine and 1,10-phenanthroline were added with both standards. Blank samples contained all the reagents, but not iron.

Since copper is present in serum at about the same amount as iron, and can also form phenanthroline complexes, it was necessary to check for possible copper interference. Competition from an easily dissociated complex such as copper(II) acetate in deionized water could be expected to be much greater than from protein-bound copper in serum. Samples containing 1-μg amounts of iron and copper were prepared in deionized water, and these samples were carried through the entire procedure. The recovery of iron indicated no significant copper interference.

Henry[11] has reported that protein precipitation can be accomplished by vigorously shaking serum with cold chloroform for about 15 min. The top layer is the protein-free filtrate, the middle layer is the chloroform–protein gel, and the bottom layer contains excess of chloroform. Since chloroform was to be used for extracting iron from serum, it was necessary to choose a protein-precipitating procedure which used chloroform, to avoid any further precipitation during the extraction step.

The methods of Caraway[12], Ramsay[13], and Trinder[14] for disruption of the iron–protein complex and precipitation of protein were investigated. Caraway's

method uses chloroform and trichloroacetic acid at room temperature to precipitate proteins. In Ramsay's method, the pH is lowered to 5 and the serum is heated for 5 min in boiling water; after cooling, chloroform is added and the mixture shaken before centrifugation. Both methods have the advantage of being reasonably quick and simple, but they were found unsuitable for this work. When the supernatant liquid from either one was shaken with chloroform, an emulsion formed which did not break even on long standing.

The protein-precipitation procedure used in Trinder's method for serum iron involves heating serum with 20% trichloracetic acid at 90–95° for 15 min. The serum is centrifuged after cooling and supernatant liquid decanted. No chloroform is used. When this supernatant liquid was shaken with chloroform, an emulsion formed making solvent extraction unworkable. It was decided to try shaking the serum with chloroform after it had cooled and before centrifugation. Centrifugation then produced three layers: a chloroform layer, a protein plug, and a protein-free supernatant liquid. When this supernatant liquid was shaken with chloroform, only a very slight emulsion was seen; it usually broke within a minute or two. With this modification, Trinder's method for protein precipitation was much better than the other two methods tested.

Ammonium acetate is usually employed in serum iron determinations to adjust the pH of acidic protein-free filtrates before color development. This compound was used in this study. Table I (Part B) shows recoveries of iron from water samples employing all the reagents which would be involved in the serum determination, including trichloroacetic acid and ammonium acetate.

The control sera used for this work were from Versatol (Warner-Chilcott, Morris Plains, New Jersey) lot number 2455121. The amount of iron present in the control sera was determined by a modification[15] of the method of Young and Hicks[16]. Recovery data for the coulometric titrations are based on this value. Recovery studies were also made on control sera to which extra iron was added. The addition, in the form of an iron(III) solution, was made to the serum sample before protein precipitation. The amount of iron added was within the normal iron-binding capacity of serum. The results of these recovery studies are shown in Table II.

TABLE II

RECOVERY OF IRON FROM CONTROL SERUM

Serum volume (ml)	*Fe content (μg)*	*Fe found (μg)*	*Recovery (%)*	*s*[a] *(μg)*	*Procedure*
A. Based on Babson and Kleinman method (ref. 15)					
0.5	0.59	0.60	102	0.10	Alternative
0.5	0.59	0.57	97	0.05	Recommended
1.0	1.18	1.30	110	0.04	Recommended
B. Based on iron added to control serum					
0.5	1.15	1.12	97	0.03	Alternative
0.5	0.47	0.48	103	0.04	Recommended

[a] Standard deviations were calculated on the basis of 3–7 trials. In most instances, more than 5 trials were used.

SUMMARY

The iron in 500 μl of serum is determined by a coulometric titration developed for ferroin. The titration step is preceded by chloroform extraction from a protein-free filtrate of serum iron as ferroin perchlorate. Evaporation of chloroform in the presence of an acetate buffer of pH 4.6 causes the ferroin perchlorate to back-extract into the aqueous layer. The ferroin is introduced into a titration cell and titrated with electrogenerated cerium(IV), a modified amperometric end-point detection system being used.

RÉSUMÉ

Le dosage de fer dans le sérum (500 μl) est déterminé par un titration coulométrique developpé pour détermination de ferroin. Le titrage est précedé par extraction du perchlorate de ferroin dans le chloroforme. Le solvant est évaporé à pH 4,6, ainsi le perchlorate rentre dans la phase aqueuse. On fait usage de Ce(IV) electrogéneré comme titrant.

ZUSAMMENFASSUNG

Eisen in 500 μl Serum wird durch eine für Ferroin entwickelte coulometrische Titration bestimmt. Vor der Titration wird das Eisen aus einem proteinfreien Serumfiltrat mit Chloroform als Ferroinperchlorat extrahiert. Durch Abdampfen des Chloroforms in Gegenwart eines Acetatpuffers von pH 4.6 wird das Ferroinperchlorat in die wässrige Schicht zurückextrahiert. Nach Überführung in eine Titrationszelle wird das Ferroin mit elektrochemisch erzeugten Cer(IV) titriert, wobei ein modifiziertes amperometrisches System für die Bestimmung des Endpunktes verwendet wird.

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A SPECIFIC BIO-ELECTROCHEMICAL SENSOR FOR HYDROGEN PEROXIDE

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Hydrogen peroxide is used in various industrial fields such as the food, textile and dye industries, because of its great ability to oxidize, bleach and sterilize. Hydrogen peroxide has been determined by volumetric or colorimetric methods, although these methods tend to be complex and time-consuming. Direct measuring methods, such as electrochemical sensors, for hydrogen peroxide would be very useful.

Enzymes are known to show excellent selectivity for their substrates. A combination of enzyme reactions and electrochemical reactions would be expected to give a highly selective electrochemical sensor. The enzyme electrodes for glucose[1, 2] and amino acids[3, 4, 5] operate on this principle; these electrodes have been prepared from polyacrylamide membranes in which the enzyme is entrapped; the enzyme has, however, difficulty in maintaining its native enzyme activity. The authors recently proposed a new electrochemical method for preparing a collagen membrane containing enzymes[6]. The enzyme–collagen membrane is *ca.* 1 μm thick, and shows an enzyme activity which is comparable to the native enzyme activity.

A catalase–collagen membrane prepared electrochemically catalyzes the reaction:

$$H_2O_2 \xrightarrow{\text{Catalase}} H_2O + \tfrac{1}{2}O_2$$

Thus hydrogen peroxide can be determined from the amount of oxygen released when the catalase–collagen membrane is placed in a hydrogen peroxide solution. Oxygen is detected by amperometric or galvanostatic methods, with an oxygen-permeable membrane such as teflon or polyethylene. In this paper, a new type of sensor specific to hydrogen peroxide is described.

EXPERIMENTAL

Materials

Catalase [$(H_2O_2)_2$, Oxidoreductase (E.C. 1-11-1-6)], obtained commercially, was dialyzed against distilled water. Hydrogen peroxide solutions were prepared from commercial 35% hydrogen peroxide by dilution with twice-distilled water.

Preparation of the catalase–collagen membrane

As previously reported[6], collagen fibrin was prepared from raw calf skin, which was minced, freed of soluble proteins with a 10% sodium chloride solution, dialyzed exhaustively against water and homogenized to a paste with 1 *M* hydro-

chloric acid. This paste was adjusted to the required concentration and pH (3.8) in order to prepare the collagen fibrin solution in water. The collagen fibrin content was determined by weighing the sample after drying it at 105° for 12 h.

The solution for electrochemical preparation of the catalase–collagen membrane contained 200 ml of 0.45% collagen fibrin solution at pH 3.8 and 20 ml of aqueous 0.45% catalase solution (collagen: catalase, 10:1). In the electrochemical preparation, a constant current of 3.2 mA was applied to this solution for 1 h, in a cell consisting of a pair of platinum anodes and a platinum cathode at 5°. The catalase–collagen membrane was formed around the cathode. This membrane was washed with water, and dried under vacuum at 5°.

Enzyme activity measurement

The enzyme activity of catalase was determined as described by Chance and Machly[7].

Assembly of the hydrogen peroxide sensor

The scheme of the hydrogen peroxide sensor is illustrated in Fig. 1. The sensor consists of a double membrane of which one layer is catalase–collagen and the other is an oxygen-permeable teflon membrane, an alkaline electrolyte, a platinum cathode and a lead anode. The double membrane is in direct contact with the platinum cathode and is tightly secured to the cell with rubber rings.

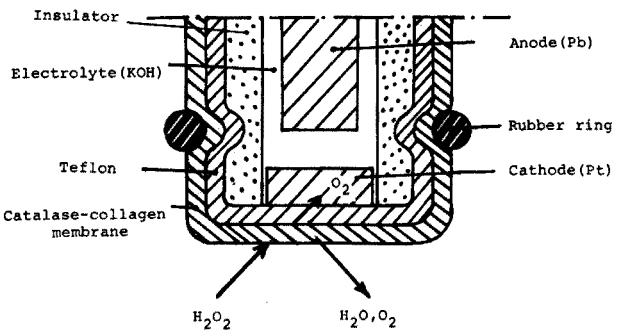


Fig. 1. Scheme of the bio-electrochemical sensor for hydrogen peroxide.

Recording of the sensor output

The block diagram system for recording the output of the sensor is illustrated in Fig. 2. The control solution was free from hydrogen peroxide and was saturated with dissolved oxygen. Both the control and sample solutions were stirred magnetically while measurements were taken.

RESULTS

Properties of the catalase–collagen membrane

The catalase–collagen membranes prepared electrochemically were 1–2 μm thick in a dried state, and were swollen in the wet state. The relative enzyme activity of the membrane was above 95%, where 100% is the enzyme activity of the catalase in solution under comparable conditions.

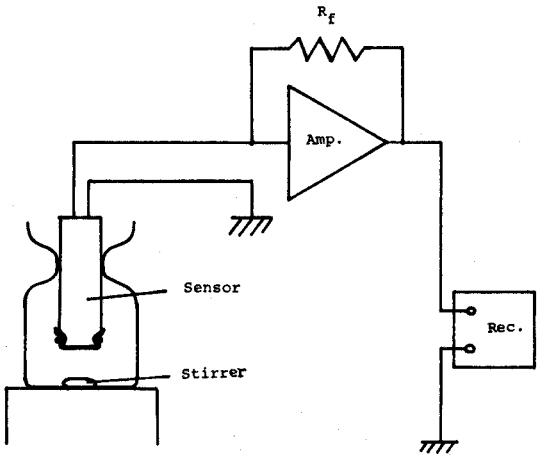
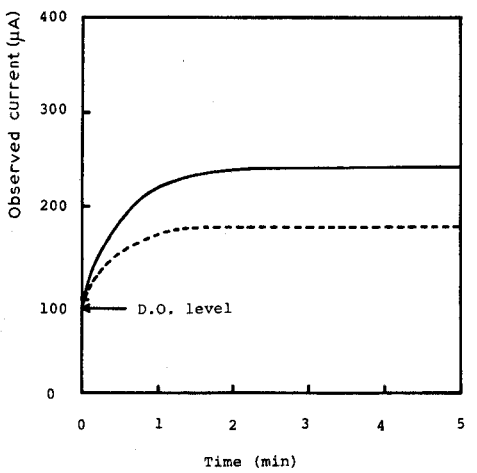


Fig. 2. Block diagram for recording the output of the sensor.

Response properties of the hydrogen peroxide sensor

In Fig. 3, the response time of the hydrogen peroxide sensor is shown for 0.5 and 1.0 mmol of hydrogen peroxide per litre at 20°. The sample solution was first saturated with oxygen gas. Saturated dissolved oxygen was responsible for the current at time 0 in the sample solution. Hydrogen peroxide was decomposed to oxygen and water by catalase entrapped in the membrane, when the sensor was inserted in the sample solution. Generation of oxygen by the decomposition of hydrogen peroxide caused oversaturation of dissolved oxygen around the membrane, which increased the output of the sensor. The output increased markedly with time, until a steady state was reached. Although the response time depended on



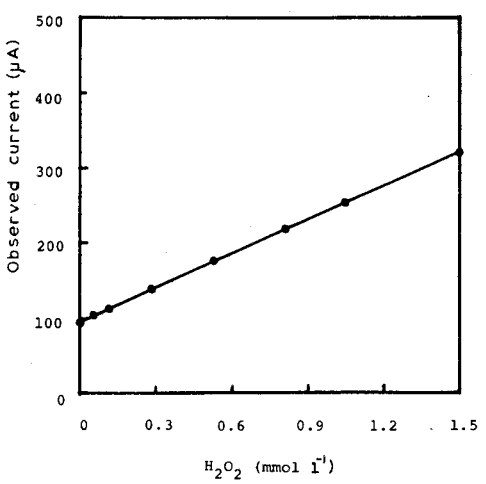


Fig. 3. Response time of the sensor for 0.5 mmol l^{-1} (---) and 1.0 mmol l^{-1} (——) of hydrogen peroxide.

Fig. 4. Calibration curve of the sensor over the concentration range 0–1.5 mmol l^{-1} at pH 6.2.

the thickness of the membrane, enzyme concentration and temperature, the steady state value was attained within 1.5 min for 0.5 mmol l^{-1} and 2 min for 1.0 mmol l^{-1} for hydrogen peroxide at 20°.

Dependence on concentration of hydrogen peroxide

The current output related to the hydrogen peroxide concentration at pH 6.2 is shown in Fig. 4 in the concentration range 0–1.5 mmol l^{-1}. The current output is defined as the steady current at 3 min after the insertion of the sensor. The oxygen generated by the hydrogen peroxide decomposition is responsible for the current, $I-I_0$, where I is the observed current for the sample solution and I_0 is the current for the saturated dissolved oxygen. When the dissolved oxygen in the sample solution was displaced with nitrogen gas, the current output could be attributed solely to the generated oxygen in the membrane, and the response curve in Fig. 4 passed through the origin. In the hydrogen peroxide concentration range above 1.5 mmol l^{-1}, the current–concentration plot curved towards the concentration axis.

The mean error from the calibration curve was determined for the standard hydrogen peroxide solutions at pH 6.2 (Table I).

TABLE 1

MEAN ERROR FROM THE CALIBRATION CURVE AT pH 6.2

H_2O_2 added (mmol l^{-1})	*H_2O_2 determined (mmol l^{-1})*	*Error (%)*	*Mean error (%)*
0.20	0.20	0	
0.30	0.30	0	
0.50	0.47	−6	
1.00	0.98	−2	
1.20	1.15	−4	
1.50	1.40	−7	−4

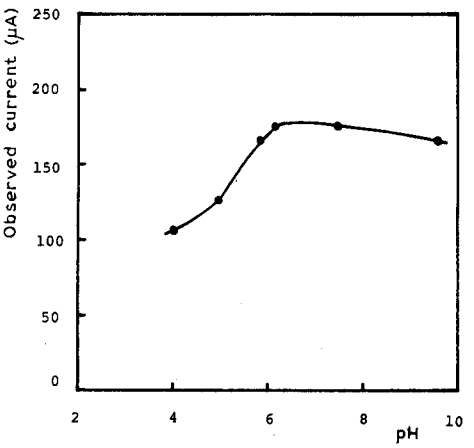


Fig. 5. Influence of pH on the output of the sensor for a hydrogen peroxide concentration of 0.5 mmol l^{-1}.

Influence of pH on the sensor output

As the enzymatic activity of catalase is markedly dependent on pH, the output of the hydrogen peroxide sensor is affected by the pH of the sample solution. The current–pH curve is shown in Fig. 5 for a 0.5 mmol l^{-1} solution of hydrogen peroxide at 20°. Buffer solutions in which the ionic strength was fixed at 0.2, were used to adjust the pH of the sample. The sample solution was saturated with dissolved oxygen before the measurement. The current output corresponds to the steady-state current 3 min after the sensor was inserted into the sample solution. The current reaches a maximum around pH 6. The current–pH curve resembles the activity–pH curve of the native catalase. Therefore the effect of pH on the current seems to be caused by the pH-dependence of the enzyme activity of catalase.

Continuous use of the sensor

In solution, the decomposition of hydrogen peroxide which is catalyzed by catalase causes the oversaturation of dissolved oxygen in the membrane of the sensor. When the sensor is placed into a solution free of hydrogen peroxide, the output of the sensor gradually decreases, but does not go to zero as a result of the oversaturation of dissolved oxygen. The recovery of the output is shown in Fig. 6 for a hydrogen peroxide solution containing 1.5 mmol l^{-1}. For Fig. 6, the sensor was placed in a hydrogen peroxide-free solution saturated by dissolved oxygen from time 0–1 min. The sensor was then immersed in a hydrogen peroxide solution (1.5 mmol l^{-1}) saturated by dissolved oxygen for up to 6 min. The sensor was then replaced in the hydrogen peroxide-free solution saturated by dissolved oxygen. It took about 2 min to register the initial output of the sensor, which is comparable to the response time of the sensor. In a more dilute hydrogen peroxide solution, the recovery time was shortened.

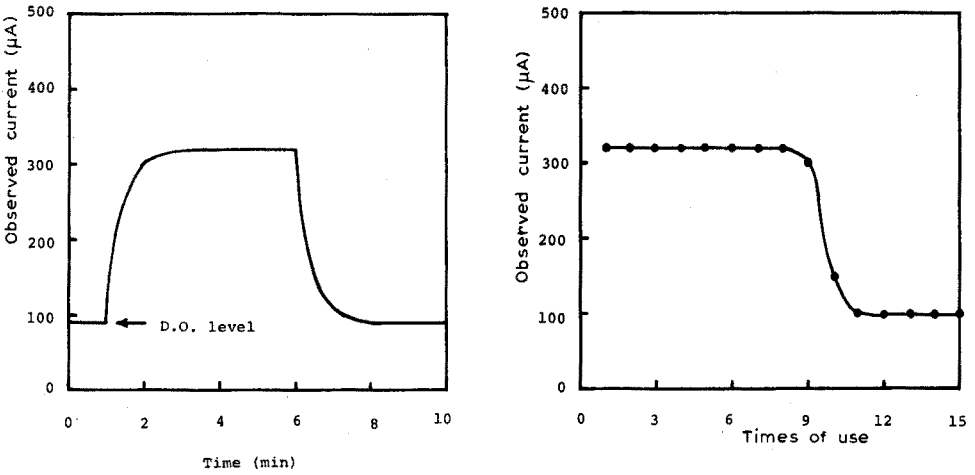


Fig. 6. Recovery time of the sensor for a 1.5 mmol l^{-1} solution of hydrogen peroxide.

Fig. 7. Output of the sensor during repeated use.

Repeated use of the sensor was tested as follows. The sensor was immersed in a hydrogen peroxide solution (1.5 mmol l^{-1}) saturated by dissolved oxygen for 4 min, and was then replaced in a hydrogen peroxide-free solution saturated by dissolved oxygen for 4 min. The above operation was repeated to determine the degradation of the sensor during repeated usage. The current during repeated use is shown in Fig. 7. Little decrease in the output current was observed until the sensor had been used for the tenth time.

DISCUSSION

The sensor consisting of a double membrane of the catalase–collagen and teflon, alkaline electrolyte and a pair of electrodes was found to function very well as a hydrogen peroxide sensor. Such a sensor may be called a "bio-electrochemical sensor" or an "enzyme membrane electrode", because the detection mechanism is a typical combination of biochemical and electrochemical reactions.

Though the precise detection mechanism of the hydrogen peroxide sensor is not yet clear, the mechanism may be explained as follows. Hydrogen peroxide near the catalase–collagen membrane of the sensor diffuses into the membrane or is adsorbed on its surface, and is decomposed to water and oxygen by the entrapped catalase. The generated oxygen diffuses to both the oxygen-permeable teflon membrane and the hydrogen peroxide solution as illustrated in Fig. 1. Oxygen which diffuses to the platinum cathode is reduced electrochemically, giving an output current. Some oxygen, of course, diffuses back into the solution. The current–concentration curve is quite linear in a hydrogen peroxide concentration range of 0–1.5 mmol l^{-1}. This indicates that the fraction ratio of oxygen diffusions into the teflon membrane and into the solution is constant in this concentration range. The ratio depends on the properties of the enzyme membrane and the teflon membrane. Therefore, the current output is determined by the enzyme activity as well as by the amount of the enzyme in the enzyme membrane, the thickness of the membranes and the diffusion coefficients of oxygen in both membranes.

An abrupt decrease in the current was found to occur after ten repeated uses. Such a decrease may be explained by a breakdown of the enzyme membrane.

The new bio-electrochemical sensor is highly selective for hydrogen peroxide and has many promising merits: (1) a convenient and direct measurement giving an electric signal, (2) a quick response, (3) applicability to coloured or opaque solutions, (4) repeated use of the analytical reagent (catalase), and (5) repeated use of the sample solution because only a trace amount of sample around the sensor is decomposed.

The authors would like to thank Prof. N. Tanaka for significant discussion and helpful suggestions.

SUMMARY

A bio-electrochemical sensor specific for hydrogen peroxide is described. The sensor consists of two membranes—a catalase–collagen membrane and a teflon

membrane—an alkaline solution, a platinum cathode and a lead anode. The catalase–collagen membrane is prepared electrochemically, the thickness being 1 μm; the enzyme activity is similar to that of native catalase. The sensor responds to hydrogen peroxide with a response time of only 1–2 min. The calibration curve is quite linear over a concentration range of 0–1.5 mmol l^{-1} for hydrogen peroxide. The utility of the sensor in continuous usage is discussed.

RÉSUMÉ

On décrit une électrode bio-électronique spécifique pour le peroxyde d'hydrogène. Elle est constituée de deux membranes—catalase–collagène et teflon—solution alcaline, avec cathode de platine et anode de plomb. La membrane catalase–collagène est préparée électrochimiquement, l'épaisseur étant de 1 μm, l'activité de l'enzyme est similaire à celle de la catalyse naissante. Le temps de réponse au peroxyde d'hydrogène n'est que de 1–2 min. La courbe de calibrage est tout à fait linéaire pour des concentrations allant de 0 à 1.5 mmol l^{-1} de peroxyde d'hydrogène. On examine les possibilités d'utilisation en continu.

ZUSAMMENFASSUNG

Es wird ein für Wasserstoffperoxid spezifischer bio-elektrochemischer Sensor beschrieben. Der Sensor besteht aus zwei Membranen—einer Katalase–Kollagen-Membran und einer Teflon-Membran—einer alkalischen Lösung, einer Platin-Kathode und einer Blei-Anode. Die Katalase–Kollagen-Membran wird elektrochemisch hergestellt; die Dicke beträgt 1 μm; die Enzymaktivität ist ähnlich der von natürlicher Katalase. Der Sensor spricht auf Wasserstoffperoxid innerhalb einer Zeit von nur 1–2 min an. Die Eichkurve für Wasserstoffperoxid ist im Konzentrationsbereich 0–1.5 mmol l^{-1} völlig geradlinig. Die Anwendbarkeit des Sensors bei kontinuierlichem Gebrauch wird diskutiert.

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THE DETERMINATION OF LOSSES IN THE FIRE ASSAY OF GOLD

PART I. CUPELLATION AND PARTING LOSSES

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The oldest known analytical procedure which is still in use today is the fire assay of gold which has been referred to since 1380 B.C.[1]. In spite of its antiquity, the interest in, and in some cases preference for, the fire assay method is based on solid reasons. Foremost amongst them is the question of analysis time; a complete fire assay can be accomplished within 3 h after receiving the sample. In contrast, wet methods requiring complete dissolution of an ore typically require 2–3 days. Multiple analyses are readily conducted by carrying through a dozen or more samples simultaneously.

The problem of obtaining a representative sample is minimized in the fire assay where 15–60 g samples are used. The fire assay can be used for samples containing from less than 1 μg to more than 1 g of gold and at the upper levels, far surpasses any instrumental technique for accuracy and precision.

The losses of gold in the fire assay procedure have been investigated by several authors at various times but studies have usually been restricted to limited areas for losses, or have neglected some of the significant variables.

A critical review of methods for the analysis of high-purity gold bullion[2] lists a variety of studies relating to gold losses in the assay process. According to these sources, losses increase with the temperature of cupellation[3], with the quantity of lead added[1], and with the concentration of base metals added[1]. Silver is cited as having a protective effect on gold losses, while varying the nature of the cupel also affects the extent of loss[4]. All of these earlier studies are deficient, however, in that they omit essential details and in that in no investigation have all the significant variables been controlled[1]. In cupellation temperature studies, for example, invariably the temperature reported is that of the muffle air, while the significant parameter is the temperature of the bead itself. While not readily obtainable, especially before the advent of the optical pyrometer, bead temperatures can vary considerably from the muffle air temperature, since the oxidation of lead occurs exothermally. Thus the bead temperature depends on the supply of oxygen as well as general air temperature. It is commonly held that the increase in gold losses on cupellation depends on lowering the viscosity of the gold. If so, this variable should depend on bead, and not air, temperature.

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While much of the earlier work is suspect for the reasons given above, several interesting studies of gold losses have been attempted more recently. In particular, a systematic study of gold losses was undertaken to determine "the magnitude of systematic errors in the standard fire assay for gold, under conditions prevailing in representative assay laboratories on the Witwatersrand"[5].

Coxon *et al.*[5] used an alloy button of lead and gold to simulate more closely the normal fire assay button. Most studies have not made this distinction and it has been assumed that unalloyed synthetic buttons were adequate for testing.

Neutron activation has been used to investigate the fire assay but this showed[6] that the losses were negligible, which was not substantiated by the assay values. Other radiochemical methods[7,8] have indicated that losses occur in the cupellation especially at the point of contact of the bead and the cupel, although experimental detail was lacking in these studies. At the micro- and nanogram levels, little work has been reported[9–11], and again these studies have been limited.

In view of the contradictory statements found in the literature, it was considered desirable to attempt to shed some light on the matter by studying gold losses under a variety of conditions with synthetic gold ores. In the process, it was hoped that the optimal conditions and procedures could be determined, and that a direct measure of the gold losses in the various stages of the complete fire assay process could be obtained. In such a manner a comparison of the losses determined directly, with the losses determined by differences from the extent of recovery could be made. The methods developed were then to be tested on an actual ore or ores.

EXPERIMENTAL

Apparatus

The equipment included a Lindberg Hevi-Duty 20 kW electric furnace, a Perkin-Elmer 306 Atomic Absorption Spectrophotometer, a Unicam SP 90 Atomic Absorption Spectrophotometer, a Unicam SP 500 Ultra-Violet-Visible Spectrophotometer, a Baird Atomic Sodium Iodide Scintillation Counter Model 530, an Epic Inc. Optix Universal Optical Pyrometer, and a Sartorius Type 1802 Microbalance. Magnesia Cupels (Leonard Light Industries, Benoni, South Africa) and crucibles (A. P. Green Refractories, Weston, Ontario, Canada) were used.

Reagents

The chemicals used were gold, unfluxed powder, and silver, precipitated powder (Johnson Matthey & Mallory); lead, 0.004-in. foil (Matheson Coleman & Bell); silica, about 240-mesh floated powder (Fisher); calcium oxide, sodium carbonate and litharge (Baker Analysed reagents, Canlab); and litharge (Anachemia reagent grade, Robson).

All acids used were Baker-analyzed reagent except hydrobromic acid which was the colorless fraction obtained by distillation of the reagent-grade acid.

Standard solutions

Gold. Approximately 2 g of the metal were dissolved in aqua regia.

Nitrous oxides were removed by repeated evaporation with hydrochloric acid in the presence of some sodium chloride. The solution was diluted to 1 l by addition of 0.1 *M* hydrochloric acid, and standardized by the hydroquinone method of Beamish *et al.*[12]. Dilute gold solutions were obtained by dilution with 0.1 *M* hydrochloric acid. For atomic absorption standards, the matrix was matched with the sample.

Silver. A stock solution containing 1000 p.p.m. of silver was prepared by accurately weighing out 1.0 g of the silver powder, and dissolving in sufficient nitric acid to make a 1-l volume *ca.* 0.1 *M* in nitric acid. Dilute silver solutions were obtained by dilution with 0.1 *M* nitric acid. For atomic absorption standards, the matrix was matched with the sample.

Lead. A stock solution containing 100.3 p.p.m. of lead was prepared by accurately weighing 100.3 mg of lead foil, dissolving in 20 ml of 5 *M* nitric acid by heating on a steam bath for several hours, and diluting to 1 l with 0.1 *M* nitric acid, to yield a stock solution 0.2 *M* in nitric acid containing 100.3 p.p.m. lead. Dilute lead solutions were made from the stock. For atomic absorption standards the matrix was matched with the sample.

Procedures

In an attempt to eliminate gold losses normally incurred in the fusion stage of the fire assay, a series of experiments was conducted on an abbreviated fire assay which has been described above as a "simulated cupellation". Thus, gold and silver powder were accurately weighed out onto a lead foil, carefully folded to encase the two in the lead, and then cupelled. The folded lead formed a cube of *ca.* 0.75-in. dimensions for 25 g of lead.

All cupellations were conducted in the same manner. The magnesia cupels were preheated in the furnace at the temperature used for cupellation. Unless noted otherwise, the temperature of cupellation was 1800°F (982°C). The lead buttons prepared as indicated above were added to the cupels as quickly as possible to prevent an excessive temperature drop. After an initial heating interval of 5 min with the draft and door closed, the lead was molten and both draft and door were opened. The door opening was controlled by placing a 0.25-in. steel plate under the door. The draft was always opened to the same extent by pulling a lever to a constant position. The resulting flow of air caused the muffle air temperature to drop *ca.* 30–50°F. Under these conditions the driving of the lead occurred at a rate of *ca.* 1 g min^{-1}. Thus a 20-g button would require a 20-min driving period. The temperature of the driving button, as measured by an optical pyrometer and taken after 5 min into the driving stage, corresponded very closely to the initial temperature of cupellation. After the driving stage was completed, the door (air supply) was closed and the cupellation finished by heating for a final 5 min. Closing the door caused the muffle air temperature to increase and ensured essentially complete removal of the lead from the silver–gold beads. The average lead residue of a series of such beads was found to be 1.4 μg.

After the cupellation was complete, the door was opened and the cupels were withdrawn slowly in two stages so as to prevent sprouting. After an initial cooling period of about 1 min, the cupels were moved to the furnace door where

the buttons were allowed to solidify. In the vast majority of beads observed, the characteristic "blick" of a lead-free bead was seen. The beads were then allowed to cool for 1 h, removed from the cupels and weighed. In the earlier experiments, the beads were cleaned of cupel material by scraping with a platinum spatula before weighing.

A blank determination for gold content of the silver powder and 25 g of lead foil indicated no detectable gold in 4 replicate samples, when tested by the modified bromoaurate spectrophotometric method[13].

RESULTS AND DISCUSSION

Effect of lead weight on cupellation losses of gold

In order to study the effect of lead weight on the cupellation loss, twelve pieces of lead foil (0.004 in.) were cut for each weight to be considered. The first series of lead buttons of 6, 12, 18 and 24 g was cupelled on 30-g magnesia cupels. The second series of 24, 32 and 40 g was cupelled on 40-g cupels. In each case, *ca.* 20–30 mg of gold was taken with about 3–4 times as much silver. With such a silver to gold ratio, a 1-h parting treatment at steam-bath temperatures with 10 ml of (1+2) nitric acid was required to obtain an adequate removal of silver. The parted bead was washed three times with hot water, annealed in a beaker over a Bunsen burner, and weighed. In almost all cases, the parted bead weighed more than the gold initially added and in addition, this difference, or surcharge, varied considerably from sample to sample. In view of the foregoing and of the report by Coxon *et al.*[5] that significant amounts of silver are retained by the parted beads, it was deemed necessary to analyse each parted bead for silver content.

To do this, the parted bead was dissolved in 3 ml of aqua regia with gentle heating. The beads reacted immediately and dissolved completely within the 1-h digestion period normally allowed. Sufficient concentrated hydrochloric acid was added to complex all of the silver, followed by dilution to an appropriate volume so that the silver concentration would be 2–10 p.p.m. In this region the atomic absorption calibration curve was linear. Standards were made up with the stock silver solution (above), and the matrix was matched with the samples.

The weight of silver found in the parted bead was then deducted from the weight of the parted bead to yield the weight of gold found or recovered. The difference between gold added and found, represented the gold loss. The results of both series of experiments (30 and 40-g cupels) are shown in Table I. It can be seen that the gold recovery varied from 99.37 to 99.39% for 30-g cupels, and from 99.37 to 99.44% for 40-g cupels. This represents a range of about 7 parts in 10,000 for all trials. Silver retained by the gold bead varied from 100 to 500 μg.

To test whether there were any significant differences between the trials, a test of significance was applied. A comparison of the results for both 30-g cupels, and 40-g cupels, indicated no significant differences at the 99% confidence level. A grouping of all experiments with 30-g cupels led to an average value which showed no significant difference from the average results with 40-g cupels at the 99% confidence level. Thus, all values of the gold loss could be

TABLE I

EFFECT OF LEAD WEIGHT ON GOLD LOSS

Lead weight (g)	*Au recovery (%)*	*Cupel size (g)*	*Au loss (%)*	*s*	*n*[a]
6.0	99.39	30	0.61	0.10	12
12.0	99.37	30	0.63	0.13	11
18.0	99.37	30	0.63	0.07	12
24.0	99.38	30	0.62	0.05	12
			Ave. loss 0.62	Ave. *s* 0.08	
24.0	99.41	40	0.59	0.08	12
32.0	99.37	40	0.63	0.08	11
40.0	99.44	40	0.56	0.10	11
			Ave. loss 0.59	Ave. *s* 0.09	
			Overall: Ave. loss 0.61		Ave. *s* 0.08

[a] Number of samples.

grouped together, giving an overall average gold loss of 0.61% with a standard deviation of 0.08%, *i.e.* a 99.39% gold recovery.

From the foregoing it would appear that losses of gold in the simulated cupellation do not vary significantly as a function of lead weight used over the range of 6–40 g, at the confidence level indicated.

Effect of silver–gold ratio on cupellation losses of gold

For convenience, the study began with a series of samples containing *ca.* 20 mg of gold and ten times as much silver. A second trial containing *ca.* 5 mg of gold and ten times as much silver was also made, to see whether the quantity of gold had any significant effect. Two more trials followed in which the silver to gold ratio was increased to 20:1 and 30:1, keeping the gold weight approximately constant at 5 mg. To enhance the tendency of the parted beads to remain coherent, thereby reducing mechanical losses, a more dilute parting acid was used. In all cases, 6 ml of (1+6) nitric acid was used with a 1-h digestion period of the beads at steam-bath temperatures. The residue was washed three times with hot water, annealed, and weighed on the microbalance. The silver content of the parted bead was determined as outlined previously.

In order to reduce the silver retained, the beads obtained from the cupellation process were flattened on a hydraulic press before parting. While a later study indicated that the silver content was reduced to some extent, the effect of using a more dilute parting acid more than offset the effect of a reduction in thickness. Thus the silver retained in this study averaged 1.46%, compared to the previous average of 1.07%. A later study showed that the temperature of the parting acid had an important bearing on this factor. The results are summarized in Table II.

A comparison of the first two trials with a constant silver–gold ratio (10:1) and varying gold weight indicates no significant difference at the 99% confidence level. A comparison of the remaining three trials at constant gold

TABLE II

EFFECT OF SILVER–GOLD RATIO ON GOLD LOSSES

Au added (mg)	*Ag:Au*	*n*[a]	*Thickness (in.)*	*s* *(in. × 10⁻³)*	*Surcharge (mg)*	*s*	*Ag retained (%)*	*s*	*Au loss (%)*	*s*
20.148	10.1:1.0	11	0.0347	1.3	0.256	0.036	1.80	0.14	0.57	0.16
5.134	10.1:1.0	11	0.0230	3.5	0.036	0.010	1.27	0.08	0.62	0.07
5.063	19.9:1.0	11	0.0290	1.3	0.043	0.008	1.59	0.14	0.68	0.13
5.514	30.1:1.0	12	0.0274	0.4	0.031	0.010	1.16	0.05	0.59	0.11

[a] Number of samples.

weight (5 mg) and varying silver–gold ratios indicates a minimal gold loss for a maximal silver–gold ratio, but a consistent pattern does not emerge and there are no significant differences at the usual confidence level of 99%. Thus, it seems safe to conclude that there is no significant effect on gold losses during cupellation and parting as a result of varying the silver–gold ratio from 10:1 to 30:1.

While no experiments were conducted at silver–gold ratios less than 10:1, the overall average gold loss of 0.61% for this complete study is identical with the gold losses for the previous study where the silver–gold ratio was about 3.5:1, although a much stronger (1+2) parting acid was used in the latter case.

Since silver–gold ratios lower than 2.5:1 are not recommended in the literature, there seemed no point in extending this study beyond the above. The average gold recovery ranged from 99.32–99.43%, a difference of only 1 part per thousand.

Effect of parting procedure on gold losses

In this study, all the samples were cupelled with $4 \times 6 \times 0.004$-in. lead foil. Since lead weight had been demonstrated to have no effect on gold losses, any variations in lead weight were of no consequence. Thus the weight chosen was an arbitrary choice somewhere within the range of lead weight normally taken for cupellation, *i.e.* 20 g. The weight of gold taken was 20–30 mg and between 6 and 7 times as much silver. In the first five procedures, the time of contact between the preheated nitric acid and the gold–silver prill was 35 min. In order to determine the effect of a second parting, trial VI involved a second parting with a more concentrated acid. An earlier attempt to reduce the silver content with a second parting had proven to be of no effect. This was in agreement with the finding of Chow[9]. However, in both earlier trials the beads had been annealed before the second parting; in procedure VI, the beads were annealed only after the second parting. The time of digestion chosen was based on the time required for action (evolution of nitrogen oxides) to cease in the most dilute acid. The time of contact for procedure VI was 15 min for each strength of acid. The parting acids were carefully decanted and the beads were washed three times with hot water, carefully annealed over a Bunsen flame while still in the 30-ml beakers used for parting, weighed and analyzed for silver content as usual. The other variable which was systematically changed was the thickness of the gold–silver bead. Beads were either not flattened at all (Procedures III and VI) or flattened to either 50 or 25 thousandth of an inch. The results are summarized in Table III, which to indicate the following generalizations:

(a) decreasing the thickness of the bead decreases the surcharge and percentage of silver retained (*cf.* I *vs.* II and III *vs.* IV);

(b) increasing the acid strength decreases the surcharge and the percentage of silver retained (*cf.* II *vs.* IV and IV *vs.* V);

(c) decreasing the thickness reduces gold losses (*cf.* I *vs.* II and III *vs.* IV);

(d) increasing acid strength (to a point) reduces gold losses (*cf.* II *vs.* IV and I *vs.* IV);

(e) increasing acid strength decreases the deviation in the silver retained (*cf.* I *vs.* II and III *vs.* VI)

(f) a second parting is effective in reducing the silver retained. (*cf.* II *vs.* VI).

TABLE III

SUMMARY OF PARTING PROCEDURES

Procedure	*Acid strength*	*Thickness (in.)*	*s*	*Surcharge (mg)*	*s*	*Ag Retained (%)*	*s*	*Au loss (%)*
I	1+6	0.0504	0.0009	0.625	0.133	3.47	0.53	0.65
II	1+6	0.0249	0.0006	0.537	0.160	3.22	0.83	0.60
III	1+4	unflattened	—	0.351	0.041	2.22	0.16	0.60
IV	1+4	0.0259	0.0008	0.297	0.056	1.82	0.07	0.50
V	1+3	0.0271	0.0014	0.200	0.047	1.81	0.19	0.93
VI	1+4 and 1+1	unflattened	—	0.061	0.018	1.03	0.08	0.74

An attempt was made at this point to determine some of the gold losses directly by atomic absorption methods. Under the conditions of analysis used, no gold was found in the parting solutions. The minimal detectable gold would have been 3 μg per sample. Analysis of surface scrapings from the cupels themselves indicated the presence of an average of 25 μg of gold. Since the total gold lost in each sample was of the order of 100 μg, considerable gold remained to be accounted for. A radiochemical procedure was planned to examine these losses later.

Volatilization

The object of this study was to determine whether gold volatilizes to a significant extent under cupellation conditions. In a preliminary experiment, the results of which are reported in Table IV, twelve beads of approximately equal size (80 mg) were made up by weighing out four samples of pure gold, four of pure silver and four of a (1+3) mixture of gold and silver. These were encased in $5 \times 6 \times 0.004$-in. lead foil and cupelled in the usual way. Since the lead foil was known to contain an average of 58 ± 4 μg of silver per g, the significant changes occurred after a cupelled bead was obtained. In 25 g of lead foil, the silver content could add as much as 1.5 mg to the bead weight if no losses occurred. This could explain, at least partially, why the gold bead obtained after cupellation was on the average 1.0 mg heavier than the gold added.

On heating the cupelled beads, some interesting changes occurred. The first heating interval was for 30 min, *i.e.* about the time required for cupellation. The gold beads systematically increased in weight by an average of 0.126 mg (s= 0.024 mg). The silver beads decreased in weight by an average of 0.162 mg (s= 0.080 mg), while the gold–silver (1:3) beads decreased in weight by 0.042 mg (s=0.018 mg). All heatings were made on the same cupel used for the cupellation of a particular bead. A second heating of the same beads for 1 h indicated an approximately proportional decrease in weight for the pure silver and the mixed beads. The gold beads, however, now began to decrease slightly (0.016 mg, s=0.024 mg) in weight. In view of the high relative uncertainty here—one of the gold beads actually increased in weight, and a mixed bead had to be rejected—the effect of heating on bead weight was examined more thoroughly.

ABLE IV

FECT OF HEATING GOLD, SILVER, AND GOLD–SILVER BEADS

Au added (mg)	*Ag added (mg)*	*Sum (mg)*	*Weight after*		*Change in weight after*	
			1st heating (mg)	*2nd heating (mg)*	*1st heating (mg)*	*2nd heating (mg)*
.578	—	81.607	81.740	81.701	+0.133	−0.039
.334	—	80.318	80.411	80.428	+0.093	+0.017
.996	—	82.017	82.143	82.139	+0.126	−0.004
.858	—	90.842	90.993	90.957	+0.151	−0.036
	Ave:	83.696	83.822	83.806	+0.126	−0.016
	s:				0.024	0.024
	82.660	81.834	81.776	81.377	−0.058	−0.399
	89.098	87.974	87.833	87.394	−0.141	−0.439
	80.288	79.416	79.177	78.721	−0.239	−0.456
	80.394	79.541	79.330	78.841	−0.211	−0.489
	Ave:	82.191	82.029	81.583	−0.162	−0.446
	s:				0.080	0.037
.765	60.207	80.766	80.698	80.684	−0.068	−0.014
.445	59.907	79.050	79.020	78.892	−0.030	−0.128
.768	60.716	81.984	81.945	81.854	−0.039	−0.091
.921	62.743	85.335	85.304	85.000	−0.031	−0.304[a]
	Ave:	81.784	81.742	81.608	−0.042	−0.078
	s:				0.018	0.058

[a] Rejected since bead formed a rootlet into the cupel on this heating.

The following tentative conclusions were made. If the relative rates of vaporization of the two metals are the same in the mixed bead as in the pure substances, then *ca.* 3% of the weight loss in the mixed bead is due to gold (*i.e.* 3 μg h^{-1}). Since the silver–gold ratio is 3:1, the loss of gold could be reasonably expected to be less. In addition, the actual cupellation process would involve only 30 min instead of 1 h, so that gold losses from volatilization would be less than 1 μg per bead.

In the second test of volatility, 12 samples of gold only were prepared and cupelled in the normal way. Again the weight of the beads increased by *ca.* 1.0 mg. Two 30-min heating periods of the cupelled beads followed. After the first such heating the weight of the gold beads increased by an average of 0.142 mg (s= 0.085). The second heating produced a slight decrease in weight of 0.011 mg on average.

In order to understand better the case of the increase in weight of the gold beads, 6 samples of high-purity gold wire and 6 samples of silver wire were cut and weighed. Each sample was placed on a clean unused cupel and heated under the same conditions as for cupellation. The weight of gold wire before and after heating remained constant within the experimental weighing error ($\pm$ 2 μg); the weight of silver wire decreased on average by 0.138 mg. It would therefore appear that the used cupel has some effect on the gold beads; possibly

some elemental lead diffused back into the gold bead, which seemed highly unlikely, but suggested a lead analysis of the gold beads. Each bead was dissolved in 6 ml of aqua regia and 10 ml of concentrated hydrochloric acid, and the solution was diluted to 100 ml. Lead standards were prepared with a matched matrix. The average lead content was 0.140 mg (s=0.048). The increase in weight of gold beads on first heating averaged 0.142 mg (s=0.085).

It would appear that the increase in weight on heating a pure gold bead is due to diffusion of lead from the cupel back into the bead. Eventually, a state of equilibrium is attained and the bead begins to lose small (0.01%) amounts of its weight on subsequent heatings. A search of the literature indicated that such an increase in weight has been observed before[14]. Hillebrand and Allen[14] left gold beads in the furnace for varying lengths of time to see whether gold losses would increase. They found that "no constant losses were observed... but curiously enough there seemed to be a slight tendency to increase in weight. To what this could be attributed is not clear." It is possible that the increase in weight of gold beads is due to another, unknown reason, and that the lead had simply not been removed from the bead in the first instance; thus the close agreement between the weight increase on heating and the weight of lead retained may be fortuitous. The matter was not considered important enough for extensive study.

On the basis of the volatility studies, it seems safe to say that gold losses via volatilization are not significant under the conditions studied.

Effect of cleaning beads

Up to this point, the beads obtained had been cleaned of all cupel material

TABLE V

EFFECT OF CLEANING UNPARTED BEADS ON GOLD LOSSES[a]

Au added (mg)	*Ag added (mg)*	*Parted bead (mg)*	*Surcharge (mg)*	*Ag retained (mg)*	*Au found (mg)*	*Au loss (mg)*
Scraped beads						
19.720	129.3	19.765	0.045	0.232	19.533	0.187
20.820	148.4	20.852	0.032	0.239	20.613	0.207
23.149	186.8	23.221	0.072	0.250	22.971	0.178
20.642	145.2	20.694	0.052	0.204	20.490	0.152
20.880	141.7	20.933	0.053	0.200	20.733	0.147
19.503	124.3	19.553	0.050	0.197	19.356	0.147
						Ave: 0.170
						s: 0.025
Unscraped beads						
21.753	139.0	21.831	0.078	0.218	21.613	0.140
21.785	141.7	21.861	0.076	0.216	21.645	0.140
23.447	148.0	23.514	0.067	0.214	23.300	0.147
18.799	116.2	18.846	0.047	0.199	18.647	0.152
20.113	138.8	20.174	0.061	0.199	19.975	0.138
23.886	158.8	23.984	0.098	0.244	23.740	0.146
						Ave: 0.144
						s: 0.005

[a] Beads were cleaned by scraping with a spatula.

by scraping with a platinum spatula. Since, however, all sources seemed to agree that the largest loss of gold in the cupellation process occurred at the point of contact between bead and cupel, it seemed highly likely that this cupel material adhering to the bead would be relatively rich in gold. It was previously established that the cupel material readily dissolved in the parting acids under the conditions (15 min with (1+4) nitric acid followed by 15 min with (1+1) nitric acid). Table V is a summary of two trials, one in which the samples were treated in the usual manner and the second in which the samples were not scraped clean.

From Table V it is evident that the gold loss is increased by 26 μg on average when the bead is scraped.

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SUMMARY

The losses in the cupellation and parting procedures of the fire assay for gold were evaluated with simulated buttons of lead foil. Variation in weight of lead, silver–gold ratio, and cupel size did not affect gold losses. Removal of cupel material from the bead increased the gold loss. Flattening the bead and increasing the parting acid strength reduced the gold losses somewhat. No evidence of losses by volatilization was observed.

RÉSUMÉ

Une étude est effectuée pour déterminer les causes de pertes en or, lors des essais par voie sèche de coupellation et lors des procédés de départ. Une variation du poids de plomb, du rapport argent/or, et de la dimension de la coupelle n'intervient pas. Le nettoyage de la perle augmente la perte en or. Par contre, le laminage de la perle et l'augmentation de la force de l'acide de départ réduisent quelque peu les pertes. Aucune évidence d'une perte par volatilisation n'a été observée.

ZUSAMMENFASSUNG

Die Verluste bei den Kupellations- und Scheideverfahren der dokimastischen Analyse auf Gold wurden mit vorgegebenen Reguli aus Bleifolie untersucht. Die Variation des Bleigewichts, des Silber–Gold-Verhältnisses und der Kupellengrösse beeinflussten die Goldverluste nicht. Die Entfernung des Kupellenmaterials von dem Korn erhöhte den Goldverlust. Das Ausplatten des Korns und die Erhöhung der Scheidesäurestärke verminderten etwas die Goldverluste. Verluste durch Verflüchtigung wurden nicht beobachtet.

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SHORT COMMUNICATION

Direct determination of cadmium in high-purity metals by flame atomic fluorescence spectrometry

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The determination of cadmium by flame atomic fluorescence spectrometry at sub-p.p.m. levels in thorium and uranium compounds after its separation from the matrix elements has already been reported[1]. This note describes the development of a method for the direct determination of cadmium at the 1-p.p.m. level in zinc, copper and indium.

Reagents and apparatus

Reagents and equipment were essentially the same as described earlier[1].

Influence of acetylene flow-rate on signal emitted at 228.8 nm

During preliminary experiments with a zinc sample of known cadmium content, the fluorescence signal at 228.8 nm was higher than expected when the zinc concentration was 5 mg ml^{-1}, while it was less at 20 mg ml^{-1} under the conditions of calibration given in the earlier communication. Further investigation showed that 1.0 *M* hydrochloric acid itself gave spurious signals (scatter), which varied with the flow-rate of acetylene. This was studied in some detail with four different electrodeless discharge tubes, prepared under practically identical conditions, each time setting the deflection for 0.1-p.p.m. cadmium solution to 100 divisions. The results are given in Table I. The scatter caused by hydrochloric acid (1.0 *M*) was negligible when a fuel-rich non-luminous flame was used. Hence, the conditions for the calibration curve were modified as follows: 0.1 p.p.m. cadmium was aspirated and the fluorescence signal was adjusted to 100 divisions by the sensitivity controls. The signal for deionized water was adjusted to 0 with the 'backing' knob. About 1.0 *M* hydrochloric acid solution was aspirated and the signal was adjusted to a minimal value by varying the acetylene flow-rate. Finally the signals of the cadmium standard and deionized water were adjusted to 100 and 0 divisions, respectively.

Effect of acidity

Cadmium solution (0.1 p.p.m.) in 0.01 *M* hydrochloric acid was aspirated into the flame under the above conditions for the calibration curve, except that the photometer reading was adjusted to 50 divisions. Cadmium solutions of the same

TABLE I

INFLUENCE OF ACETYLENE FLOW-RATE ON THE SCATTER SIGNAL OF 1.0 *M* HYDROCHLORIC ACID

(Air flow-rate, 15 p.s.i. on the dial (6 l min^{-1}). Slit, 300 μm. 100 divisions deflection for 0.1 p.p.m. Cd at 228.8 nm)

Acetylene flow-rate		*Scatter signal*			
Reading on rotameter	*l* min^{-1}	*EDT–1*	*EDT–2*	*EDT–3*	*EDT–4*
1.7	0.8	10	12	17	40
2.0	1.0	22	20	30	70
2.5	1.2	4	1	0	3
3.0	1.5	3	1	0	0
3.5	1.9	3	1	0	1

TABLE II

EFFECT OF ACIDITY ON THE FLUORESCENCE SIGNAL OF CADMIUM IN PREMIX BURNER

(Air, 15 p.s.i. Acetylene, 2.5 on the rotameter)

Acidity	*Fluorescence signal at various cadmium concentrations*			
	0.10 $\mu g\ ml^{-1}$	*1.0 $\mu g\ ml^{-1}$*	*10 $\mu g\ ml^{-1}$*	*100 $\mu g\ ml^{-1}$*
0.01 *M* HCl	50[a]	50[a]	50[a]	50[a]
0.10 *M* HCl	52	50	50	50
1.0 *M* HCl	52	48	51	51
3.0 *M* HCl	49	47	50	56
5.0 *M* HCl	47	43	49	62
8.0 *M* HCl	40	40	47	67
1.0 *M* HNO_3	52	50	51	48
5.0 *M* HNO_3	47	47	50	57
0.5 *M* H_2SO_4	51	51	51	53
2.5 *M* H_2SO_4	42	50	47	81

[a] Fluorescence signal adjusted to this value in each case.

concentration but at different acidities (also nitric and sulphuric acids) were aspirated and the fluorescence signals were measured. These experiments were repeated with cadmium solutions of 1.0, 10.0 and 100 p.p.m. The results given in Table II show that a maximal acidity of 1 *M* has no influence on the fluorescence signal of cadmium over a wide range of concentration.

Influence of other ions

Mixtures of cadmium (0.1 p.p.m.) with other cations at 1000 p.p.m. were aspirated under the experimental conditions mentioned above. No interference was observed from Al, Ca, Cr, Cu, Fe^{3+}, Hg^{2+}, In, K, Mg, Mn^{2+}, Na, Ni, TiO^{2+},

Tl^{3+}, Zn and ZrO^{2+}, the fluorescence signal remaining at 50 ± 2 divisions. Cobalt (above 100 p.p.m.) and aluminium (above 1000 p.p.m.) interfered, causing high results.

Determination of cadmium at the 1-p.p.m. level in OFHC copper, pure indium and zinc

Since so few cations interfered, the direct determination of cadmium at the 1-p.p.m. level was attempted in the case of copper, indium and zinc by aspirating solutions containing 10 mg of metal per ml. In the case of copper, 1.00 g of OFHC copper was dissolved in the minimal amount of concentrated nitric acid and the solution was made up to 50 ml. In the absence of a high-purity indium sample, cadmium-free indium solution (10 mg In ml^{-1}) was prepared by extraction into Amberlite LA-1 from hydrochloric acid medium[2]. In the case of zinc, an NBS standard sample (SRM 683) was dissolved in the minimum of hydrochloric acid and the solution was diluted to a suitable volume. When the standard addition technique was applied to zinc, the cadmium originally present was determined by the proposed method and then known amounts of cadmium were added. The results are given in Table III. When the concentration of zinc reached the 20-mg ml^{-1} level, the cadmium signal was slightly suppressed.

TABLE III

DIRECT DETERMINATION OF CADMIUM IN COPPER, INDIUM AND ZINC

Sample	*Concentration of solution (mg ml^{-1})*	*Cadmium added (μg ml^{-1})*	*Cadmium obtained (μg ml^{-1})*
Copper (OFHC)	10.0	0	0
Copper (OFHC)	10.0	0.01	0.01
Copper (OFHC)	10.0	0.02	0.02
Indium (purified)	10.0	0	0
Indium (purified)	10.0	0.02	0.02
Indium (purified)	10.0	0.04	0.039
Indium (purified)	10.0	0.06	0.057
Indium (purified)	10.0	0.08	0.078
Zinc (NBS SRM 683)	9.0	0	0.011[a]
Zinc (NBS SRM 683)	12.3	0	0.015[a]
Zinc (NBS SRM 683)	11.5	0	0.014[a]
Zinc (zone-refined)	10.0	0	0.008
Zinc (zone-refined)	10.0	0.020	0.028
Zinc (zone-refined)	10.0	0.040	0.048

[a] This works out to 1.2 p.p.m. against a certificate value of 1.1 p.p.m.

Discussion

Hydrochloric acid (1.0 *M*) gives significant scattering at 228.8 nm in a fuel-lean flame; this is stable and reproducible during measurement but not in different sets of experiments and with different discharge tubes. The effect is negligible in a fuel-rich non-luminous flame. Using a total combustion burner, Bratzel *et al.*[3] reported that increase of hydrochloric acid concentration suppressed the fluorescence signal of cadmium at lower concentrations of cadmium (< 10

p.p.m.) but enhanced it at 100 p.p.m. The present study (Table II) with a premix burner showed similar effects, but only above 1.0 *M* acid.

Dagnall *et al.*[4] showed that as many as 40 cations do not interfere in the determination of 1.0 p.p.m. cadmium, when they are present in 100-fold amounts. In order to exploit the high sensitivity of the atomic fluorescence of cadmium for its direct determination in high-purity metals, the behaviour of some cations at 1000 p.p.m. levels on the determination of 0.1 p.p.m. of cadmium was studied. Cobalt (above 100 p.p.m.) and aluminium (about 1000 p.p.m.) were found to interfere, while all other metal ions studied did not.

This study has shown that cadmium can be directly determined at the 1-p.p.m. level in those metals which do not interfere at 10 mg ml^{-1} levels.

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SHORT COMMUNICATION

Simultaneous multi-element atomic emission flame spectrometry with an image vidicon detector

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Simultaneous measurement of several elements by emission optical spectrometry has been achieved in the past primarily by means of the so-called direct readers which consist of a separate channel for each spectral line[1]. The source of excitation in such studies has been some form of a.c. spark. Atomic emission flame spectrometry, on the other hand, has generally been used for single-channel measurements and has been shown in many cases[2,3], to be more precise and accurate than spark emission spectrometry. Multi-element instrumentation for flame emission spectrometry has been described by several workers, some of the past systems having been reviewed by Vickers and Winefordner[2], Mavrodineanu and Boiteux[3], and Morrison[4], but such instrumentation is quite expensive, non-versatile, and of limited use except for a small range of sample types. Multiplexing methods, *i.e.* the simultaneous measurement of many spectral components with the same detector, have been utilized in the i.r. region but have received little use in the u.v.–visible region of the electromagnetic spectrum. Multiplexed or multichannel spectrometers[5–7] would seem to be suited to atomic flame spectrometric methods, especially for low-background atomizers (some flames and furnaces).

Margoches[8] was the first to describe the use of a TV camera in spark emission spectrometry, in which an echelle grating was coupled with a TV camera tube detector with associated electronics and computer processor. It would seem that the use of a similar detection system (and simpler optics) would be of great benefit for simultaneous multi-element detection in atomic flame spectrometry, especially atomic emission and fluorescence. Recently, a review article appeared on multi-element flame spectrometry[9], which emphasized the need for more research in this area.

Arrays of photo-diodes and photo-transistors as multichannel detectors in flame emission spectrometry have been studied by Boumans and Brouwer[10] for detecting small concentrations of barium in the presence of an excess of calcium. Analytical curves for potassium, rubidium, and calcium in flame atomic emission and atomic absorption have been given by Horlick and Codding[11], with the use of self-scanning linear silicon photo-diode arrays as detectors. However, the emission sensitivity with the oxyhydrogen flame was extremely poor.

In this communication, the analytical possibilities of a silicon diode array vidicon camera tube with enhanced u.v. response will be used for the detection of the atomic emission of several mixtures of elements with rather complex emission spectra, nebulized in the nitrous oxide–acetylene flame. The optical multi-channel analyzer and diode array vidicon used has been described in more detail by others[9,12–14]. The unit has several useful characteristics including broad spectral range (200–1100 nm), high quantum efficiency (>20% in the regions of 250 and 1000 nm, and >50% in the range 350–800 nm), linear response over a large range of fluxes ($\simeq 10^3$), low geometric distortion, and the possibility of storing and subsequently subtracting the background from the data spectrum.

TABLE I

DESCRIPTION OF THE EXPERIMENTAL SET-UP

Flame	Pre-mix laminar flow nebulizer burner (Model 303-0110, Perkin Elmer Corp., Norwalk, Conn.) with capillary burner[17] provided with an argon sheath for flame shielding and separation.
Monochromator	0.22-m, *f*/4 Czerny-Turner monochromator (Model 1650, Spex Industries, Inc., Metucher, N.J.) 100-μm entrance slit and a 300-cm focal length output mirror were used. The spectral range covered was about 25 nm.
Detector and associated electronics	U.v. enhanced silicon diode array vidicon camera tube, the output of which was processed by an optical multi-channel analyzer (Model 1200, SSR Instruments, Santa Monica, Calif.). The 500 channels of spectral information were displayed on an oscilloscope or recorded on an X–Y recorder.
Standard solutions	All solutions were prepared from reagent grade chemicals; 1000 p.p.m. of K as KCl was added as an ionization suppressant.

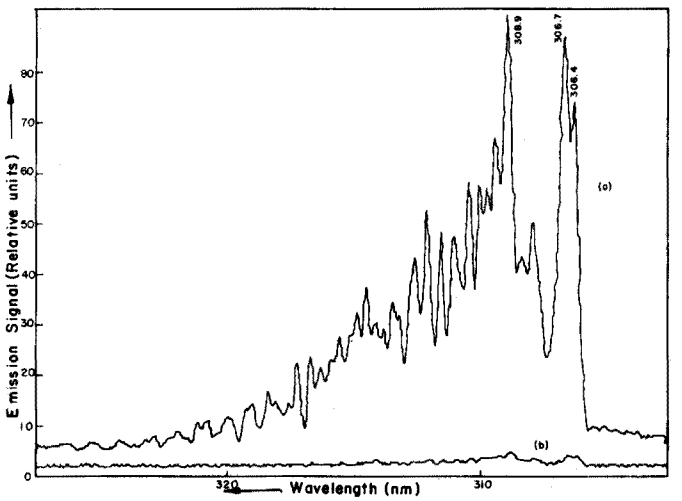

Fig. 1. Usefulness of flame separation in minimizing the OH emission in the nitrous oxide–acetylene flame. (a) Unseparated flame; (b) argon-separated flame.

The experimental set-up is given in Table I. The nitrous oxide–acetylene flame was used because of its great excitation capabilities for a very large number of elements[15,16] despite its high background in some regions of the spectrum. Flame separation is extremely useful in minimizing the strong OH emission (Fig. 1). The background subtracting capability can be of definite analytical usefulness in some specific cases (Fig. 2). Flame emission spectra for a number

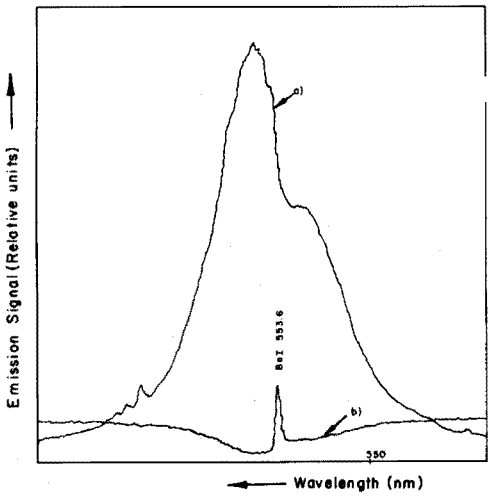

Fig. 2. Identification of the line emission of barium (1 p.p.m.) in the presence of excess calcium (500 p.p.m.) and 1000 p.p.m. of potassium. (a) Spectrum obtained nebulizing Ca + Ba + K. The strong CaOH molecular emission does not permit the identification of the barium line at 553.6 nm; (b) same spectrum with background subtraction. The background solution contained Ca and K alone. The barium atomic emission is now clearly visible.

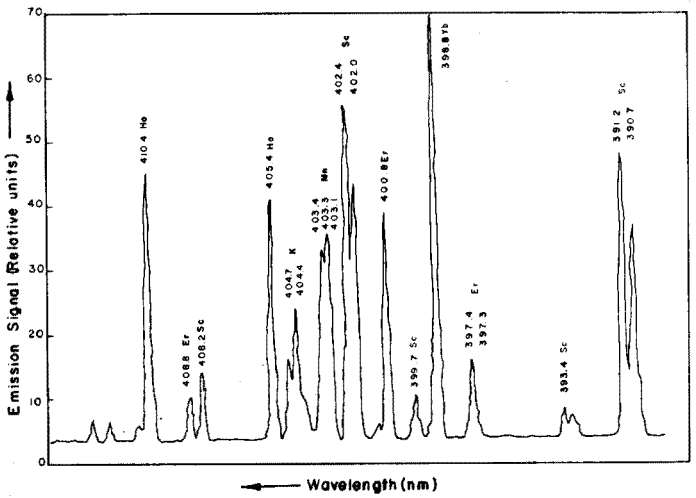

Fig. 3. Emission spectrum of aqueous solution containing erbium (100 p.p.m.), holmium (50 p.p.m.), manganese (10 p.p.m.), scandium (50 p.p.m.), and ytterbium (5 p.p.m.) nebulized in the unseparated N_2O–C_2H_2 flame with 1000 p.p.m. of KCl to suppress ionization. Conditions of OMA: 2 delays and 250 presets. Flame conditions: N_2O, 14.5 l min^{-1}; C_2H_2, 6.3 l min^{-1}.

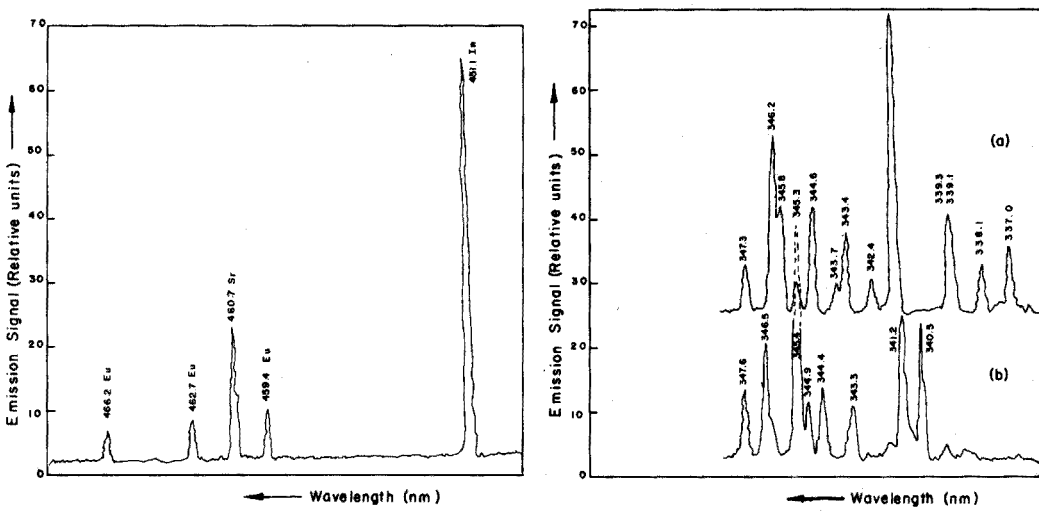

Fig. 4. Emission spectrum of aqueous solution containing indium (50 p.p.m.), europium (1 p.p.m.), and strontium (0.5 p.p.m.) nebulized in the unseparated nitrous oxide–acetylene flame. All other conditions as for Fig. 3.

Fig. 5. Emission spectra of aqueous solution containing (a) nickel (500 p.p.m.) and (b) cobalt (500 p.p.m.) nebulized in the unseparated nitrous oxide–acetylene flame. All other conditions as for Fig. 3.

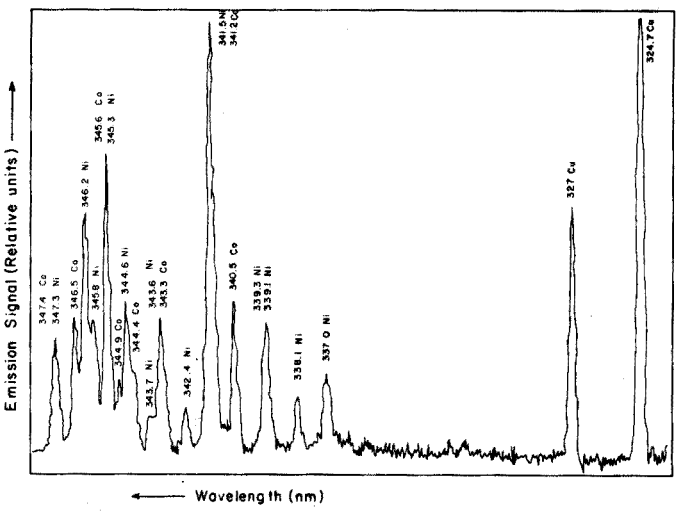

Fig. 6. Emission spectra of aqueous solution containing nickel (100 p.p.m.), cobalt (100 p.p.m.), and copper (10 p.p.m.) nebulized in the unseparated nitrous oxide–acetylene flame. All other conditions as for Fig. 3.

of elements obtained by nebulizing aqueous solutions of several concentrations of various mixtures are shown in Figs. 3–6. The same flame conditions were maintained for all spectra, and therefore the limits of detection presented in Table II for eleven elements (Co, Cu, Er, Eu, Ho, In, Mn, Ni, Se, Sr, and Yb) were not individually optimized with regards to the flame stoichiometry and height of

TABLE II

LIMITS OF DETECTION FOR SEVERAL ELEMENTS WITH THE SILICON DIODE ARRAY VIDICON CAMERA TUBE AND THE NITROUS OXIDE–ACETYLENE FLAME

Element	*Wavelength (nm)*	*Detection limit*[a]	
		This work	*Ref. 18*
Co	345.4	8.	0.05
Cu	324.7	0.2	
	327.4		0.01
Er	400.8	0.9	0.04
Eu	459.4	0.01	0.0006
Ho	405.4	0.5	0.02
	410.4	0.4	
In	451.1	0.1	0.001
Mn	403.1	0.02	0.005
Ni	341.5	5.	0.6
Sc	391.2	0.4	0.03
	402.4	0.3	
Sr	460.7	0.003	0.0002
Yb	398.8	0.04	0.002

[a] Defined as that concentration giving a signal to (rms) noise ratio of 2.

observation. The possibility of occurrence of spectral interferences for the simultaneous determination of the elements can be easily seen in Figs. 5 and 6.

From these preliminary results it is apparent that the nitrous oxide–acetylene flame results in analytically useful emission when such a flame is coupled with a diode array vidicon detector. Simultaneous multi-element analysis of practical samples seems to be a real possibility and further work on the present system is justified. Future studies will also involve replacing the image vidicon with a Silicon Intensifier Target tube and using the more sensitive TV tube for both flame atomic emission and fluorescence spectrometry.

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SHORT COMMUNICATION

The analysis of native gold by atomic absorption spectrometry*

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A knowledge of the composition of native gold has useful applications in prospecting and ore genesis studies; and the determination of trace impurities in gold is often necessary to estimate its fineness. Methods involving fire assays and wet chemical analysis are time-consuming, requiring highly skilled analysts. Owing to matrix effects, the determination of traces of platinum-group metals in native gold by spectrophotometry becomes very complicated and difficult.

As data for platinum-group metals in native gold are not available in the literature, presumably because of the lack of suitable procedures for their determination, it was thought worthwhile to develop a rapid method. Atomic absorption has proven its value in the determination of traces of precious metals in matrices where the major matrix material is platinum (native platinum and sperrylite[1]), or silver (native silver[2]), or a combination of elements such as osmium, iridium and ruthenium (osmiridium[1]). After investigation with synthetic solutions, this method was extended to the determination of noble and common metals in several specimens of placer nugget gold obtained from British Columbia and the Yukon Territory.

Experimental

Apparatus and reagents. These have been recorded in earlier papers[1, 2].

Procedure. Weigh 125–150 mg of placer nugget gold and digest with hot aqua regia to decompose the material. Evaporate on the steam bath, warm the residue with dilute hydrochloric acid and filter (Filtrate A). Wash with dilute hydrochloric acid followed by water. Dissolve silver chloride by washing the residue on the funnel with 15% (v/v) ammonia solution and collect in a 100-ml volumetric flask (Filtrate B). Ignite the paper and residue in a muffle furnace.

If the residue is less than 2 mg, mix with 50 mg of finely powdered sodium chloride in a porcelain crucible (Coors 00000) and chlorinate at 700°. If the residue exceeds 2 mg, repeat the attack with several portions of dilute nitric acid until the major portion has dissolved. Evaporate (steam bath), precipitate silver chloride with hydrochloric acid and filter. Combine the filtrate A, dissolve silver chloride

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in 15% ammonia solution as before, and combine with filtrate B. Ignite the paper and residue, mix with 50 mg of sodium chloride and chlorinate at 700°. Treat the chlorinated product with dilute hydrochloric acid and filter. Combine the filtrate with filtrate A, dissolve the residue in 15% ammonia solution and combine with filtrate B. Make up the volume of filtrate A to 50 ml with water, and that of filtrate B to 100 ml with 15% ammonia solution.

Silver. Determine by atomic absorption from 5-ml aliquots of solutions A and B, A being diluted to 10 ml and buffered with 1% lanthanum, and B diluted to 100 ml with 7% (v/v) ammonia solution.

Magnesium, calcium, iron, nickel, copper and zinc. Determine by atomic absorption in the above 1% lanthanum buffered solution.

Gold. Determine by atomic absorption after mixing a 0.5-ml aliquot of solution A with 5 ml of the copper–cadmium spectroscopic buffer[1] and diluting to 25 ml with water.

Platinum, palladium, rhodium, iridium and ruthenium. Determine by atomic absorption after evaporating a 40-ml aliquot of solution A to dryness on the steam bath, dissolving the residue in 1 ml of the copper–cadmium buffer and diluting to 5 ml in a volumetric flask, The total concentration of gold in the final volume should not exceed 26,000 p.p.m.

Results and discussion

A study with synthetic solutions of gold and platinum-group metals showed that gold could be tolerated up to 26,000 p.p.m. in the atomic absorption determination of traces of platinum-group metals (see Table I).

TABLE I

ATOMIC ABSORPTION STUDY OF THE RECOVERY OF PLATINUM-GROUP METALS FRC SYNTHETIC SOLUTIONS APPROXIMATING THE COMPOSITION OF PLACER NUGGET GO

Elements present in synthetic soln. (mg)[a]	*Pt (μg)*		*Pd (μg)*		*Rh (μg)*		*Ir (μg)*		*Ru (μg)*	
	Taken	*Found*	*Taken*	*Found*	*Taken*	*Found*	*Taken*	*Found*	*Taken*	*Found*
1. Au = 159.75; NaCl = 56.0	20	17	20	20	20	20	20	25	—	
2. Au = 102.52; NaCl = 56.0	10	10	5	5	5	5	10	10	—	
3. Au = 159.9; NaCl = 52.0	20	14	20	20	20	20	20	22	20	20
4. Au = 122.5; NaCl = 45.0	10	9	10	10	5	5	10	12	13	13

[a] Plus specified amounts of platinum-group metals.

If the native gold contains very minute traces of the platinum-group metals, an enrichment by separation of gold would perhaps be useful to increase the sensitivity of determination. However, when gold was separated from synthetic solutions of precious metals by quinhydrone precipitation[3], some losses of traces

of platinum-group metals were noted, hence some other separation method is necessary.

The recovery of gold by atomic absorption procedure was checked by direct gravimetric determinations involving quinhydrone precipitation, and the results were found to agree with each other. The advantage of the atomic absorption method obviously lies in the rapidity of the determination.

The results of analyses of six specimens of placer nugget gold are given in Tables II and III.

TABLE II

ANALYSIS OF PLACER NUGGET GOLD BY ATOMIC ABSORPTION SPECTROMETRY

(All results given as percentages)

Element	*Specimen no.*[a]					
	1	*2*	*3*	*4*	*5*	*6*
Au	83.7_6	87.2_7	82.7_3	80.1_7	72.0_9	74.4_0
Ag	15.5_9	11.9_3	15.8_3	18.9_5	27.3_0	24.6_0
Mg	0.01	0.01	0.01	0.01	0.02	0.02
Ca	0.05	0.13	0.04	0.10	0.05	0.07
Fe	0.17	0.38	0.28	0.06	0.05	0.10
Ni	0.01	<0.01	0.01	<0.01	0.01	0.02
Cu	0.03	0.04	0.03	0.02	0.02	0.02
Zn	0.03	0.02	0.01	0.02	0.03	0.02
Insol.	0.23	0.00	0.64	0.54	0.36	0.64
Total	99.8_8	99.7_8	99.5_8	99.8_7	99.9_3	99.8_9

[a] (1) Placer nugget gold, Dublin Gulch, Yukon Territory, Canada; (2) placer nugget gold, Lowhee Creek, British Columbia, Canada; (3) placer nugget gold, California Creek, British Columbia, Canada; (4) placer nugget gold, Dominion Creek, Yukon Territory, Canada; (5) placer nugget gold, Bear Creek, Yukon Territory, Canada; (6) placer nugget gold, Eldorado, Yukon Territory, Canada.

TABLE III

ATOMIC ABSORPTION DETERMINATION OF THE PLATINUM-GROUP METALS IN PLACER NUGGET GOLD

(All results given as p.p.m.; average of two determinations)

Specimen no.[a]	*Ru*	*Rh*	*Pd*	*Ir*	*Pt*
1	14	7	14	25	41
2	6	5	14	44	43
3	7	7	18	35	29
4	27	5	17	45	36
5	13	7	12	45	50
6	13	8	20	30	39

[a] See footnote to Table II for specimen identification.

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SHORT COMMUNICATION

Spectrophotometric determination of osmium with rubeanic acid

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(Received 25th September 1973)

Rubeanic acid has been proposed[1] as a sensitive reagent for the photometric determination of osmium(VIII) at pH 6–7 after a full colour development period of 1 h; Beer's law was claimed to be obeyed from 0.03 to 0.75 mg per 50 ml. However, in a spectrophotometric study this pH range was found to give a very insensitive reaction even with 8 p.p.m. of osmium. Moreover, the earlier workers did not study the procedure in detail, so that a re-investigation of the reaction seemed desirable. Herein is described a method for the determination of osmium-(VIII) in which it is converted to osmium(VI) by ethanol used as a solvent for the reagent. The brown insoluble complex formed dissolves in the presence of pyridine, and ultimately forms a brownish-violet colour in hydrochloric acid medium. In 4–8 *M* hydrochloric acid solutions, colour development is instantaneous and the complex with maximum absorption at 490 nm obeys Beer's law from 6–18 p.p.m. with an optimal range[2] of 6–15 p.p.m. The sensitivity[3] and relative analysis error[4] are, respectively, 0.0255 μg cm^{-2} and 2.72%. For full development of colour intensity, 12 p.p.m. of osmium require 1.5 ml of ethanolic 0.1% (w/v) reagent solution. The colour remains stable for 4 h. The complex is only partially extracted into amyl or n-butyl alcohol, and at least four extractions are needed for its full extraction into tri-n-butylphosphate, where it shows a hump at 470–490 nm with lower intensity than that obtained in ethanolic solution. Osmium(IV) reacts to form a different complex, and the colour reaction under the same conditions as that for osmium(VIII) is very insensitive.

Apparatus and reagents

A Unicam SP 600 spectrophotometer was used with 10-mm glass cells.

A standard osmium(VIII) solution was prepared as suggested by Ayres and Wells[5]; the solution was standardized iodimetrically[6]. A standard osmium(VI) solution was obtained by reducing the standard osmium(VIII) solution with ethanol.

To prepare a standard osmium(IV) solution, an accurately weighed quantity of potassium hexachloroosmate (Johnson and Matthey) was dissolved in 1 *M* hydrochloric acid.

Standard solutions of diverse cations were prepared from their chlorides or nitrates, and of anions from their sodium or ammonium salts; the strengths were determined by standard methods.

A 0.1% (w/v) solution of rubeanic acid (G.R.E., Merck) in ethanol was used. All reagents used were of the highest available purity.

Absorbance spectra

To measured aliquots of osmium(VIII) and (IV) were added 2 ml of pyridine followed by 2 ml of the reagent solution. The acidity of each of the solutions was adjusted to 6 *M* with concentrated hydrochloric acid. The solutions were made up to 25 ml with ethanol, and the absorbances were measured immediately against a reagent blank.

In extraction studies, the colour with osmium(VIII) was developed in a separatory funnel as described above, and the solution was extracted four times with tri-n-butyl phosphate, the extracts being transferred to a 25-ml volumetric flask. The extract was diluted to volume with the same solvent and the absorbances were measured against a reagent blank.

The osmium(VIII) complex in ethanol showed maximal absorbance at 490 nm, whereas osmium(IV) showed no peak. The tri-n-butyl phosphate extract showed a hump at 470–490 nm with lower intensity than obtained in ethanolic solution (Fig. 1).

Composition of the complex

The composition of the complex was studied for both osmium(VIII) and (VI) by Job's method[7] of continuous variations and by the mole ratio method[8]. Job's method indicated that both osmium(VIII) and (VI) form the same 1:2 complex with the reagent (Fig. 2). The mole ratio method confirmed this finding. From these observations it may be concluded that osmium(VIII) is first reduced to osmium(VI) by ethanol in the reagent solution and the colour is actually produced by osmium(VI).

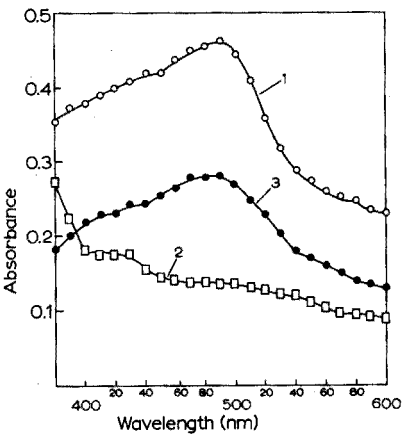

Fig. 1. Absorbance spectra: (1) 12 p.p.m. osmium(VIII) in ethanol; (2) 18 p.p.m. osmium(IV) in ethanol; (3) 9 p.p.m. osmium(VIII) in tri-n-butyl phosphate.

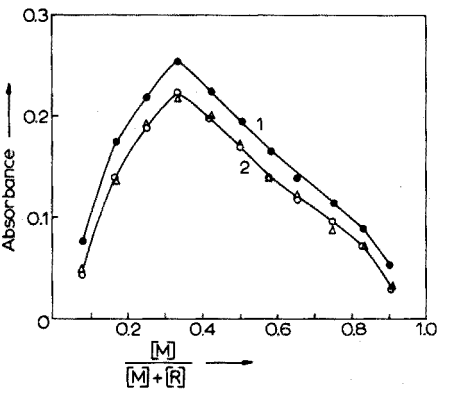

Fig. 2. Job's method for the osmium–rubeanic acid complex. (1), [Os(VIII)] = [reagent] = $5 \cdot 10^{-4}$ *M*. (2): (△), [Os(VI)] = [reagent] = $3.156 \cdot 10^{-4}$ *M*; (○), [Os(VIII)] = [reagent] = $3.156 \cdot 10^{-4}$ *M*.

Degree of dissociation and dissociation constant

From the value of the degree of dissociation, α, the dissociation constant of the complex was calculated, using the equation of Harvey and Manning[9], to be $1.54 \cdot 10^{-8}$. The dissociation constant was also calculated from a study of the absorbances of mixtures of osmium(VIII) and reagent solutions. The dissociation constant, calculated as described by Majumdar and Sen[10], was $2.83 \cdot 10^{-8}$ (Table I).

TABLE I

DISSOCIATION CONSTANT FROM JOB'S EQUATION

(See ref. 10)

Concn. osmium(VIII) (M)	*Concn. reagent (M)*	*m*	*n*	*p*	*X*	*K*
$3.156 \cdot 10^{-4}$	$1.578 \cdot 10^{-3}$	1	2	5	0.42	$4.036 \cdot 10^{-8}$
$2.0 \cdot 10^{-4}$	$1.0 \cdot 10^{-3}$	1	2	5	0.42	$1.622 \cdot 10^{-8}$
						Mean $K = 2.83 \cdot 10^{-8}$

Effect of diverse ions

To evaluate the tolerance limits of different ions, solutions containing a definite quantity of osmium(VIII) were prepared with varying amounts of other ions; the procedure was as described above.

It was found that the system tolerated the presence of the following ions (in p.p.m.): Rh^{3+} (20), Ir^{4+} (100), Fe^{3+} (100), UO_2^{2+} (100), Th^{4+} (400), Ti^{4+} (400), Mo^{6+} (400), As^{5+} (400), Bi^{3+} (400), Pb^{2+} (200), Cd^{2+} (400), Mn^{2+} (400), Sb^{3+} (400), Cr^{3+} (100), Zr^{4+} (400), Hg^{2+} (100), Mg^{2+} (400), Be^{2+} (400), Zn^{2+} (400), La^{3+} (400), Ce^{3+} (400), Au^{3+} (200), citrate (400), tartrate (400), oxalate (400), EDTA (400) and the rare earths (400). Ions like Cu^{2+}, Ni^{2+}, Co^{2+}, V^{5+}, Pd^{2+}, Ru^{3+}, Pt^{4+} and Ag^{+} interfered. However, the interference from these ions may be avoided by prior distillation of osmium as the tetroxide.

Distillation and determination of osmium and subsequent determination

In a distillation apparatus[11], a solution containing 8 p.p.m. of osmium(VIII) was mixed with solutions of the ions (in p.p.m.) Cu^{2+} (400), Ni^{2+} (400), Co^{2+} (400), V^{5+} (400), Pd^{2+} (100), Ru^{3+} (100), Pt^{4+} (100) and Ag^{+} (100). The mixture was treated with 20 ml of 6 *M* nitric acid and was distilled slowly in a current of air for 20 min. The distillate was absorbed in 7.0 ml of 0.07 *M* potassium hydroxide solution. In the distillate the colour was developed and measured as described before. Duplicate experiments showed that the average recovery of osmium was 7.8 p.p.m., *i.e.* 97.5%.

The author wishes to express his deep sense of gratitude to Prof. Dr. A. K. Majumdar, Professor of Inorganic and Analytical Chemistry, Jadavpur University, for his keen interest and valuable suggestions.

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Analytica Chimica Acta, 69 (1974) 469–472

SHORT COMMUNICATION

Photometric titration of beryllium(II) with 5-sulfosalicylic acid

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Several indirect titrations of beryllium have been described in the literature but no direct complexiметric titrations of this element have been reported. This is due to the fact that the available chelating agents which form 1:1 chelates with most metal ions do not rapidly form a complex with beryllium. However, a great number of complexing agents form very stable 1:2 complexes with this metal, and these have found extensive use in spectrophotometry; β-diketones and some hydroxy acids should be mentioned. Some of these compounds seem to be suitable as titrant for beryllium as was shown by Florence and Farrar[1] who determined beryllium by back-titrating the excess of sulfosalicyclic acid added to the sample with a standard beryllium sulphate solution. The end-point was detected photometrically by means of arsenazo as metallochromic indicator. A considerably lower limit of detection can generally be obtained with linear, self-indicating titration systems. Photometric indication is the obvious means for end-point detection in this case as well. Acetylacetone and other β-diketones are not suitable as titrants, as the absorption spectra of the ligand and the complexes are very similar at the pH values suitable for titrations.

5-Sulfosalicylic acid seems to be suitable for a direct titration as well, for the absorption curves of the two complexes BeL_1 and BeL_2 are shifted towards longer wavelengths with respect to the ligand[2] at pH values suitable for titration. The spectra are given in Fig. 1. The stability constants of the complexes have been determined by Banks and Singh[3], who report $\log K_1 = 11.5$ for BeL and $\log K_2 = 8.8$ for BeL_2. These values are larger than for many other metal ions. The two dissociation constants for the carboxylic and phenolic protons are $pK_1 = 2.8$ and $pK_2 = 11.7$. These data suggest that a selective photometric titration of beryllium(II) with 5-sulfosalicylic acid should be possible.

In recent papers[4,5], the conditions for a successful complexiметric titration based on 1:2 complex formation were derived. With photometric indication, no end-point can be expected for the titration of beryllium(II) with sulfosalicylic acid at the point of 1:1 complex formation, for the ratio of the molar absorptivities of BeL_2 and BeL is about 2 at all wavelengths. At 325 nm (Fig. 1), the chelates absorb much more strongly than the ligand, so that at

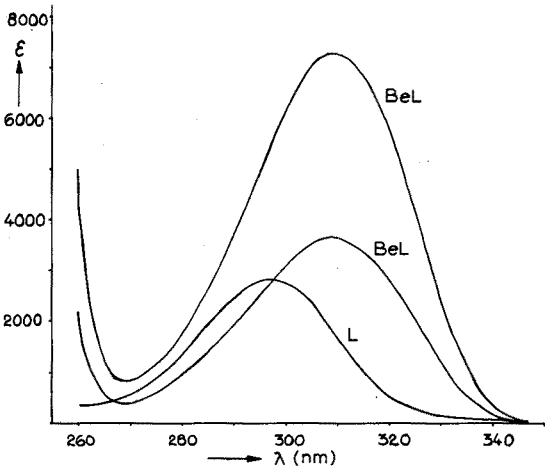

Fig. 1. Absorption spectra of L, BeL and BeL_2 (L = 5-sulfosalicylic acid) at pH 8.8 (0.1 *M* ammonia–ammonium chloride buffer).

this wavelength an end-point can be expected corresponding to 1:2 complex formation, provided that certain requirements involving the product of the conditional stability constants K'_1 and K'_2 and the concentration of beryllium(II) are fulfilled[4].

The conditional stability constants can only be calculated roughly, because of the complicated interaction between beryllium(II) and hydroxyl ions; mononuclear as well as several polynuclear complexes are mentioned in the literature, but the stability constant data are rather incomplete. As only dilute solutions of beryllium(II) were to be analyzed, it seemed reasonable to ignore the occurrence of polynuclear complexes. For the 1:1 complex, $BeOH^+$, a value of $\log K_1 = 8.3$ has been mentioned several times in the literature; for $\log \beta_2$, values ranging from 14 to 17 are to be found. With these values, the conditional stability constants

$$\log K'_1 = \log K_1 - \log \alpha_{L(H)} - \log \alpha_{Be(OH)},$$

and

$$\log K'_2 = \log K_2 - \log \alpha_{L(H)}$$

were calculated as a function of pH. The results are summarized in Table I for β_2 values of 10^{14} and 10^{17}. The titration error[5] is determined by $Z_2 = K'_2 c_{Be}$ when $K'_1 \gg K'_2$ but by $Z_1 Z_2 = K'_1 K'_2 c_{Be}^2$ when $K'_1 \ll K'_2$. From Table I it can be concluded that the optimal pH for the titration will be about 9–10. This is in agreement with the experimental results.

Experimental

Reagents and equipment. Analytical grade reagents were used, as well as doubly distilled water. Beryllium sulfate tetrahydrate was used to prepare beryllium(II) solutions. Titrations were carried out in the pH range 8.8–9.3 with 0.1 *M* ammonia–ammonium chloride buffer. A Zeiss PMQ II spectrophotometer provided with 10-ml titration cells, fitting in the cell holder, was used.

TABLE I

CONDITIONAL STABILITY CONSTANTS

pH	$\log \alpha_{L(H)}$	$\log \alpha_{Be(OH)}$		$\log K'_1$		$\log K'_2$	$\log \beta'_2 = \log K'_1 K'_2$	
		$\log \beta_2 = 14$[a]	$\log \beta_2 = 17$[a]	14	17		14	17
5	6.7	0	0	4.8	4.8	2.1	6.9	6.9
6	5.7	0.5	1.1	5.3	4.7	3.1	8.4	7.8
7	4.7	1.3	3	5.5	3.8	4.1	9.6	7.9
8	3.7	2.5	5	5.3	2.8	5.1	10.4	7.9
9	2.7	4.1	7	4.7	1.8	6.1	10.8	7.9
10	1.7	6	9	3.8	0.8	7.1	10.9	7.9
11	0.8	8	11	2.7	−0.3	8.0	10.7	7.7
12	0.2	10	13	1.3	−1.7	8.6	9.9	6.9

[a] $\beta_2 = K^{2OH}_{M(OH)_2}$.

Absorbance measurements were carried out at 325 nm. The titrant was added from a 0.5-ml piston-type burette.

Preliminary tests. The first attempts to titrate beryllium(II) with sulfosalicylic acid were unsuccessful; the reaction appeared to be very slow, and attempts to speed it up by increasing the temperature failed. It was thought that the slow reaction was due to the formation at pH 9 of beryllium(II)–hydroxo complexes, which react slowly with the ligand. This was confirmed by the fact that the formation of the complex was strongly influenced by the order of mixing the reagents. When beryllium(II), sulfosalicylic acid and buffer were mixed in this order, the complex was formed immediately and the absorbance remained unchanged on standing. However, when the order of mixing was changed to beryllium(II), buffer, sulfosalicylic acid, the absorbance did not reach a constant value over a long period of time. Therefore, the titration procedure was altered in the following way: beryllium(II) and sulfosalicylic acid were mixed in slightly acidic solution in about the ratio 1:1.2; then the solution was buffered at the proper pH value of about 9, and the titration was completed with sulfosalicylic acid, correct end-points being obtained. Obviously, the formation of the 1:1 complex in slightly acidic solution prevents the formation of hydroxo complexes.

Various other metal ions react with the ligand under the experimental conditions or cause precipitation of hydroxides, hence EDTA or a similar compound must be added as masking agent. Heating of the slightly acidic solution containing beryllium(II), the ligand, interfering metal ions and EDTA may be necessary in order to attain equilibrium. The following procedure was found to be the most satisfactory for titrations at the limit of determination, which was *ca.* 10^{-4} *M* beryllium(II).

Recommended procedure. Take 10 ml of the slightly acidic sample solution containing about 10 μg of beryllium(II); add EDTA in excess with respect to interfering metal ions; add sufficient titrant to give a ratio of *ca.* 1:1.2 of beryllium(II) to 5-sulfosalicylic acid. Heat the solution to 50° for 5 min, cool and

buffer the solution at pH 9. Titrate with sulfosalicylic acid solution. Take absorbance readings at 325 nm, at least 4 before and 3 after the equivalence point. Wait 3 min after each addition of the titrant. The end-point is found by the intersection of the two straight parts of the titration curve.

Results and discussion

Some results of determinations of beryllium(II) alone and in the presence of other metal ions supposed to complex with the ligand are given in Table II. Iron(III) interferes because of the large absorption by the iron(III)–EDTA complex at 325 nm.

TABLE II

DETERMINATION OF BERYLLIUM(II) IN THE PRESENCE OF SOME METAL IONS

(In all cases, 9.01 μg of beryllium(II) was taken; *ca.* 100 μg of each of the other metal ions were used)

Other ions	*Be(II) found (μg)*	*No. of detus.*	*S_r (%)*
—	9.05	4	0.3
Cu(II)	9.26	4	0.3
Ni(II)	9.07	3	0.3
Al(III)	9.42	3	0.7
Mn(II)	8.86	3	0.7
Cu(II), Ni(II), Al(III), Mn(II)	9.37	3	0.5

The optimal pH value of 9 expected from Table I corresponds to that found experimentally. The values of log β'_2 at this pH value predict that the limit of determination is about 10^{-3} *M*. In practice, successful determinations could be carried out at the 10^{-4} *M* level. Obviously, the beryllium(II)–hydroxo complexes play a less important rôle than would be expected on the basis of the available data on their stability. This is probably caused by the slow formation of these complexes at low concentrations. The titration appears to be rather selective. However, the selectivity can be further increased by removing interfering metal ions such as iron(III) by electrolysis at a mercury cathode, beryllium(II) remaining in the solution.

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SHORT COMMUNICATION

Surfactant-selective electrodes

Part II. The use of perm-selective membranes

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Some recent publications have been concerned with the measurement of surfactant activities by means of electrochemical cells containing perm-selective ion-exchange membranes[1–3]. The use of such cells in micellar solutions would have advantages over liquid ion-exchanger membrane electrodes, where the possibility of solubilization of the membrane must be considered[4,5]. However, it is likely that the use of perm-selective membranes could be restricted by poor selectivity[6] and by the non-reversible adsorption or exchange of surfactants[7,8]. Furthermore, it has been noted that perm-selective membranes used for determination of surfactant activities may not be totally in the surfactant form after the usual conditioning procedure[2]. Accordingly, tests have been carried out to determine the rates of exchange of an homologous series of sodium n-alkyl sulphates with Asahi membranes, and to optimize the operating conditions in electrochemical cells.

Experimental

The homologous series of sodium n-alkyl sulphates (n = 6–12) was synthesized by Palmer Research Ltd., Mostyn, Flint; purities >99% were established by standard methods. Sodium ethyl sulphate and sodium nitrate were AR grade, used as supplied. Ammonium perfluoro-octanoate (purity >99%) was synthesized in this laboratory by Dr. G. Tiddy. Conductivity water was used to make up all solutions. Samples of Asahi anion-exchange membrane CA-1, were conditioned by soaking in sodium chloride solution for one month, followed by thorough washing with water. All measurements were carried out at 21° ± 0.5°.

Exchange studies with surfactant ions

The kinetics of exchange was established by monitoring the release of chloride ion with time from 0.1 g of membrane into 100 ml of well-stirred 0.02 *M* surfactant solution. This concentration of chloride ion was found from the change in e.m.f. of a previously calibrated Ag/AgCl electrode immersed in the solution, with a reference calomel electrode via a sodium sulphate salt bridge. Figure 1 gives plots of percentage conversion of the membrane to the surfactant form *vs.* $\log_{10}$ time. Data obtained for solutions of sodium nitrate and ammonium perfluoro-

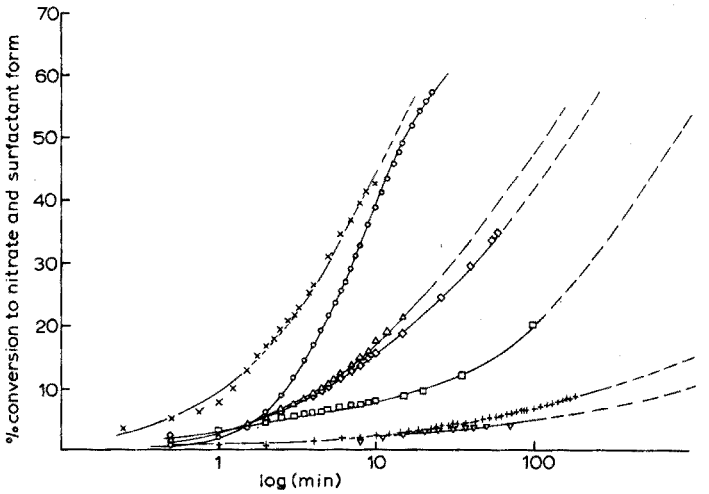

Fig 1. Kinetics of exchange of surfactant for chloride ion with Asahi membrane CA-1. (×) sodium nitrate; (○) sodium ethylsulphate; (△) sodium hexylsulphate; (◇) sodium octylsulphate; (□) sodium decylsulphate; (+) sodium dodecylsulphate; (▽) ammonium perfluoro-octanoate.

octanoate (which has a similar anionic size to dodecylsulphate anion) are given for comparison. The rate of exchange of surfactant ions is slow compared with nitrate ion, decreasing markedly with increase in alkyl chain-length.

In general, the kinetics of ion exchange in solid membranes is governed by two rate-limiting factors, the diffusion of counter-ions within the membrane matrix (particle diffusion), and the diffusion of counter-ions across the liquid film immediately adjacent to the membrane surface (film diffusion). For the exchange of counter-ions of similar size to the membrane pores, particle diffusion is the rate-determining step, and the time taken for 50% conversion ($t_{\frac{1}{2}}$) is inversely proportional to the counter-ion diffusion coefficient in the membrane matrix[6]. For exchange of counter-ions which are small compared to the membrane pores, film diffusion is the rate-determining step. For exchange from a 0.02 *M* solution of counter-ions, it has been shown that $t_{\frac{1}{2}}$ is *ca.* 20 min when film diffusion is rate-determining[9]. From Fig. 1, it appears that exchange of chloride ions for nitrate and ethylsulphate ions is "film diffusion" controlled ($t_{\frac{1}{2}} = 14$ and 16 min, respectively), whilst the sluggish exchange for decyl- and dodecylsulphate (estimated $t_{\frac{1}{2}}$ values are 800 and 10^4 min, respectively) almost certainly reflects slow "particle diffusion" within the membrane matrix. The intermediate $t_{\frac{1}{2}}$ values for hexyl- and octylsulphate indicate that these anions also undergo somewhat restricted diffusion within the membrane matrix.

These results confirm the suspicions of Botre *et al.*[2] that their membranes were not fully equilibrated with dodecylsulphate ion. Therefore, concentration cells containing these membranes between two surfactant solutions, give rise to potentials which are not due solely to diffusion of surfactant across the membrane.

Surfactant concentration cells

The form of the cell[10], which was made of Perspex, is shown schematically below:

;, Hg_2Cl_2	5 *M* NH_4NO_3	0.005 *M*	C A I	Test	C K I	0.005 *M*	5 *M* NH_4NO_3	Hg, Hg_2Cl_2
ıt. KCl	Agar gel	SDS		solution		SDS	Agar gel	sat. KCl

where CKI and CAI are cation- and anion-exchange membranes, respectively. The three compartments could be stirred synchronously at about 2 rev s^{-1}. The potential of the cell with change in test solution concentration, was determined with a Radiometer pH M52 digital millivoltmeter. Operating temperature was $21° \pm 0.5°$.

If the membranes in this cell act in a reversible fashion to the species of interest, then the potential developed is a function of the mean activities $a\pm$ of neutral electrolyte in each compartment. Since the reference compartments are at equal concentrations the potential is given by:

$$E = \frac{2RT}{F}\left[\frac{\ln a\pm \text{ (test solution)}}{a\pm \text{ (ref solution)}}\right]$$

Initially, experiments were performed with sodium chloride solutions and membranes which had been soaked in 0.1 *M* sodium chloride solution for 96 h before use. With change in concentration of the test solution, the cell potential reached a value stable to ± 0.2 mV within 2 min. The response of the cell was near-theoretical (*i.e.* $2\times$ Nernstian response $\equiv 116$ mV/decade change in sodium chloride concentration).

When the same experiment was carried out with sodium dodecylsulphate (SDS) solutions and membranes soaked in 0.02 *M* SDS solution for 96 h before use, change in SDS concentration in the test solution produced an initial rapid change in potential (*ca.* 1 min), followed by a slow drift in potential for about 25 min, with all three solutions stirred, or for about 45 min, with only the test solution stirred. The mode of stirring did not affect the final potential values, which were stable to ± 1.5 mV and ± 0.5 mV, respectively. Figure 2 shows a plot

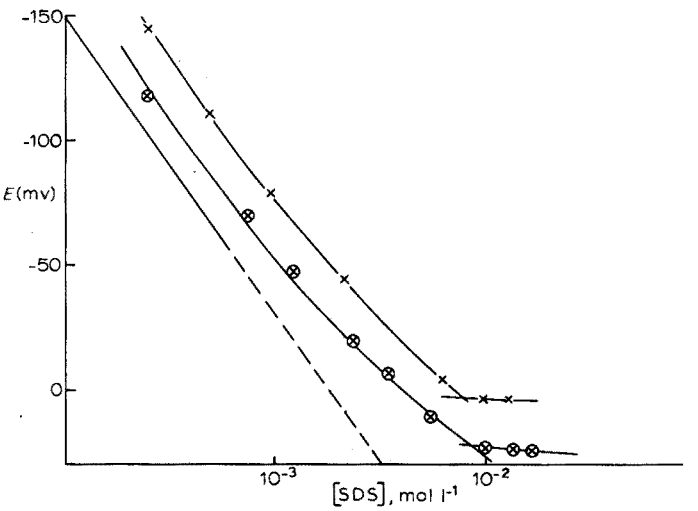

Fig. 2. Effect of membrane soaking time with a three-compartment cell. (⊗) Membrane equilibrated for 96 h; (×) membrane equilibrated for 24 h; (---) theoretical slope.

of the final potentials obtained together with analogous data obtained with membranes soaked for only 24 h. Clearly, there is considerable difference in the final potential values obtained with these different soaking conditions. Furthermore, when compared with the ideal Nernstian slope, reduced response (curvature) is evident, especially above 10^{-3} *M*. Replotting in terms of activities did not reduce the curvature. The break at *ca.* $8 \cdot 10^{-3}$ *M* is due to the onset of micellization, and is within the accepted concentration limits.

Similar data (Fig. 3) were obtained over a wider range of membrane soaking times, with the cationic half-cell replaced by a sodium-selective electrode (EIL 33C) dipping directly into the test solution. Only the cell incorporating the membranes soaked for the shortest time, *i.e.* 30 min, exhibited linear near-theoretical response below the c.m.c.

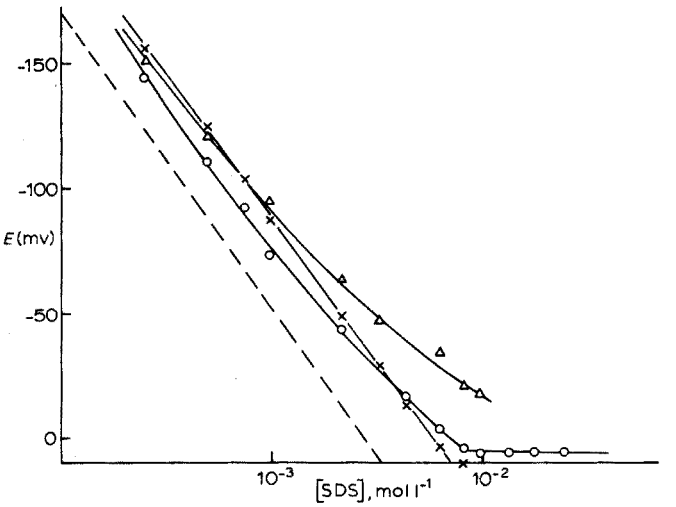

Fig. 3. Effect of membrane soaking time with a two-compartment cell. (△) Membrane equilibrated for two weeks; (○) membrane equilibrated for 96 h; (×) membrane equilibrated for 30 min; (---) theoretical slope.

From the above results, it is apparent that linear Nernstian response in surfactant solutions is obtained only with short pre-soaking times. From Fig. 1, an upper limit of 5% conversion to the surfactant form can be set for 30-min soaking, rising to probably about 20% for an overnight soak, which appears to be a general conditioning soaking time[1–3]. These estimates will be considerably reduced if the conditioning solution is not stirred. It is likely therefore that the curvature observed with membranes soaked for the longer times results from progressive irreversible sorption or exchange of surfactant and that the observed potential is due primarily to exchange of chloride ion for surfactant ion. Thus the linearity below the c.m.c. observed by other workers is probably due to the membrane remaining primarily in the chloride form throughout the conditioning procedure. The low c.m.c. value reported by Botre *et al.*[2] (outside the accepted limits) may possibly be due to neglect of any curvature at higher concentrations.

Conclusion

These results must cast serious doubt on the thermodynamic validity of using perm-selective membranes to determine surfactant activities. Furthermore, the potentials are slow to reach equilibrium values, so that the use of these membranes is time-consuming and unsuitable for routine work. A further drawback is, of course, that they are non-selective, and hence cannot be used in mixed electrolyte solutions. These types of cells seem to have no practical advantages over cells based on liquid ion-exchanger membranes.

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SHORT COMMUNICATION

The monothio derivatives of 1,1,1-trifluoro-5,5-dimethylhexane-2,4-dione and 1,1,1,2,2-pentafluoro-6,6-dimethylheptane-3,5-dione

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The fluorinated β-diketones have been used as reagents in analysis for many metals by gas chromatography. Recently, it has been shown that monothio derivatives of the fluorinated β-diketones are effective reagents for metal analysis by gas chromatography[1,2].

1,1,1-Trifluoro-5,5-dimethylhexane-2,4-dione (H(thd)) and 1,1,1,2,2-pentafluoro-6,6-dimethylheptane-3,5-dione (H(phd)) were synthesized by Pullen[3] and Belcher *et al.*[4] H(thd) was used by Blair[5] in the solvent extraction of some lanthanides. The lead(II) complexes of the above reagents were examined by gas chromatography[4]. Belcher *et al.*[1] synthesized the monothio derivatives of H(thd) and H(phd), *i.e.* T-H(thd) and T-H(phd), but did not isolate them as pure reagents. In the present work, the synthesis and purification of analytically useful quantities of T-H(thd) and T-H(phd) is reported, and the pure products are characterized.

Synthesis

A similar procedure to that of Belcher *et al.*[4] was used in the preparation of H(thd) and H(phd). However, sodium hydride was used as a 57% dispersion in mineral oil instead of the pure powdered reagent. A 52% yield was obtained.

The preparative procedure for T-H(thd) and T-H(phd) was as follows: absolute ethanol (1.6 l) was mixed with 40 ml of β-diketone in a 3-l round-bottom flask that was flamed and flushed with dry nitrogen. The flask was equipped with a mechanical stirrer and placed in a dry ice and alcohol bath at −75 to −80°. Dry hydrogen sulfide and hydrogen chloride were passed alternately for 15 min each time. The reaction mixture volume had increased by 900 ml after 2 h, at which time the reaction mixture was left undisturbed overnight, to warm slowly to room temperature.

Isolation

The red-orange reaction mixture was poured into 2.4 l of cold distilled water, and was extracted with hexane (4 × 200 ml). The extracts were combined, washed twice with 400 ml of water, and dried over anhydrous magnesium sulfate. The solvent was evaporated with a rotary evaporator. A 25-cm still packed with

helices was used to separate the β-diketone, which has a lower boiling point than the monothio derivative. The residue was transferred to a micro still and the product was distilled at 35–38 torr. No apparent decomposition of the product was observed after one month.

Instrumental

Mass spectra were obtained on the AEI-MS-9, i.r. spectra on a Perkin-Elmer Model 137 Spectrophotometer, and n.m.r. spectra with a Varian A-60 for H^1 *versus* TMS. Carbon, hydrogen, and sulfur analyses were performed by MHW Laboratories, Garden City, Mich.

Results

A yield of 50% was obtained for T-H(thd) and 32% for T-H(phd). Elemental analyses gave the results which follow. T-H(thd): calculated for $(CH_3)_3CCSCH_2COCF_3$: 45.29% C, 5.23% H, 15.08% S; found 45.03% C, 5.01% H, 14.89% S; T-H(phd): calculated for $(CH_3)_3CCSCH_2COC_2F_5$: 41.23% C, 4.23% H, 12.21% S; found: 41.44% C, 4.19% H, 12.07% S. The boiling points were 87° at 35 mm and 94° at 39 mm for T-H(thd) and T-H(phd), respectively.

Infrared spectra were obtained on neat samples of T-H(thd) and T-H(phd) with NaCl discs (Fig. 1). The broad band at 4.1 μm is due to the S–H stretching absorption of the thioenol[6]. The four characteristic bands of monothio derivatives according to Chaston *et al.*[7] are at 6.0–6.9, 6.1–6.5, 7.9–8.4, and 11.9–12.4 μm.

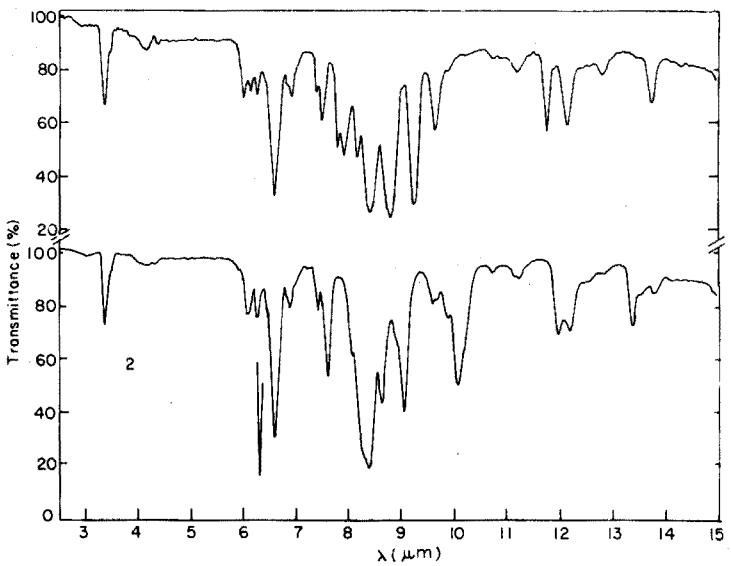

Fig. 1. The i.r. spectrum of (1) T-H(thd) and (2) T-H(phd).

The n.m.r. spectra gave three singlet peaks (Fig. 2) at 1.36, 6.74, and 10.80 p.p.m. for T-H(thd) and 1.36, 6.76, and 10.92 p.p.m. for T-H(phd) with an area ratio of *ca.* 9:1:1, indicating that both compounds exist *ca.* 100% in the enol or thioenol form, as was found for the monothio derivative of 1,1,1,2,2,3,3-heptafluoro-7,7-

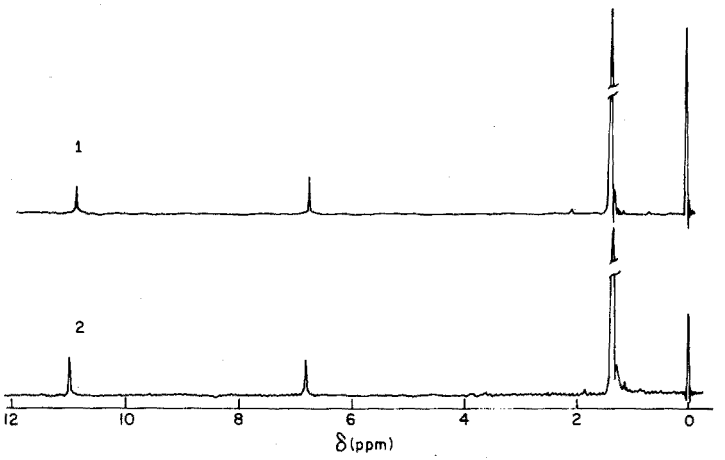

Fig. 2. The n.m.r. spectrum of (1) T-H(thd) and (2) T-H(phd).

dimethyloctane-4,6-dione[8]. The corresponding n.m.r. spectra of the parent β-diketones gave the following three singlet peaks at 1.24, 6.12, and 14.74 p.p.m. for H(thd) and 1.26, 6.22 and 14.86 p.p.m. for H(phd) with an area ratio of 9:1:1.

The parent β-diketones and monothio derivatives were examined by gas chromatography. Almost symmetrical peaks for H(thd) and H(phd) were found at retention times of 1.6 and 1.3 min and for T-H(thd) and T-H(phd) at 4.5 and 3.6 min under the following conditions: a 3.5 ft. × 6 mm-o.d. glass column containing 20% β,β'-oxydipropionitrile on Chromosorb W at 96°; injection port temperature, 140°; katharometer detector temperature, 145°; bridge current, 100 mA; helium gas flow, 59 ml min^{-1}; an F and M Model 720 instrument was used.

The mass spectrum of both monothio derivatives showed the common peak m/*e* 57 (100%) due to the t-butyl group. The maximum peaks, at m/*e* 212 (20%) and 262 (17%), were due to T-H(thd) and T-H(phd), respectively. The presence of $(CH_3)_3CC{=}S^{\oplus}$ was confirmed at m/*e* 101 (*ca.* 3% for each compound).

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Analytica Chimica Acta, 69 (1974) 481–483

SHORT COMMUNICATION

Derivative method for chemical confirmation of the identity of aflatoxin B_1

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(Received 16th July 1973)

The official method of the Association of Official Analytical Chemists (AOAC) for chemical confirmation of aflatoxin B_1 by derivative formation[1] involves the preparation of a water adduct by treatment either with a mixture of formic acid and thionyl chloride or with trifluoracetic acid, and the preparation of a mixture of epimeric acetates by reaction with acetic acid and thionyl chloride. With better knowledge of the ultimate structures, a much simplified method for preparation of both derivatives was developed[2] and has been included as one of the official methods for 'Preparation of Derivatives'[1]. That method involves treating the aflatoxin-containing extract with concentrated hydrochloric acid and water to yield the water adduct and with concentrated hydrochloric acid and acetic anhydride to yield the two acetates. The derivatives of aflatoxin B_1 in each case are determined by thin-layer chromatography.

In this communication, a method for forming a derivative of aflatoxin B_1, for chemical confirmation that can be carried out on a silica thin-layer chromatogram, is reported. The method involves treating an aflatoxin-containing extract on a chromatogram with a solution of 2,4-dinitrophenylhydrazine (DNPH). The chromatogram can then be eluted with a normal eluent, used for separating aflatoxins, to detect the aflatoxin B_1 derivative.

Experimental

To prepare the DNPH derivative of authentic aflatoxin B_1, aflatoxin B_1 (Calbiochem; 1 mg) in ethanol (400 μl) was reacted with a solution of DNPH (50 μl; 1.3 mg in ethanol–sulfuric acid–water) as described by Fuson *et al.*[3]. The precipitate which formed after about 10 min was separated from the supernatant liquid by filtration and washed twice with ethanol. The precipitate, which contained the DNPH derivative of aflatoxin B_1, was dissolved in benzene and used as a side marker when authentic aflatoxin B_1 was chromatographed.

To investigate the preparation of the DNPH derivative of aflatoxin B_1 on a chromatogram, aflatoxin standard (5 or 10 μl), which contains 5 μg of B_1 and G_1 and 1.5 μg of B_2 and G_2 per ml of benzene–acetonitrile (98+2) and which is suitable for t.l.c. analysis (supplied by SRRL, USDA, ARS, New Orleans, La.), was applied to a silica thin-layer chromatoplate (sil G-25 HR; Macherey–Nagel). The chromatoplate was chromatographed two-dimensionally in the dark

at room temperature in an unlined tank, with chloroform–acetone (85+15) (eluent I) in the first direction. Portions of aflatoxin standard were used as side-markers for both directions and the DNPH derivative of authentic aflatoxin B_1 was used as a side-marker in the second direction. After the chromatogram had been eluted in the first direction, it was allowed to dry at room temperature for 30 min. Then the portions of the chromatoplate that contained aflatoxin standard side-markers, one for each direction, and aflatoxin standard being two-dimensionally chromatographed, were sprayed with a solution of DNPH [0.2 g in 1 ml of hydrochloric acid (d 1.19) plus 100 ml of ethanol]. The chromatoplate was then set aside in the dark at room temperature for 60–90 min before being chromatographed in the second direction with benzene (IX) followed by eluent I. The compounds were observed under u.v. light (365 nm). All of the operations were performed in the dark.

Results and discussion

After aflatoxin B_1 has been sprayed with DNPH solution on a silica thin-layer chromatoplate, it is converted to one major new compound and about three trace compounds, with only a trace of aflatoxin B_1 remaining unreacted (Fig. 1). The fluorescent color and intensity of fluorescence of the major derivative appears to be similar to that of the original aflatoxin B_1. The portion of the chromatogram which contains aflatoxin B_1 turns pale yellow after being sprayed with DNPH

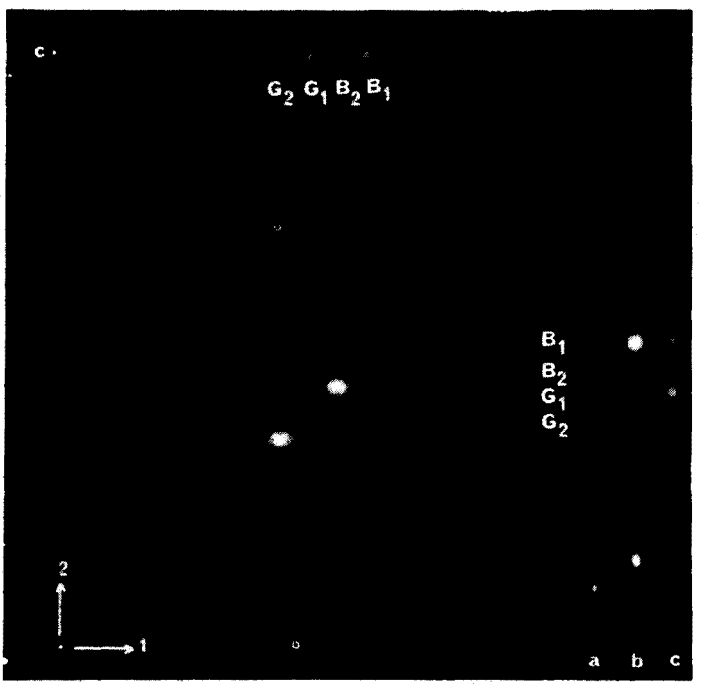

Fig. 1. Thin-layer chromatogram on silica of aflatoxin standard chromatographed in two dimensions. Portions of the chromatogram were sprayed with DNPH solution before elution in the second direction (see text). (a) Aflatoxin standard, DNPH treatment before elution. (b) DNPH derivatives of authentic aflatoxin B_1. (c) Aflatoxin standard, no treatment.

solution. (Chromatography in the second direction moves the unreacted DNPH to the front allowing observation of any visible color formation.) It, therefore, appears that aflatoxin B_1 can easily be reacted with DNPH on a chromatogram, to form a derivative to confirm the presence of aflatoxin B_1 in a sample.

To determine aflatoxin in a sample, two portions of the sample to be analyzed could be applied to a chromatoplate along with aflatoxin B_1 and several portions of aflatoxin standard. Next, one portion of the sample and one of authentic aflatoxin B_1 would be sprayed with DNPH solution and chromatography could be performed in the usual way. If the sample to be analyzed contained pigments that would interfere with the detection of the derivative in one dimension, a portion of the sample could be chromatographed two-dimensionally, that portion suspected of containing aflatoxin B_1 being sprayed with DNPH solution before elution of the chromatogram in the second direction.

In Fig. 1, it can be seen that aflatoxin G_1, as well as aflatoxin B_1, forms a major derivative when treated with DNPH, but aflatoxin B_2 and G_2 do not. Such an observation suggests that DNPH, under acidic conditions, does not react with the carbonyl group in ring E (see Fig. 2) of B_1 and B_2. The major derivative may be formed by reaction of DNPH with the aldehyde that could be formed if ring A in B_1 is opened under acidic conditions.

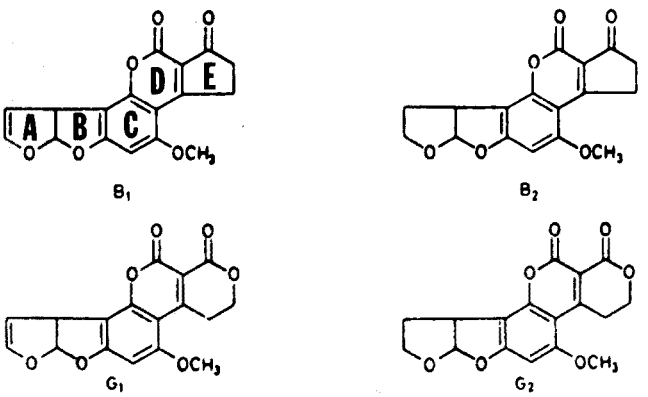

Fig. 2. Structural formulae for aflatoxin B_1, B_2, G_1, and G_2.

In conclusion, the derivative method for chemical confirmation of the identity of aflatoxin B_1 described here appears to provide a convenient, fast and reliable method for confirming the identity of aflatoxin B_1.

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Analytica Chimica Acta, 69 (1974) 484–487

SHORT COMMUNICATION

Sulphuric–perchloric acid digestion of plant material for nitrogen determination

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(Received 24th September 1973)

The Kjeldahl method is extensively used for the determination of organic nitrogen in plant and soil samples[1]. Despite much research effort, however, the methods most commonly employed retain several disadvantages. The digestion process is time-consuming (normally requiring as long as 5 h[2]) and requires considerable attention. Moreover, the usual catalyst mixtures (apart from pure selenium)[3] render the digest unsuitable for the determination of potassium, or sodium and many trace elements; this factor may be important if only small samples are available.

Among the relatively powerful oxidizing agents which can be used to accelerate the digestion, perchloric acid is attractive if used without additional catalysts, since a single digest can then be used for determinations of major nutrients (N, P, K, Mg, and Ca) and of selected trace elements. This use of perchloric acid has been critically reviewed[4]. Unfortunately, when perchloric–sulphuric mixtures are used, nitrogen losses can occur unless the amount of perchloric acid is carefully controlled[1,4–6]. The method had been criticized because of the difficulty in adding the exact amount of perchloric acid to complete oxidation. Willard and Cake[6] and Wicks and Firminger[4] commented on the problems of balancing complete oxidation with perchloric acid against nitrogen losses. John[7] has shown that, under carefully optimized conditions, dropwise addition of perchloric acid to plant samples predigested with concentrated sulphuric acid yields reliable results, but subsequent studies[8] indicated that three conditions must be fulfilled: (i) the amount of perchloric acid added must be optimized for each sample type and sample mass or sample mass/acid volume ratio; (ii) the sulphuric acid digest must be cooled before perchloric acid is added; and (iii) the solution must be heated only slightly after the addition of perchloric acid. Other authors[9] have also observed that vigorous boiling at this final stage causes substantial loss of nitrogen.

Careful examination of earlier reports indicates that perchloric acid can cause loss of nitrogen only when conditions of high temperature and high local concentration of perchloric acid occur simultaneously. Close temperature control is not practicable in routine work, hence the only controllable parameter remaining is the local concentration of perchloric acid. Even the formation of droplets of quite concentrated perchloric acid which can occur as soon as heating and

refluxing are recommenced must be avoided. This may be achieved by substantial dilution of the perchloric acid with concentrated sulphuric acid before its addition, and by adding a sufficiently small amount of perchloric acid. The possibility of nitrogen loss may thus be avoided at all stages in the digestion process, but a significant time-saving is still achieved in comparison with standard methods.

Experimental procedure

Weigh 100 ± 0.1 mg of ground oven-dried plant material into a clean dry 100-ml Kjeldahl flask: add 5 ml of concentrated sulphuric acid (reagent grade) and 2–3 glass beads, swirl the flask and leave to stand for 20 min. Heat over a very low Bunsen flame, and slowly increase the heating rate until the solution boils after a total period of 10–15 min. Continue to boil gently for a further 20 min. Do not heat so strongly that the edges of the flame touch the flask wall above the liquid level or loss of ammonium will occur. Remove the source of heat momentarily, and add 1 ml of a 4% (v/v) solution of perchloric acid (62%) in concentrated sulphuric acid to the digest. Swirl the flask and heat at the same rate used in the previous stage for a further 10 min (the digest should clear within 3–5 min). Cool, transfer the digest quantitatively to a 100-ml volumetric flask, and dilute to the mark with distilled water. (If the digest is to be used for the determination of the other elements, it may be necessary to weigh the Kjeldahl flask before adding the sample, and to add concentrated sulphuric acid dropwise before dilution to give a constant mass of acid in sample digests and standards.) Transfer a 20-ml aliquot of the digest to a Hoskins apparatus, make strongly alkaline with *ca.* 10 *M* sodium hydroxide, and distil, collecting the ammonia in boric acid–indicator solution. Titrate with 0.01 or 0.02 *M* standard hydrochloric acid or sulphuric acid.

Results and discussion

Effect of time. The importance of timing was investigated for the three stages of the digestion procedure. Duplicate samples of five plant materials and of reagent blanks were heated after preheating times of 5, 15, and 20 min. The recoveries of nitrogen (compared with a standard method[2]) varied between 71% and 86% for the shortest period, but were always quantitative after 15–20-min standing.

Duplicate ground sub-samples of kale, Norway spruce needles and Calluna shoots were also boiled gently for periods of 0, 5, 10, 20, 30, and 60 min, after the recommended standing period of 20 min, before addition of the perchloric–sulphuric acid mixture and heating for a further 10 min. In each case, quantitative recovery of nitrogen was obtained within 10 min, and no increase was found on further heating. Initially, losses of nitrogen of 2–4% were found at longer heating periods, but these were later traced to slightly excessive heating rates (*cf.* Procedure). Eventually, a heating period of only 20 min was chosen; this allows a safety margin for complete oxidation, but minimizes the risk of nitrogen loss.

To examine the importance of heating time after the addition of the sulphuric–perchloric acid mixture, duplicate samples of Calluna shoots were heated for 0, 5, 10, 15, 20 and 25 min after clearing. Prolonged gentle heating at this stage was unnecessary, but caused no significant error. A final heating period of

10 min was selected to ensure complete oxidation.

Effect of perchloric acid concentration. Initial experiments were carried out by adding 1 ml of 4% or 0.4% (v/v) perchloric acid in sulphuric acid to duplicate samples of seven different plant materials. The latter concentration of perchloric acid was inadequate for most of the samples chosen, and 4% perchloric acid was chosen: this corresponds to the addition of only 40 µl of perchloric acid (62%), which is less than that used by other investigators, and is insufficient to cause localized high concentration. Stronger (5% and 9%) perchloric acid solutions were also satisfactory, but unnecessary for the samples investigated.

Comparison with standard method. The proposed method and a standard Kjeldahl method[2] were applied to several samples, and the proposed method was also applied to samples which had been analysed elsewhere (Tables I and II). Good agreement was obtained between the methods used. The figures shown for the ryegrass samples in Table II correspond to the difference between total N and nitrate N, so the proposed method clearly excludes nitrate N.

TABLE I

COMPARISON OF PROPOSED AND STANDARD METHODS

Sample	*%N, standard method*		*%N, proposed method*				
	Rep. A	*Rep. B*	*Rep. A*	*Rep. B*	*Mean of 12 results*[b]	*s*	s_r *(%)*
Pinus radiata (n)[a]	1.75	1.72	1.74	1.75	—	—	—
Pinus radiata (n)	1.76	1.77	1.74	1.74	—	—	—
Pinus sylvestris (n)	1.37	1.39	1.39	1.37	—	—	—
Pinus contorta (n)	1.12	1.13	1.14	1.15	—	—	—
Picea abies (n)	1.27	1.25	1.24	1.27	1.253	0.0161	1.3
Picea sitchensis (n)	1.29	1.29	1.30	1.30	—	—	—
Pseudotsuga (n)	1.24	1.22	1.23	1.26	—	—	—
Abies procera (n)	0.76	0.79	0.76	0.76	—	—	—
Abies grandis (n)	1.39	1.37	1.36	1.37	—	—	—
Calluna vulgaris (w)	0.31	0.32	0.30	0.30	0.31	0.0165	5.3
Calluna vulgaris (s)	1.06	1.05	1.05	1.06	1.023	0.0325	3.2
Fagus sylvatica (l)	1.32	1.34	1.34	1.30	—	—	—
Lolium perenne (l)	1.98	—	1.96	—	—	—	—
Phleum pratense (l)	1.80	—	1.81	—	—	—	—
Festuca rubra (l)	1.87	—	1.89	—	—	—	—

[a] n, Needles; w, woody stems; s, young shoots; l, leaves.
[b] For estimate of precision.

Precision of proposed method. To obtain an indication of the precision of the method, 12 subsamples of six samples were analysed by the proposed method (see final columns of Tables I and II). The greater spread of results at the two lowest levels of nitrogen (0.3–1.0% N) is probably due to the greater significance of titration errors and to the fact that the Calluna samples were more coarsely ground than the other samples. This could lead both to sampling errors for 100-mg subsamples, and to insufficient time for complete acid attack during the digestion.

TABLE II

ANALYSIS OF STANDARD SAMPLES

Sample	*Source*[a]	*%N*	*Proposed method*				
			No. of detns.	*Mean %N*	*Difference (%)*	*s*	*s_r (%)*
Barley	M.A.F.F.	2.01[b]	5	2.00	−0.5	—	—
Wheatings	M.A.F.F.	2.90[b]	12[c]	2.91	+0.3	0.0338	1.2
Grass	M.A.F.F.	1.94[b]	5	1.93	−0.5	—	—
Standard kale	Reading	4.32[b]	12[c]	4.31	−0.3	0.0431	1.0
Freeze-dried ryegrass	Roth.	2.05	6	2.08	+1.5	—	—
Freeze-dried ryegrass	Roth.	2.72	6	2.67	−1.8	—	—
Freeze-dried ryegrass	Roth.	3.00	6	2.93	−2.3	—	—
Freeze-dried ryegrass	Roth.	3.46	6	3.51	+1.4	—	—
Freeze-dried ryegrass	Roth.	3.78	6	3.78	0.0	—	—
Freeze-dried ryegrass	Roth.	4.57	6	4.53	−0.9	—	—
Freeze-dried ryegrass	Roth.	4.88	12[c]	4.86	−0.4	0.055	1.1

[a] M.A.F.F.: Nutrition Department, A.D.A.S., Shardlow; Reading: International Kale sample, University of Reading; Roth.: Rothamstead Experimental Station.
[b] Mean of several determinations.
[c] For estimate of precision.

A slight improvement in precision was obtained when these samples were reground and passed through a 0.2-mm sieve. Distillation and titration of a standard ammonium sulphate solution corresponding to the ammonium concentration in the digest of the Calluna stems also led to a standard deviation only slightly better than that of the original analyses. The precision could, however, be considerably improved by using a Mettler automatic titrator and titrating to constant pH.

The authors are indebted to Professor J. Tinsley for useful discussion, to Mr. R. E. Collier (A.D.A.S., M.A.F.F., Shardlow), Dr. Bowen (University of Reading) and Dr. Nowakowski (Rothamstead Experimental Station), for the kind provision of analysed samples; and to the Ministry of Agriculture, Fisheries and Food for the award of a research studentship to one of us (I.R.W.).

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Analytica Chimica Acta, 69 (1974) 488

ANNOUNCEMENTS

Short Summer Courses in X-Ray Powder Diffraction and X-Ray Spectrometry, State University of New York at Albany.

A one-week course in modern x-ray powder diffraction will be offered during June 17–21, 1974. The course will be tutorial in nature and will develop the basic theory and practical applications starting from elementary considerations. No previous knowledge or experience is required. Emphasis will be placed on the principles and practice of instrumentation, identification of powder patterns on both qualitative and quantitative basis and practical considerations on the use of the several indices as well as computer retrieval. Equal time will be devoted to lectures and laboratory-problem solving sessions. A suitable amount of time will be set aside for discussion of individual problems. The registration fee is $325.00 payable in advance.

A two-week course in modern x-ray spectrometry will be offered during June 3–14, 1974. The course will develop the basic theory and techniques starting from elementary principles. No previous knowledge or experience are required. The first week will cover basic principles and techniques and the second week will continue with further fundamentals and practical applications. Emphasis in the second week will be placed on advanced principles and techniques, absorption-enhancement corrections by mathematical methods, computer automation of modern x-ray spectrometers and energy-dispersive methods. Equal time will be devoted to lectures and laboratory-problem solving sessions. Registration may be made for one week, either week, at a registration fee of $325.00. or for the entire two-week session at a registration fee of $600.00, payable in advance.

For further information on these courses, please communicate with: Professor Henry Chessin, State University of New York at Albany, Department of Physics, 1400 Washington Avenue, Albany, New York 12222.

Journées de Calorimétrie et d'Analyse Thermique

The forthcoming "Journées de Calorimétrie et d'Analyse Thermique", the annual meeting of the French Association of Calorimetry and Thermal Analysis (AFCAT), organized in 1974 with the scientific collaboration of the Group of Experimental Thermodynamics (GTE), will be held at the University of Rennes (France), on May 9 and 10, 1974. The scientific programme will consist of four sessions:

Sessions 1, 2: Calibration in Calorimetry and Thermal Analysis.

Session 3: Determination, by calorimetry and thermal analysis, of thermodynamic data related to phase changes.

Session 4: Calorimetry and Thermal Analysis.

The registration fee will amount to 110 FF. This includes the cost of the volume of preprints. All inquiries should be sent to the organizers, J. P. Auffredic and C. Carel, at the following address: Secrétariat des Journées de Calorimetrie et d'Analyse Thermique, Laboratoire de Cristallochimie, U.E.R. "S.P.M", Université de Rennes I, Avenue du Général Leclerc, 35031 Rennes Cedex, France.

Analytica Chimica Acta, 69 (1974) 489

BOOK REVIEWS

H. Bennett and R. A. Reed, *The Accuracy of Industrial Ceramic Analysis: Second Survey*, Special Publ. 80, 1973, British Ceramic Research Association, Stoke-on-Trent ST4 7LQ, England, 26 pp., price £2.50.

A statistical survey of the results obtained for fourteen different types of sample common in the ceramics industry, by the various analytical methods used in forty firms, is described. Some recommendations are outlined.

W. Westland and B. S. Cooper, *Analytical Methods in Use in Non-ferrous Mining and Metallurgy: A Selective Review*, Institution of Mining and Metallurgy, London, 1973, viii + 54 pp., price £3.50.

Applications of atomic absorption and emission spectrometry, and of X-ray methods in the industry are first discussed. The determination of the elements by classical and modern techniques is then reviewed.

Standard Reference Materials—1973 Catalog, N.B.S. Special Publ. 260, April 1973, U.S. Government Printing Office, Washington, D.C. 20402, 96 pp., price $ 1.25.

This catalog contains full information on the standards of certified chemical composition, standards of certified physical properties, engineering type standards, research materials and general materials which are currently available from N.B.S.

PUBLICATIONS RECEIVED

Codata newsletter 9 and 10, Committee on Data for Science and Technology, I.C.S.U., ICSU/CODATA Central Office, 6 Frankfurt/Main, West Germany.

Proceedings of Third International Codata Conference on Generation, Compilation, Evaluation and Dissemination of Data for Science and Technology, June 1972, Codata Central Office, 6 Frankfurt/Main, West Germany, 1973, 100 pp., price DM 30.–.

AUTHOR INDEX

ANALYTICA CHIMICA ACTA, VOL. 69 (1974)

SUBJECT INDEX

(Continued from page 4 of cover)

CONTENTS

(continued on inst