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THE DETERMINATION OF PHENOBARBITAL AND DIPHENYLHYDANTOIN IN BLOOD BY DIFFERENTIAL PULSE POLAROGRAPHY

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(Received 3rd October 1972)

The determination of phenobarbital and diphenylhydantoin in blood has mainly been accomplished by spectrophotometric and more recently by gas-chromatographic techniques. Spectrophotometric methods for the determination of phenobarbital were reported by Lous[1] and Goldbaum[2]. Spectrophotometric methods for the determination of diphenylhydantoin using the u.v. absorbance of the compound[3,4], the Bratton–Marshall chromophore[5,6] [formed by nitration, reduction, diazotization and coupling with N-(1-naphthyl)ethylenediamine dihydrochloride] and the benzophenone[7–9] (formed by alkaline permanganate oxidation) have been reported.

Gas-chromatographic techniques involve the formation of derivatives to obtain analytically-usable symmetrical peaks. Diphenylhydantoin has been converted to a methoxy derivative[10,11] and a trimethylsilyl derivative[12] and analyzed in this manner. Kupferberg[13] and Van Meter *et al.*[14] have analyzed blood containing phenobarbital, diphenylhydantoin and pyrimidone by gas chromatography by means of their respective methoxy derivatives.

The principle of controlled nitration is applicable to the analysis of a wide variety of phenyl-ring-containing drugs which may lack functional groups amenable to other methods of analysis. The present work involves the controlled nitration and polarographic procedures as outlined by de Silva and Hackman[15] in the determination of Glibornuride in blood. The polarography of the nitro-derivatives of phenobarbital and diphenylhydantoin (Fig. 1) enabled these compounds to be determined with a sensitivity of 1–2 μg ml^{-1} in blood, which is similar to that of the gas-chromatographic assays reported.

EXPERIMENTAL

Reagents

All reagents used were of analytical-grade purity (>99%) and were used without any further purification.

1 M Phosphate buffer (pH 7.0). Mix 390 ml of 1 *M* potassium dihydrogenphosphate and 610 ml of 1 *M* dipotassium monohydrogenphosphate. Check the pH and adjust to 7.0 with either solution as necessary.

Chloroform, hexanes (mixture of isomers) (Fischer), methanol, isopropanol (J. T. Baker), and absolute ethanol (Pharmoco). Ethyl acetate was of spectroquality grade (Matheson, Coleman and Bell).

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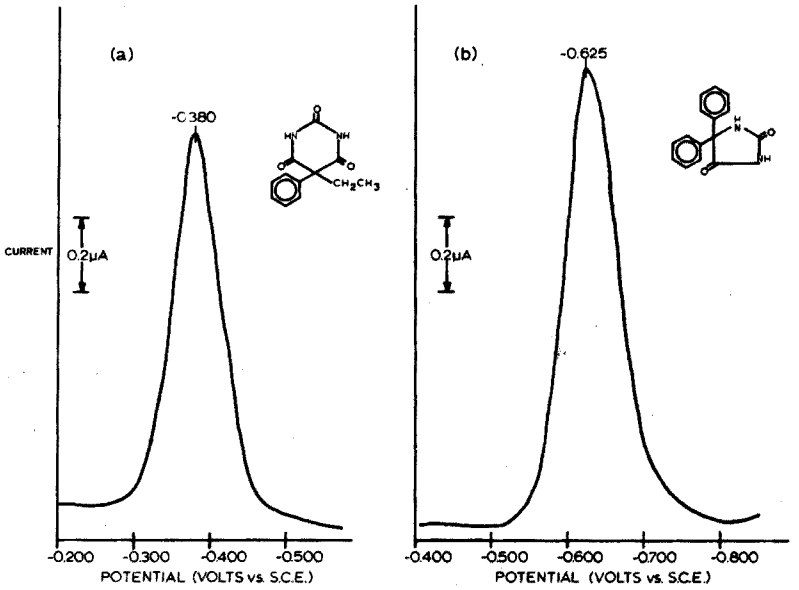


Fig. 1. Polarograms of (a) nitro-derivative of 20 μg of phenobarbital (supporting electrolyte 1 *M* phosphate buffer pH 7) and (b) nitro-derivative of 15 μg of diphenylhydantoin (supporting electrolyte 0.1 *M* NaOH). Differential pulse mode with a (−)50 mV pulse applied to a 0.5-s drop using a scan rate of 5 mV s^{-1}.

Nitration mixture. Dissolve 5 g of potassium nitrate in 50 ml of concentrated sulfuric acid. Store in a refrigerator. Prepare freshly weekly because of limited shelf life.

Standard solutions. Phenobarbital (5-ethyl-5-phenylbarbituric acid; U.S.P. grade, Merck & Co.) and diphenylhydantoin (phenytoin or 5,5-diphenylhydantoin; Matheson, Coleman and Bell) were used. Dissolve 10.0 mg of phenobarbital or diphenylhydantoin in 100 ml of absolute ethanol to give stock solutions containing 100 μg ml^{-1}. Dilute 1 ml of each stock solution to 10 ml with ethanol to give working solutions containing 10.0 μg ml^{-1}. Suitable aliquots of these solutions are added to blood as internal standards.

Parameters for polarographic analysis

A P.A.R. Model 171 Polarographic Analyzer (Princeton Applied Research Corporation) equipped with a P.A.R. Model 172 Drop Timer and electrode assembly was used.

A three-electrode cell containing a dropping mercury electrode (D.M.E.; capillary tube of inside diameter 0.05–0.08 mm; E. H. Sargent #S-29417) as the indicator electrode, a saturated calomel electrode (S.C.E.; fiber junction calomel; Beckman #39178) as the reference electrode, and a platinum wire as the auxiliary electrode[15]. The drop mass was 2.32 mg s^{-1} ($m^{\frac{2}{3}} t^{\frac{1}{3}} = 1.5613$), and the drop rate was 2 drops s^{-1}.

Polarography was performed in the differential pulse polarographic mode with a (−)50 mV pulse being applied at a 0.5-s drop[16]. Scans were performed from

(−)0.200 V to (−)0.600 V and (−)0.400 V to (−)0.900 V *vs.* S.C.E. for the phenobarbital and diphenylhydantoin, respectively, at a scan rate of 5 mV s^{-1} with a full scan range of 1.5 V. The usual required current sensitivity was 1, 2 or 5 μA full-scale deflection.

Determination of phenobarbital or diphenylhydantoin in blood

To a 15-ml conical glass-stoppered centrifuge tube add 1 ml of whole blood or serum sample, 1 ml of 1 *M* phosphate buffer (pH 7) and 5 ml of chloroform. Stopper the tube (seal stopper with a drop of distilled water), shake on a reciprocating shaker for 10 min at a moderate speed and then centrifuge for 5 min at 2000 rev min^{-1}. Along with the samples, process a 1-ml specimen of control whole blood or serum, and separate 1-ml specimens of control whole blood or serum containing 5.0, 10.0, and 20.0 μg of phenobarbital or diphenylhydantoin as internal standards; prepare these by adding 0.5, 1.0 and 2.0 ml of the working standards to 15-ml conical centrifuge tubes, evaporating the ethanolic solution to dryness in a 65° water bath under a stream of nitrogen and then adding 1 ml of control specimen to the residue. Carefully remove the lower chloroform layer (*ca.* 4.8 ml) with a 5-ml hypodermic syringe (Becton, Dickinson & Company, Rutherford, N.J., Catalog #2141) equipped with a 20-gauge, 6-in cannula (from the same Company) and transfer this layer to another 15-ml conical glass stoppered centrifuge tube. (In order to ensure that only chloroform is drawn into the syringe, draw a small amount of air into the syringe before insertion into the sample. This air bubble is then expelled when the cannula is inserted into the chloroform phase thus discharging any aqueous phase drawn into the cannula back into the upper layer.) Reextract the sample with a second 5-ml portion of chloroform as described above. Combine the two extracts in a 15-ml conical centrifuge tube, and evaporate to dryness in a 65° water bath under a stream of nitrogen. Dissolve the residues in 3.0 ml of methanol and 2.5 ml of aqueous 0.25 *N* hydrochloric acid by mixing on a Vortex super-mixer. Back-wash the methanolic acid phase with 5.0 ml of (isomeric) hexanes by shaking for 10 min at moderate speed on a reciprocating shaker, centrifuging for 5 min at 2000 rev min^{-1} and aspirating off the hexane layer. Repeat the back-wash step with another 5 ml of hexane. Add 5 ml of chloroform to the hexane-washed methanolic acid solution, seal the stopper in the usual manner, shake on a reciprocating shaker for 10 min at moderate speed, and then centrifuge for 5 min at 2000 rev min^{-1}. Transfer the lower chloroform layer as previously described to a 15-ml conical centrifuge tube. Re-extract the methanolic acid solution with 5 ml of chloroform and combine with first chloroform extract. The combined chloroform extract is evaporated to dryness in a 65° water bath under a stream of nitrogen. (Note: if both diphenylhydantoin and phenobarbital are present in the original sample a thin-layer chromatographic step is included in the assay at this point, see below.) To the residue add 0.3 ml of the nitration mixture (at room temperature). Mix on the Vortex super-mixer for 10 s and then stopper. At this point in the analysis include a set of external standards of 5.0, 10.0 and 20.0 μg of phenobarbital or diphenylhydantoin for the determination of percentage recovery, prepared as described for the internal standards. Dissolve the residue of the ethanolic solutions in 0.3 ml of the nitration mixture. Allow the tubes to stand at room temperature for 1 h to complete the nitration reaction. Carefully evacuate the nitration mixture in

each tube under vacuum (*ca.* 50 mm Hg) for 15 min to remove any residual nitrous acid present as NO_2 vapor. This step is performed in a vacuum desiccator containing lime as the desiccant, with a dry ice–acetone solvent trap connected between the desiccator and the vacuum pump to trap the volatile acid vapors. Carefully add 2.7 ml of distilled water to each tube, mix well on the super-mixer and evacuate from a water line aspirator for 1 min. Add 5 ml of ethyl acetate to each tube, stopper and extract the nitro-derivative of the compound on a reciprocating shaker for 10 min. Centrifuge the samples for 5 min at 2000 rev min^{-1} and transfer the ethyl acetate supernate to a 15-ml conical centrifuge tube. Repeat the extraction with another 5 ml of ethyl acetate and combine the extracts.

Evaporate the combined ethyl acetate extract to dryness in a 75° water bath under a stream of nitrogen. To the residues containing the nitro-derivative of phenobarbital, add 5 ml of 1 *M* phosphate buffer (pH 7.0); add 5 ml of 0.1 *M* sodium hydroxide to those containing diphenylhydantoin. Mix well on the Vortex super-mixer and deoxygenate the samples for 5 min with nitrogen gas bubbled in through a coarse-porosity micro filter stick (Scientific Glass Apparatus Inc., Bloomfield, N.J.). Transfer the deoxygenated sample to the polarographic cell[15] and analyse the samples for phenobarbital between (−)0.200 V and (−)0.600 V *vs.* S.C.E., and the samples for diphenylhydantoin between (−)0.400 V and (−)0.900 V *vs.* S.C.E. using the differential-pulse mode of operation, and the polarographic parameters described above. The polarograms are recorded on the X-Y recorder and show analytical peaks at $E_p = (-)0.380$ V and (−)0.625 V *vs.* S.C.E. for phenobarbital and diphenylhydantoin respectively.

Thin-layer chromatographic separation of samples containing a mixture of phenobarbital and diphenylhydantoin

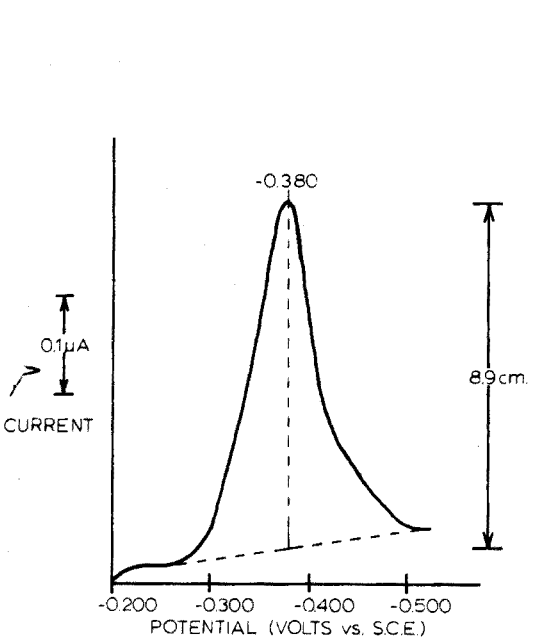
Dissolve the residues from the combined chloroform extracts in 100 μl of acetone and transfer quantitatively onto a 20 × 20 cm Brinkman (F_{254}) silica gel G chromatoplate. Develop the plate in a vapor-saturated chamber using isopropanol: chloroform: 25% aqueous ammonia (38:57:5) until the solvent front has ascended 15 cm. Examine the plate under short wave u.v., and identify the areas on the silica gel corresponding to phenobarbital ($R_F = 0.53$) and diphenylhydantoin ($R_F = 0.65$) by the R_F values of 10 μg of authentic standards run alongside the sample extracts. Scrape off these areas and transfer to a 15-ml centrifuge tube to which 5 ml of ethanol is added. Slurry the tubes on a Vortex mixer for *ca.* 1 min, and centrifuge for 5 min at 2000 rev min^{-1} to spin down the silica gel. Transfer the ethanol supernate to another 15-ml conical centrifuge tube and evaporate to dryness. The respective residues are then nitrated and polarographed as described above.

Calculations

Calculate the resultant currents from the polarographic reduction of nitrated phenobarbital and diphenylhydantoin on the basis of their peak heights measured at (−)0.380 V and (−)0.625 V *vs.* S.C.E., respectively, as shown in Fig. 2. A correction for the control or nitration blank is necessary (Fig. 3). The resultant current is calculated as follows:

$$(h_s r_s - h_c r_c)/25.4 \text{ cm} = \mu A_s$$

where h_s = peak height of sample (in cm),
h_c = peak height of control (in cm),
r_s = current scale of sample (in μA),
r_c = current scale of control (in μA),
μA_s = total resultant current sample corrected for control (in μA),
25.4 cm = full-scale deflection.



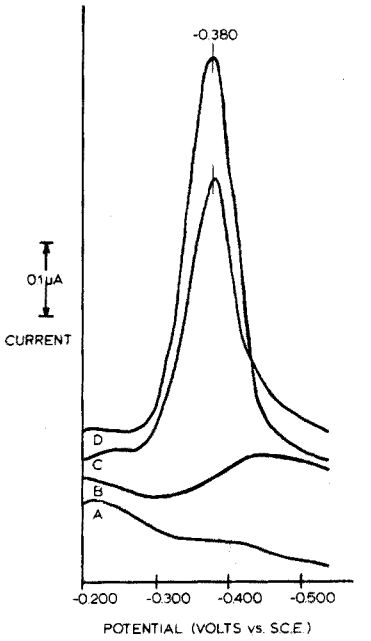


Fig. 2. Sample calculation of the resultant current for phenobarbital. (8.9 cm/25.4 cm) × 1 μA = 0.350 μA.

Fig. 3. Polarograms of nitrated phenobarbital. (A) Reagent blank; (B) blood control; (C) internal standard 10 μg; (D) external standard 10 μg.

The concentration of phenobarbital and diphenylhydantoin is calculated on the basis of the resultant current of the internal standard in the usual way. The current (μA) per μg of the internal standards can then be compared directly to that of the external standards to obtain percent recovery. Alternatively, the percent recovery can be determined from the slope values ($\mu A\ \mu g^{-1}$) of standard curves (Fig. 4).

RESULTS AND DISCUSSIONS

Investigation of analytical parameters

Nitration conditions. The optimal temperature for nitration was determined by reacting 10 μg of phenobarbital and diphenylhydantoin for a period of 1 h at 0°, 25°, 65°, and 105°, and observing the conditions which yielded one nitro-derivative of high sensitivity ($\mu A\ \mu g^{-1}$). Polarographic analysis (Figs. 5A and 5B) showed that nitration at either 0° or 25° yielded essentially one nitro-derivative of high sensitivity for both compounds. A time-course study on nitration at 25° was conducted

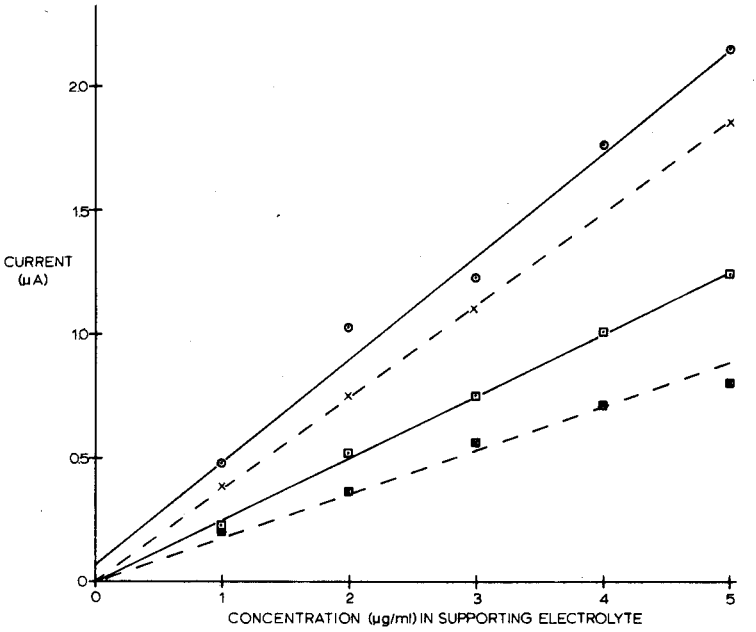


Fig. 4. Polarographic calibration curves for phenobarbital and diphenylhydantoin. (□) External phenobarbital; (⊠) internal phenobarbital; (○) external diphenylhydantoin; (×) internal diphenylhydantoin.

on 20 μg of each compound. The data indicated that nitration for 1 h was sufficient for an optimal yield of the nitro-derivatives.

Choice of supporting electrolyte. Nitro-derivatives of 10-μg samples of each compound, were analysed by polarography in 1 *M* phosphate buffers ranging from pH 1.5 to 12.8, and at pH 14 in 1 *M* sodium hydroxide. Graphs of E_p (V *vs.* S.C.E.) *versus* pH (Fig. 6) indicated that both derivatives were reduced at more negative potentials with increase in pH. At pH 14, it was possible to distinguish a mixture of the nitro-derivatives of phenobarbital and diphenylhydantoin; there was a 180 mV separation of the peaks. However, the sensitivity ($\mu A\ \mu g^{-1}$) for phenobarbital was extremely low at this pH. Although the diffusion current, I_D (μA) *vs.* pH plot (Fig. 7) indicated that the highest sensitivity for diphenylhydantoin could be obtained at pH 3.0, the need for exhaustive deaeration to remove all traces of oxygen precluded its use. The use of pH 5.5 buffer for polarography of both compounds appeared to be best but problems were caused by impurities in the supporting electrolyte in the potential range [(−)0.280 V *vs.* S.C.E.] of interest. Interferences from biological material co-extracted from blood were minimized by the use of 1 *M* phosphate buffer (pH 7.0), and 0.1 *M* sodium hydroxide for the polarography of the nitro-derivatives of phenobarbital and diphenylhydantoin, respectively.

Polarography of the nitro-derivatives of the two compounds indicated that the sensitivity ($\mu A\ \mu g^{-1}$) for diphenylhydantoin was nearly twice that for phenobarbital, indicating a significant difference in the chemical structure of the two derivatives. Rapid-scan d.c. polarography was performed on 20-μg samples of

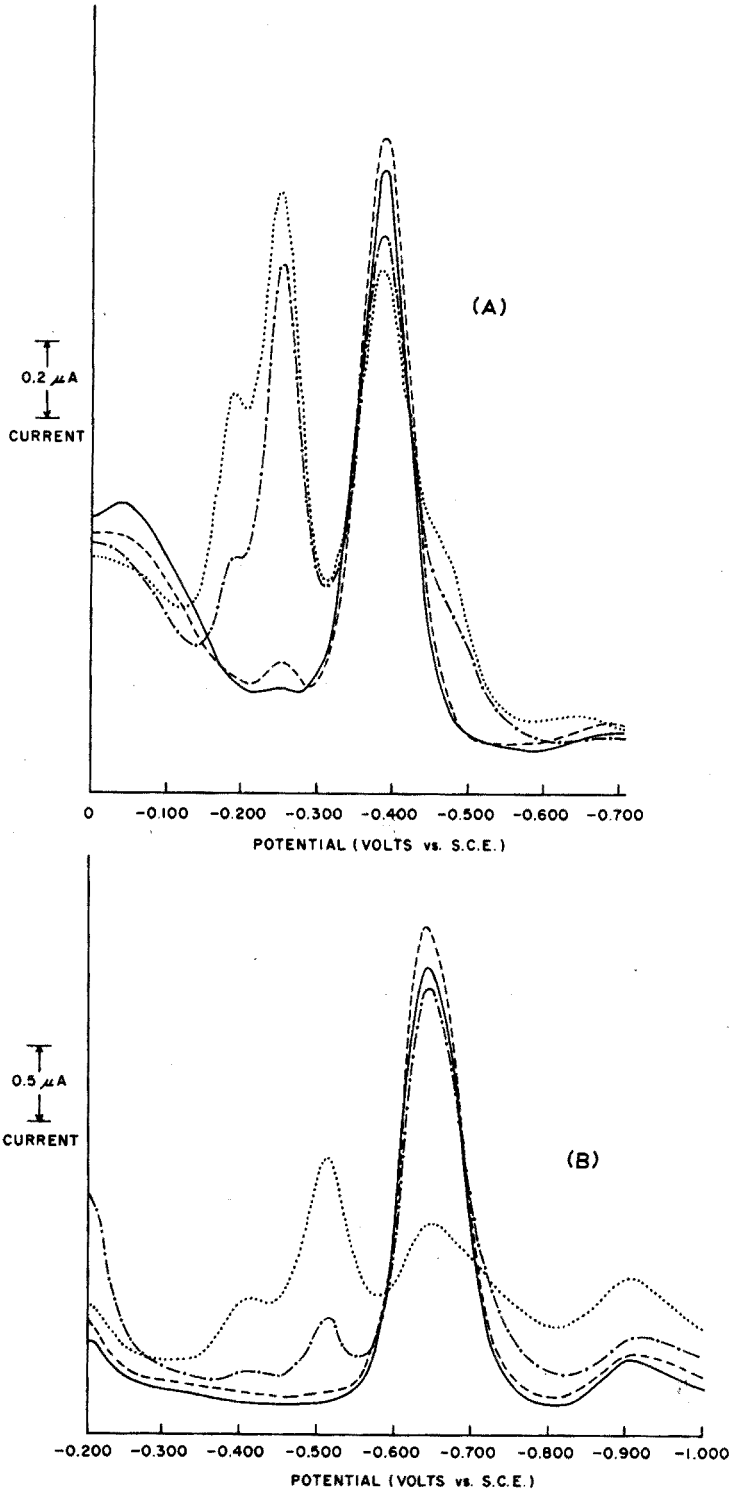


Fig. 5. Effect of nitration temperature on phenobarbital (A) and diphenylhydantoin (B). (——) 0°; (– – – –) 25°; (—·—·) 65°; (·····) 105°.

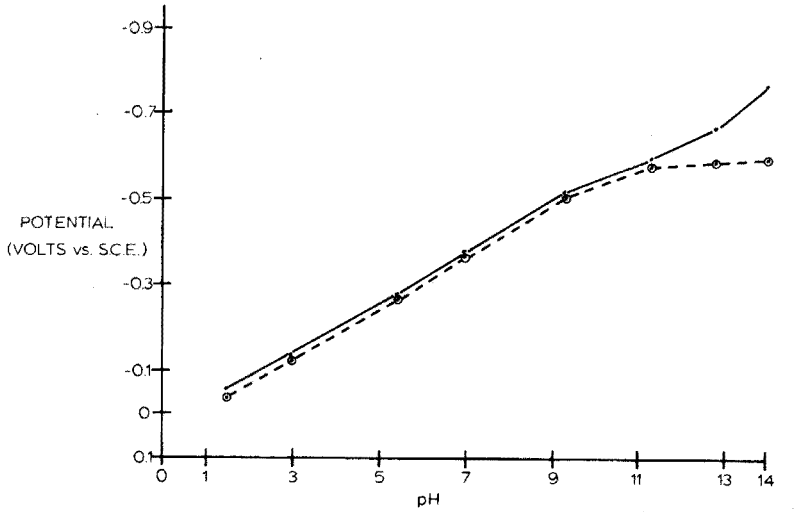


Fig. 6. Effect of pH on E_p for nitro-derivatives of phenobarbital (——) and diphenylhydantoin (– – – – –).

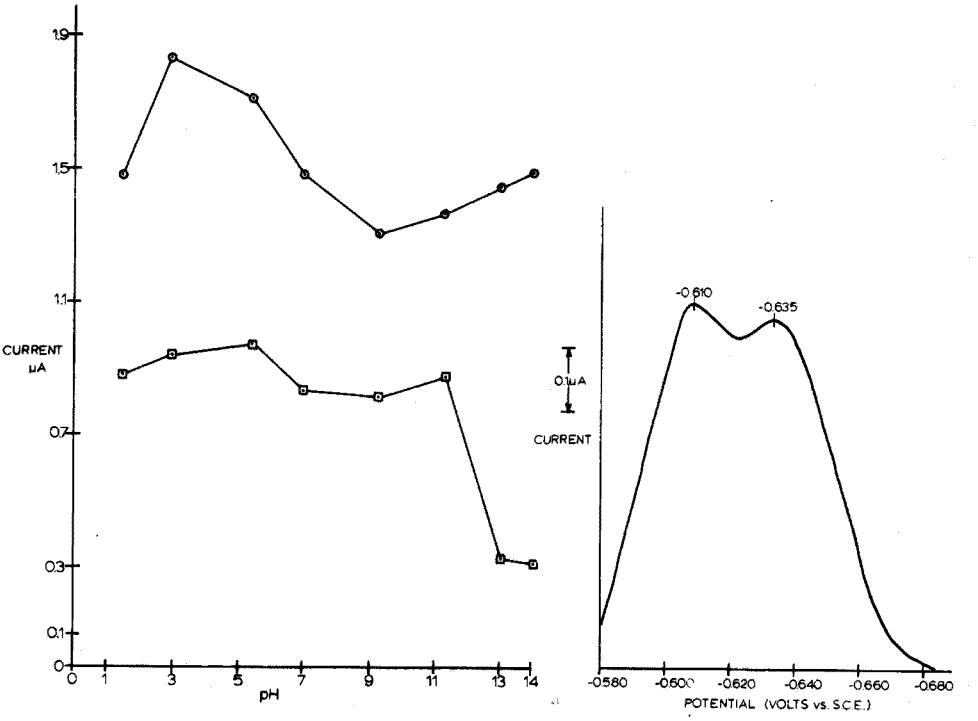


Fig. 7. Effect of pH on I_D for nitro-derivatives of phenobarbital (□) and diphenylhydantoin (○).

Fig. 8. Rapid-scan d.c. polarogram of 20 μg of diphenylhydantoin (scan from (−)0.540 V to (−)0.700 V *vs.* S.C.E., at 100 mV s^{-1}, with a 0.3-V scan range in 1.0 *M* NaOH).

nitrated diphenylhydantoin at a hanging mercury drop electrode. The samples were scanned from (−)0.540 V to (−)0.700 V *vs.* S.C.E. in 1 *M* sodium hydroxide, at a rate of 100 mV s^{-1} and a scan range of 0.3 V. The polarograms showed two distinct peaks for the derivative at $E_p = (-)0.610$ V and (−)0.635 V *vs.* S.C.E., respectively (Fig. 8). This is probably because diphenylhydantoin is nitrated in both phenyl rings; the two reducible nitro-groups would account for the two-fold increase in sensitivity over nitrated phenobarbital. It is assumed that both compounds are nitrated in the *m*-position[17].

Sample preparation. Considerable problems were encountered in the nitration of phenobarbital and diphenylhydantoin in blood extracts because of coextracted impurities. Optimal extractability was established by extraction into ethyl acetate, diethyl ether, benzene, and chloroform from blood buffered to pH 1, 3, 5, 7 and 9. Chloroform extracts of blood at pH 7 gave the highest recoveries. However, this extract could not be nitrated directly because of interferences from co-extracted impurities. It was found that sample "clean-up" of the initial chloroform extract was needed to perform direct nitration. The hexane back-wash step included in this assay is a modification of the "clean-up" procedure reported for diphenylhydantoin and phenobarbital by Kupferberg[13].

The quantitative analysis of a mixture of the two compounds is based on a modification of a t.l.c. separation step reported[18] for diphenylhydantoin and phenobarbital, which provides sufficient resolution of both compounds. Absolute ethanol was a better solvent for elution from the t.l.c. plate than either ethanol: 0.1 *M* HCl (50:50), ethanol: 0.1 *M* HCl (90:10), 0.1 *M* HCl or 1 *M* HCl. Elution by rapid slurrying with a mixer was more efficient than agitation on a reciprocating shaker.

The finalized assay has an overall recovery from blood of 72.3%±6.5 (s_r) and 76.7%±2.3 (s_r) for phenobarbital and diphenylhydantoin, respectively. The sensitivity limit of the assay for both compounds is 1–2 μg ml^{-1} of blood with a 1-ml sample per assay and a 2:1 sample to blank diffusion current (I_D) value as a limit of detectability. In the determination of the compounds in a mixture which includes the t.l.c. separation step, the overall recovery from blood declines to 47.7%±4.6 (s_r) and 41.1%±3.6 (s_r) for phenobarbital and diphenylhydantoin, respectively. This is due to the poor recovery from the silica gel which is of the order of 73.6%±4.5 (s_r) for phenobarbital and 65.4%±3.9 (s_r) for diphenylhydantoin. The sensitivity limit for both compounds under these conditions is approximately 2 μg ml^{-1} of blood.

Selectivity of the assay

The assay is specific for either phenobarbital or diphenylhydantoin when administered singly. Concomitant administration of the two drugs requires the inclusion of the t.l.c. step. The selectivity of the assay will be affected by the concomitant administration of other drugs which extract under the above-mentioned conditions and contain a nitratable phenyl ring such as pyrimidone, benzodiazepines and phenothiazenes, or others such as nitroimidazoles which contain a reducible nitro-group in the molecule. If interfering drugs are known to be present, then two-dimensional t.l.c. is required to ensure specificity.

Application of the method to biological specimens

Phenobarbital levels were measured in two patients after treatment with

TABLE I

DETERMINATION OF PHENOBARBITAL AND DIPHENYLHYDANTOIN IN HUMAN BLOOD SPECIMENS

Sample	*Phenobarbital ($\mu g\ ml^{-1}$)*	*Diphenylhydantoin ($\mu g\ ml^{-1}$)*
1[a]	5.7	—
2[a]	3.4	—
3[b]	22.2	6.8
4[b]	—	6.6
5[b]	—	6.2

[a] Analyses for single component.
[b] Analyses for mixture, with additional 2-dimensional t.l.c.

phenobarbital (samples 1 and 2), and phenobarbital in combination with diphenylhydantoin and diazepam (sample 3). Blood samples (#4 and #5) were also received from another patient receiving diphenylhydantoin and pyrimidone, a phenobarbital analog. The results (Table I) demonstrate the feasibility of the method. Samples 1 and 2 were analyzed by the method described for single component analysis, whereas the analysis of Sample 3 included the t.l.c. separation step. A two-dimensional t.l.c. system with chloroform:acetone:isopropanol (80:6:4) for the second development was used to separate further diazepam from the phenobarbital and diphenylhydantoin. Diazepam was determined from the same t.l.c. plate. Samples 4 and 5 which contained a mixture of diphenylhydantoin and pyrimidone were analyzed for diphenylhydantoin after two-dimensional t.l.c. with chloroform:methanol (80:20) for the second development.

SUMMARY

A sensitive differential pulse polarographic assay was developed for the determination of phenobarbital or diphenylhydantoin in blood. The assay involves the selective extraction of the compound into chloroform from whole blood buffered to pH 7.0. After suitable "clean-up" of the sample, each compound is nitrated in 10% potassium nitrate in sulfuric acid at 25° for 1 h. The nitro-derivatives are extracted into ethyl acetate, and the residues are dissolved in 1 *M* phosphate buffer (pH 7.0) or 0.1 *M* sodium hydroxide for phenobarbital and diphenylhydantoin, respectively; the solutions are deoxygenated, and analyzed by differential pulse polarography. The overall recovery of phenobarbital and diphenylhydantoin from blood was $72.3\% \pm 6.5$ (s_r) and 76.7 ± 2.3 (s_r) respectively. The sensitivity limit is 1–2 $\mu g\ ml^{-1}$ of blood for both compounds. A modified assay for the determination of both compounds in blood with t.l.c. separation was also developed.

RÉSUMÉ

On propose une méthode polarographique différentielle sensible pour le dosage de phénobarbital ou de diphénylhydantoïne dans le sang. Elle consiste en une extraction sélective dans le chloroforme, à partir du sang total, tamponné à pH 7.0. On procède ensuite à une nitration par le nitrate de potassium en milieu

acide sulfurique. Les nitrodérivés sont extraits dans l'acétate d'éthyle. Les résidus sont finalement dissous dans un tampon phosphate (pH 7.0) et analysés par polarographie. La limite de sensibilité est de 1 à 2 μg ml^{-1} de sang pour les deux composés.

ZUSAMMENFASSUNG

Für die Bestimmung von Phenobarbital und Diphenylhydantoin in Blut wurde eine empfindliche Methode der Differential-Pulse-Polarographie entwickelt. Bei dem Verfahren wird die Verbindung aus dem auf pH 7.0 gepufferten Blut mit Chloroform selektiv extrahiert. Nach einer geeigneten Vorbehandlung der Probe wird jede Verbindung mit 10% Kaliumnitrat in Schwefelsäure 1 h lang bei 25° nitriert. Die Nitro-Derivate werden mit Äthylacetat extrahiert. Der Rückstand wird im Falle von Phenobarbital in 1 *M* Phosphatpuffer (pH 7.0) und im Falle von Diphenylhydantoin in 0.1 *M* Natriumhydroxid gelöst; die Lösungen werden nach Entfernung des Sauerstoffs durch Differential-Pulse-Polarographie analysiert. Der insgesamt aus Blut erfasste Anteil von Phenobarbital und Diphenylhydantoin betrug 72.3% ± 6.5 (s_r) bzw. 76.7% ± 2.3. Die Empfindlichkeitsgrenze für beide Verbindungen in Blut ist 1–2 μg ml^{-1}. Ein abgewandeltes Verfahren für die Bestimmung beider Verbindungen in Blut unter Anwendung einer dünnschichtchromatographischen Trennung wurde ebenfalls entwickelt.

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ANION EXCHANGE OF SOME ELEMENTS IN ACETIC ACID–HYDROCHLORIC ACID MEDIUM

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(Received 14th October 1972)

In previous work[1–3], the adsorption of 65 elements from aqueous acetic acid solutions on the strong base anion-exchange resin Dowex 1-X8 (100–200 mesh) has been studied. Although the physicochemical interpretation of the results remains uncertain, the usefulness of acetic acid as an eluent for group and individual anion-exchange separations has been clearly demonstrated[2].

In the work described below a more detailed investigation of the nature of the metal complexes that were strongly adsorbed from concentrated acetic acid solutions was carried out. First, the adsorbabilities of some elements from aqueous acetic acid solutions on Dowex 1-X8 resin in the acetate form (RAc) and in the chloride form (RCl) were compared. The ions under investigation were: Cd(II), Ni(II), Mn(II), Ga(III), La(III), Mo(VI) and bromide. Secondly, the distribution coefficients of Ni(II), Mn(II) and Cd(II) on RAc resin were determined as a function of the hydrochloric acid concentration in the aqueous acetic acid solutions. Finally, the results of these experiments were applied to achieve a less cumbersome adsorption step in the anion-exchange separation procedure in acetic acid medium. This was demonstrated for the Ni(II)–Mn(II)–Cd(II) separation.

EXPERIMENTAL

Chromatographic methods

The resin used was the strong base anion exchanger Dowex 1-X8, 100–200 mesh, chloride or acetate form. The exchanger, available in the chloride form, was purified by a column conditioning procedure with sodium hydroxide and hydrochloric acid[4]. The resin bed was eventually conditioned with glacial acetic acid, followed by thorough rinsing with water and drying *in vacuo* over phosphorus pentoxide. The total exchange capacity of this dry RCl-resin was evaluated[5] as being 3.72 meq g^{-1}. The specific bed volume at 25° varied from 2.171 to 2.392 ml g^{-1} of dry resin in water and 17.0 *M* acetic acid, respectively[2]. For the conversion into the acetate form, a RCl-resin column, after being purified as mentioned above, was washed with 75 bed volumes of 1 *M* sodium acetate, whereafter it was treated with anhydrous acetic acid and dried as described above. The dry RAc-resin obtained had a total exchange capacity[5] of 3.44 meq g^{-1}, and the residual chloride content was evaluated by a non-destructive neutron activation analysis[6] as being

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0.012 meq g^{-1} of dry resin. The specific bed volume at 25° ranged from 2.377 to 2.622 ml g^{-1} of dry resin in water and 17.0 *M* acetic acid, respectively[5]. In one case, namely in the anion-exchange separation of Ni(II)–Mn(II)–Cd(II), use was made of Dowex 1-X8, 200–400 mesh, chloride form. This resin was pretreated in the same way as described above.

The adsorbabilities of the elements are expressed as weight distribution coefficients K_d (amount of ion per g of dry resin/amount of ion per ml of solution). They were determined by batch equilibration[7]. Weighed amounts of dry resin were agitated with known volumes of acetic acid or acetic–hydrochloric acid solutions, containing the elements under investigation. After the resin particles had been filtered off, the concentration of the element in the liquid phase was compared to the initial concentration. The adsorption data were obtained under conditions of low loading ($\leqslant 1\%$). An equilibrium time of 15 h (overnight) was found to be sufficient in all cases. All experiments were carried out at 25°.

The anion-exchange separations were performed on resin columns of 8 mm diameter and 75 mm height. The elements were added as 1 ml of solution, containing $4 \cdot 10^{-2}$ mmol of nickel(II), $4 \cdot 10^{-3}$ mmol of manganese(II) and $2 \cdot 10^{-2}$ mmol of cadmium(II). The adsorption and elution rates were kept constant at 2 ml min^{-1} cm^{-2} and 4 ml min^{-1} cm^{-2}, respectively. The eluates were collected in fractions of 64 drops (*ca.* 1 ml) with the aid of an automatic fraction collector. The elution of an element was considered as quantitative as soon as less than 0.1% of the original amount was left on the column. All experiments were performed at 25°.

Analytical methods and radioactive tracers

All analyses were carried out radiometrically. As all the tracers used in this work were γ-emitting nuclides, the activities were measured by integral counting in a NaI(Tl) well-type detector. For the purity control, especially during the anion-exchange separations, Ge(Li) γ-spectrometry was used[8]. Activity measurements of series of samples were done with the aid of an automatic sample changer. In some cases of transient equilibria (^{99}Mo–^{99m}Tc, ^{115}Cd–^{115m}In), the establishment of the mother–daughter equilibrium had to be taken into account.

The ^{54}Mn isotope was obtained by ion-exchange separation from an iron target irradiated in the BR-2 reactor[9]. The other isotopes were prepared by neutron irradiation of appropriate target materials in the Thetis reactor at a neutron flux of 10^{12} n cm^{-2} s^{-1}.

The tracer solutions were prepared as described earlier[2]. For the anion-exchange separations, the irradiated target compounds ($NiCO_3 \cdot 2Ni(OH)_2 \cdot 4aq$; $MnCO_3$; CdO) were dissolved in 100 μl of 12 *M* hydrochloric acid and diluted with 10 ml of aqueous acetic acid solution.

RESULTS

Comparative study of RCl- and RAc-resin

The influence of the exchangeable anionic groups of the resin on the adsorbability of the elements was examined by comparing the K_d values for the systems: aqueous acetic acid/RCl and aqueous acetic acid/RAc. The ions investigated

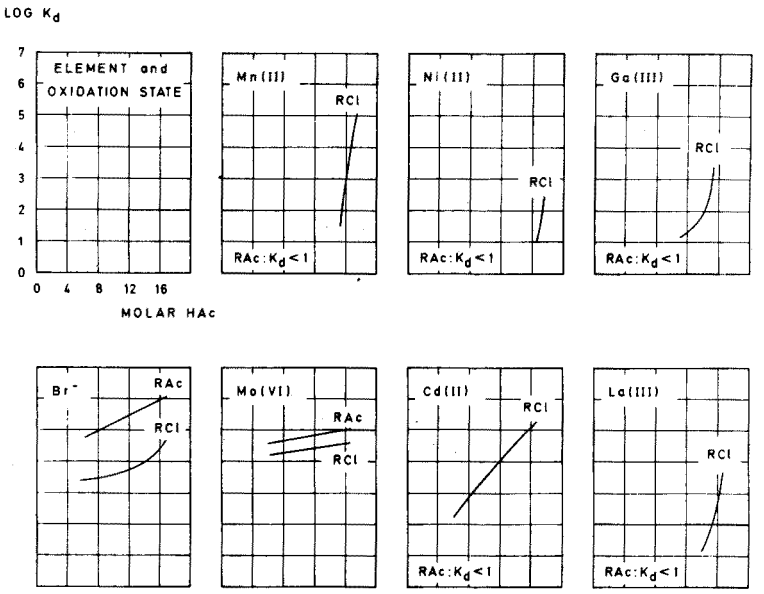

Fig. 1. Comparison of K_d values from acetic acid solutions on RCl- and RAc-resin.

were: Cd(II), Ni(II), Mn(II), Ga(III), La(III), Mo(VI) and bromide. The experiments were performed with 100 mg of dry resin, shaken with 26 ml of tracer solution of the desired acetic acid concentration.

From the results (Fig. 1), some striking conclusions can be drawn.

1. Over the whole molarity range of acetic acid, Cd(II), Ni(II), Mn(II), Ga(III) and La(III) show a negligible adsorption for the RAc-resin. But these elements are strongly adsorbed from concentrated acetic acid on the RCl-resin, the K_d values sharply increasing with the acetic acid concentration. In an earlier qualitative physicochemical interpretation[2] of the adsorption behaviour of the elements from acetic acid solutions on RCl-resin, it was suggested that the formation of metal acetate complexes was responsible for the high K_d values in concentrated acetic acid solutions (with a low dielectric constant: $\varepsilon_{H_2O} = 80.4$, $\varepsilon_{HAc} = 6.2$). However, the results of Fig. 1 show that the chloride ions introduced in the system from the RCl-resin, are required for high adsorption. Consequently, it can be stated that the increasing affinity of these elements for the RCl-resin in highly concentrated acetic acid solutions is due to the enhanced formation and adsorption of metal–chloride or metal–acetate–chloride complexes.

2. The adsorbability of typical anions, such as molybdate and bromide, is higher for RAc- than for RCl-resin. This holds for the whole molarity range of acetic acid. These results are quite logical, since the affinity of the acetate anion for the anion exchanger is lower than that of the chloride anion (selectivity coefficient $E_{Cl^-}^{Ac^-} \leqslant 1$) so that it should be easier for the molybdate or the bromide anion to exchange with the acetate counter ions.

Adsorption from aqueous acetic–hydrochloric acid solutions

The adsorbability of Ni(II), Mn(II) and Cd(II) on RAc-resin from aqueous

acetic–hydrochloric acid solutions was examined as a function of the concentration of hydrochloric acid. The batch experiments were carried out by shaking 500 mg of dry RAc-resin with 100 ml of aqueous acetic acid solution, 1 ml of metal tracer solution and 1 ml of aqueous hydrochloric acid solution. The final hydrochloric acid concentrations varied from $2 \cdot 10^{-4}$ *M* to 0.12 *M* for nickel(II) and manganese(II), and from $2 \cdot 10^{-3}$ to 0.6 *M* for cadmium(II). The metal concentrations were $5 \cdot 10^{-3}$, 10^{-5} and 10^{-2} mmol of metal per meq of dry resin for Ni(II), Mn(II) and Cd(II), respectively. The experiments were carried out for different acetic acid concentrations: 17.2 and 16.5 *M* acetic acid for nickel(II); 17.2, 16.5 and 16.0 *M* acetic acid for manganese(II); 16.0 and 7.9 *M* acetic acid for cadmium(II). The results are plotted as K_d *versus* meq Cl^-/g dry RAc-resin.

For comparison, the K_d values on RCl-resin are also indicated on the graphs, with an abscissa value of 3.44 meq Cl^- g^{-1}. Indeed, this value corresponds to the total exchange capacity of the RAc-resin (RAc-resin virtually completely loaded with chloride ions). The numerical values are summarized in Table I.

TABLE I

COMPARISON OF K_d VALUES ON RCl AND RAc + 3.44 meq Cl^- g^{-1} OF DRY RESIN

MH RAc K_d						
	Ni(II)		*Mn(II)*		*Cd(II)*	
	RCl	*RAc +3.44 meq* Cl^- g^{-1}	*RCl*	*RAc +3.44 meq* Cl^- g^{-1}	*RCl*	*RAc +3.44 meq* Cl^- g^{-1}
7.87	n.a.[a]	n.a.	—	—	$7.9 \cdot 10^2$	$8.5 \cdot 10^2$
16.0	n.a.	n.a.	$1.8 \cdot 10^3$	$1.6 \cdot 10^3$	$1.3 \cdot 10^5$	$1.5 \cdot 10^5$
16.5	n.a.	n.a.	$7.2 \cdot 10^3$	$7.7 \cdot 10^3$	—	—
17.2	35	39	$8.1 \cdot 10^4$	$7.0 \cdot 10^4$	—	—

[a] Not adsorbed.

Nickel(II) (Fig. 2). For an acetic acid molarity of 17.2 *M*, the adsorbability of nickel(II) was found to be negligible below 1 meq Cl^- g^{-1} of dry RAc-resin. For higher chloride concentrations, K_d increased sharply, reaching a maximum value of 300 at 4 meq Cl g^{-1} of dry RAc-resin. Above this chloride content, the K_d value decreased slowly to a value of 110 at 24 meq Cl^- g^{-1} dry RAc-resin. The K_d value for a chloride content of 3.44 meq Cl^- g^{-1} of dry RAc-resin, as experimentally determined on RCl-resin, coincided with the adsorption isotherm described.

For 16.5 *M* acetic acid, no adsorption ($K_d \leqslant 1$) was found over the whole chloride concentration range. Further, nickel(II) was not adsorbed on RCl-resin from 16.5 *M* acetic acid.

Manganese(II) (Fig. 3). Over the whole chloride concentration range, the K_d curves followed the sequence K_d (16.0 *M*) $\leqslant K_d$(16.5 *M*) $\leqslant K_d$(17.2 *M*). For 16.0 *M* acetic acid, K_d sharply increased up to 3.6 meq Cl^- g^{-1} of dry RAc-resin, whereafter the adsorption curve became considerably less steep.

For 16.5 and 17.2 *M* acetic acid an analogous steeply rising adsorption

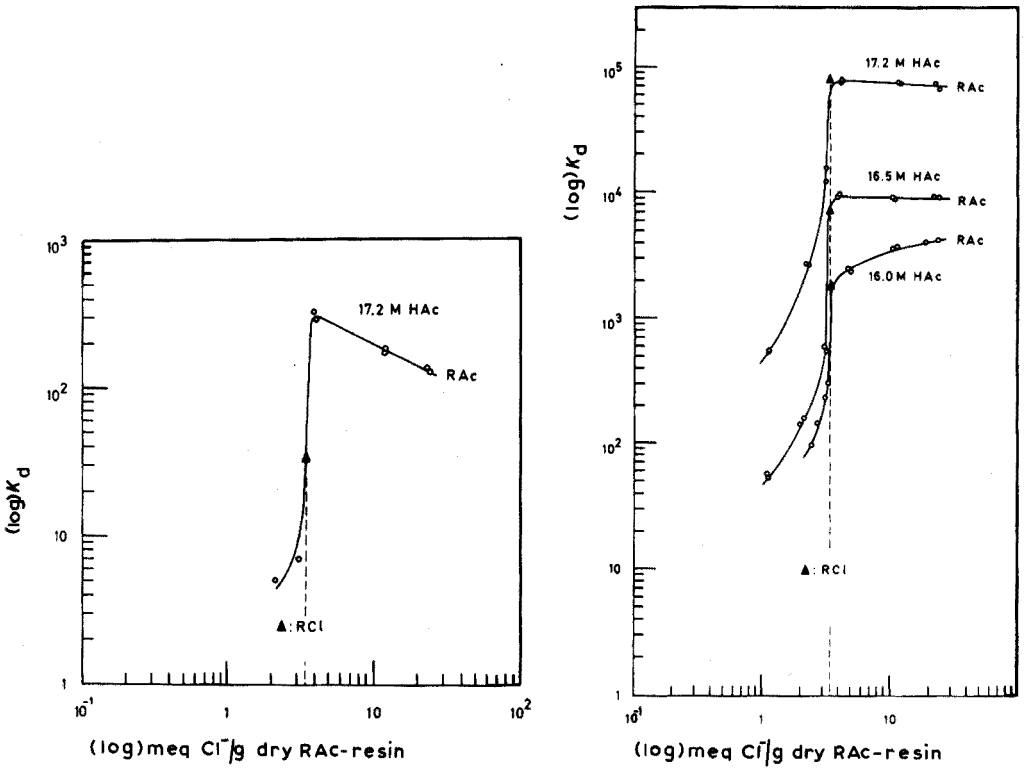

Fig. 2. K_d values of nickel(II) on RAc-resin for 17.2 *M* acetic acid as a function of hydrochloric acid concentration. (▲) K_d values experimentally determined on RCl-resin (see text).

Fig. 3. K_d values of manganese(II) on RAc-resin for 16.0 *M*, 16.5 *M* and 17.2 *M* acetic acid as a function of hydrochloric acid concentration. (▲) K_d values experimentally determined on RCl-resin (see text).

function was observed up to 3.7 meq Cl^- g^{-1} of dry RAc-resin. For higher chloride contents, K_d decreased slowly, this part of the curve being almost horizontal for 16.5 *M* acetic acid. For the three acetic acid molarities under investigation, the experimentally determined K_d values on RCl-resin, located at 3.44 meq Cl^- g^{-1} of dry RAc-resin, coincided quite well with the adsorption isotherms on RAc-resin.

Cadmium(II) (Fig. 4). For 7.87 *M* acetic acid the adsorption isotherm of cadmium(II) showed a rather sharp increase up to 11 meq Cl^- g^{-1} of dry RAc-resin, although this effect was not as pronounced as in the cases of nickel(II) and manganese(II). At higher chloride contents, K_d increased slowly.

The adsorption function for 16.0 *M* acetic acid was very similar, but the K_d values were considerably higher. The curve showed a maximum at 13 meq Cl^- g^{-1} of dry RAc-resin, whereafter it decreased quite rapidly. For both acetic acid molarities, the experimentally determined K_d values for RCl-resin corresponded reasonably with those interpolated from the adsorption curves for RAc-resin at a chloride content of 3.44 meq Cl^- g^{-1} of dry RAc-resin.

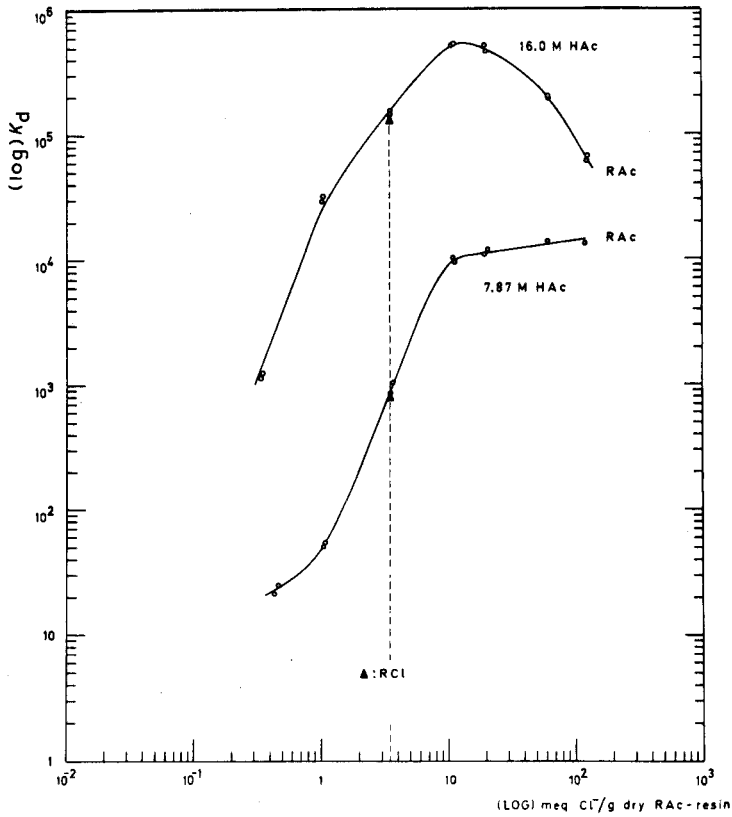

Fig. 4. K_d values of cadmium(II) on RAc-resin for 7.87 *M* and 16.0 *M* acetic acid as a function of hydrochloric acid concentration. (▲) K_d values experimentally determined on RCl-resin (see text).

Conclusions. From the results of Figs. 2–4 and especially from the good agreement found between the K_d values on RCl-resin and on RAc-resin + 3.44 meq Cl^- g^{-1}, it can be concluded that the chloride anions, introduced by the RCl-resin, are responsible for the high adsorbability of the elements on RCl-resin from concentrated acetic acid solutions. From the above-mentioned equivalence between RCl and RAc + 3.44 meq Cl^- g^{-1} it is obvious that, in the acetic acid–resin–metal ion system, it is unimportant whether the complex-forming chloride anions are introduced by the resin or by the solvent. In a qualitative way this can be explained by the very strong affinity of the chloride ions for the RAc-resin, with the result that in the presence of an equivalent amount of chloride anions in the solvent, the RAc-resin is almost quantitatively converted to the chloride form, thus behaving like a RCl-resin.

According to the theory of Fronaeus[10], one qualitative conclusion can be drawn with certainty from the adsorption isotherms presented in Figs. 2–4. The occurrence of a maximum of the K_d value, plotted as a function of the electrolyte (Cl^-) concentration, proves undoubtedly the formation of anionic metal complexes. However, the absence of a maximum in certain K_d curves is no proof of the

absence of anionic complexes[11]. Consequently, it is obvious that, for a given acetic acid concentration, an increasing chloride content gives rise to the gradual formation of cationic, neutral and anionic metal–chloride or metal–chloride–acetate complexes. A study of the exact composition of these complexes will be published in due course. That these anionic complexes are formed even in the case of nickel(II), for which no such phenomena have been hitherto observed in aqueous electrolyte

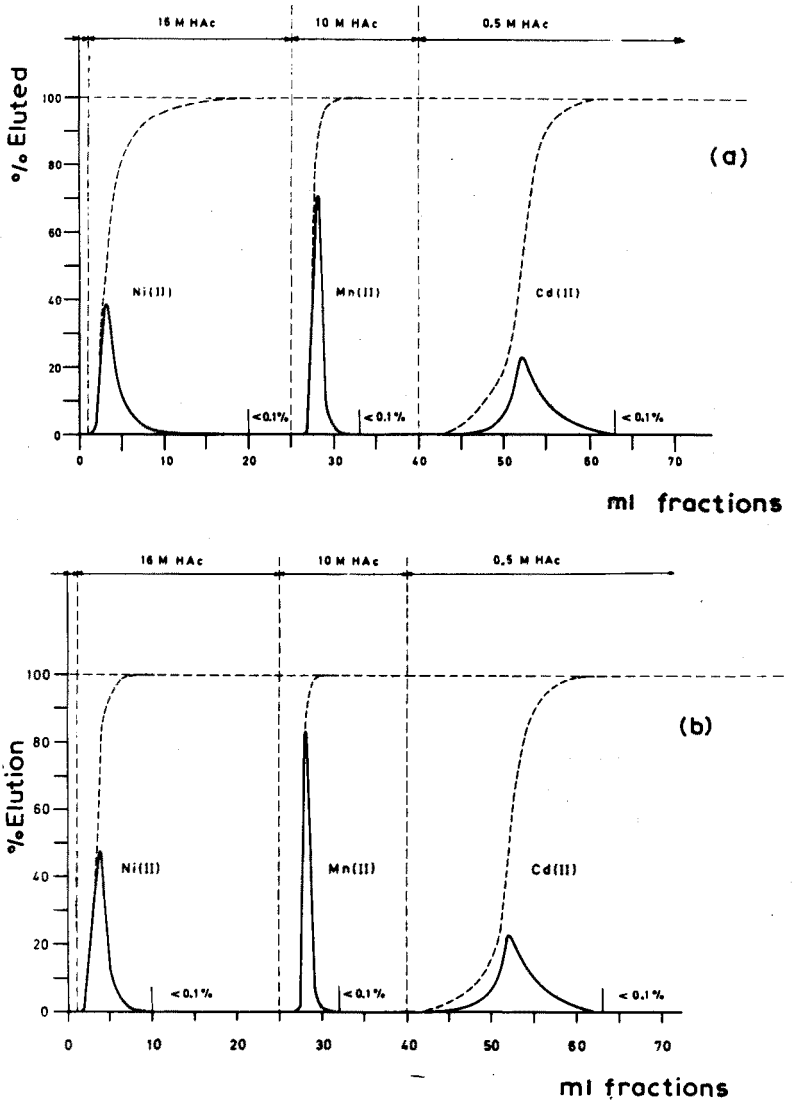

Fig. 5. Separation of Ni(II)–Mn(II)–Cd(II) on Dowex 1-X8 in the chloride form. (a) 100–200 mesh; (b) 200–400 mesh. Resin conditioned with 16 *M* acetic acid. Column dimensions: 8 mm diameter × 75 mm height. Adsorption rate: 2 ml min^{-1} cm^{-2}. Elution rate: 4 ml min^{-1} cm^{-2}. Adsorbed quantities: Ni(II), $4 \cdot 10^{-2}$ mmol; Mn(II), $4 \cdot 10^{-3}$ mmol; Cd(II), $2 \cdot 10^{-2}$ mmol. (——) % eluted per fraction; (------) total % eluted.

solutions, is most probably due to the low dielectric constant of the highly concentrated acetic acid solutions. Analogous behaviour has been reported for other organic solvents with low dielectric constants[12,13].

From the steeply rising adsorption functions in the neighbourhood of 3.44 meq Cl^- g^{-1} of dry RAc-resin, especially in the case of nickel(II) and manganese(II), it is not surprising that the determination of the K_d values for a given acetic acid concentration on RCl-resins with a slightly different total exchange capacity can show a considerable spread of results. Indeed, for nickel(II) at 17.2 *M* acetic acid, K_d values from 30 to 200 were found when different batches of resin were used.

ANION-EXCHANGE SEPARATION OF Ni(II)–Mn(II)–Cd(II)

In an earlier paper[2], the separation of elements in acetic acid medium was performed with initial aqueous metal ion–acetic acid solutions. Although excellent results were obtained, the dissolution of metal compounds in acetic acid was a rather troublesome manipulation.

As the K_d values decrease only slowly, or not at all, for chloride contents higher than 3.44 meq Cl^- g^{-1} of dry RAc resin (Figs. 2–4), it is obvious that the elements to be separated can as well be dissolved in aqueous hydrochloric acid, after which the solution can be mixed with acetic acid up to the desired composition. When the correct final acetic and hydrochloric acid concentrations are chosen for the adsorbing solution, the anion-exchange separation can be performed without appreciable loss of selectivity. For the sake of simplicity, an RCl-column can be used, and the adsorbed elements can then be eluted with aqueous acetic acid solutions.

In order to prove the usefulness of the above-mentioned simplified adsorption step the anion-exchange separation of Ni(II)–Mn(II)–Cd(II) was tested. The final composition of the adsorbed solution was 16.0 *M* acetic acid and 1.2 *M* hydrochloric acid; 1 ml of the solution was pipetted on to the top of the RCl-resin column, and the elements were separated with the eluents shown in Fig. 5a. The results proved that the separations were excellent, with quantitative yields for the three elements. However, from the elution curves of Fig. 5a, it is apparent that considerable tailing occurred, especially when highly concentrated acetic acid solutions were used as eluent. Therefore the separation was repeated on Dowex 1-X8, 200–400 mesh (Fig. 5b). With these finer resin beads, tailing was reduced markedly, nickel(II) being eluted quantitatively with 10 ml of 16 *M* acetic acid, instead of the 20 ml needed with the 100–200 mesh resin.

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SUMMARY

A comparative study of the adsorbability of Cd(II), Ni(II), Mn(II), Ga(III), La(III), Mo(VI) and bromide from aqueous acetic acid solutions on Dowex 1-X8,

100–200 mesh, in the acetate and chloride forms, proved that chloride ions are indispensable for high adsorption from concentrated acetic acid solutions. A study of the adsorption isotherms of Ni(II), Mn(II) and Cd(II) on the acetate-form resin from acetic acid–hydrochloric acid solutions, showed that these elements form anionic complexes. The K_d values on RCl-exchanger, for a given acetic acid concentration, were highly dependent on the total exchange capacity of the resin. A simplified anion-exchange separation procedure in aqueous acetic acid was developed, with an adsorption step from a mixture of acetic and hydrochloric acids.

RÉSUMÉ

Une étude comparative de l'adsorbabilité de Cd(II), Ni(II), Mn(II), Ga(III), La(III), Mo(VI) et bromure en solutions acétiques, sur Dowex 1-X8, 100–200 mesh, sous forme acétate et chlorure démontre que la présence des ions chlorures est indispensable pour une forte adsorption de solutions acétiques concentrées. On propose une méthode simplifiée de séparation sur échangeur d'anions dans l'acide acétique, en solutions aqueuse.

ZUSAMMENFASSUNG

Eine vergleichende Untersuchung der Adsorbierbarkeit von Cd(II), Ni(II), Mn(II), Ga(III), La(III), Mo(VI) und Bromid aus wässrigen Essigsäurelösungen an Dowex 1-X8, 100–200 mesh, in der Acetat- und Chloridform ergab, dass Chloridionen für eine hohe Adsorption aus konzentrierten Essigsäurelösungen unerlässlich sind. Die Isothermen für die -Adsorption von Ni(II), Mn(II) und Cd(II) aus Essigsäure–Salzsäure-Lösungen an dem Harz in der Acetatform zeigen, dass diese Elemente anionische Komplexe bilden. Die K_d-Werte am RCl-Austauscher für eine gegebene Essigsäurekonzentration hingen stark von der Gesamt-Austauschkapazität des Harzes ab. Ein vereinfachtes Verfahren der Anionenaustauschtrennung in wässriger Essigsäure unter Verwendung eines Adsorptionsschrittes in einem Gemisch von Essigsäure und Salzsäure wurde entwickelt.

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SYSTEMATIC ERRORS IN 14-MeV NEUTRON ACTIVATION ANALYSIS FOR OXYGEN

PART I. NEUTRON AND γ-RAY ATTENUATION EFFECTS

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Neutron and γ-ray attenuation is known to be a potential source of errors in 14-MeV neutron activation analysis. Several authors have proposed methods to correct for these effects. Such corrections have been determined experimentally[1] or deduced from theoretical considerations combined with experimental facts[2–5] or have resulted from purely theoretical considerations[6]. The present paper discusses the neutron and γ-ray attenuation processes in terms of experimental results and is limited to the reaction $^{16}O(n, p)^{16}N$.

Neutron attenuation

It is reasonable to assume that 14-MeV neutron attenuation is described by an exponential absorption law. For a homogeneous monodirectional flux traversing a sample, one can write:

$$\phi/\phi_0 = \exp(-\Sigma d) \tag{1}$$

where ϕ = neutron flux (n cm^{-2} s^{-1}) after passing through a sample of thickness d (cm);
ϕ_0 = neutron flux without a sample present;
Σ = macroscopic cross-section for 14-MeV neutrons (cm^{-1}), to be defined below.

The macroscopic cross-section Σ (cm^{-1}) can be calculated from

$$\Sigma = \frac{\sigma \delta N_A}{A} \tag{2}$$

where σ = microscopic cross-section for 14-MeV neutrons (cm^2);
δ = density (g cm^{-3});
N_A = Avogadro's number;
A = atomic mass.

The problem remains which cross-section should be used in the above equa-

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tions. Several possibilities have been proposed. Anders and Briden[7] chose σ_T, the total cross-section, which represents all interaction processes that may result from the collision of a neutron with an atom, since "even a single interaction will, for small samples, eliminate from the effective flux a neutron which is capable of initiating the activation of interest, *e.g.* $^{16}O(n, p)^{16}N$ in the deeper layers of the sample". Adams *et al.*[8] concluded that this is not correct in the case of elastic scattering with heavy nuclei. Elastic scattering of 14-MeV neutrons changes only the direction of motion of the neutron although the angles are quite small; the elastic scattering of fast neutrons is indeed so strongly peaked in the forward direction that the neutrons act as if there were no scattering at all. Light nuclei ($A < 12$) also cause a small energy loss in an elastic collision, but the number of collisions required to decrease the neutron energy below the threshold of the $^{16}O(n, p)^{16}N$ reaction (10.2 MeV) is rather high (10 in the case of aluminium). Except in extremely large samples, multiple interaction can be considered as being negligible.

From these arguments, it follows that σ_x, the non-elastic scattering cross-section, should be more appropriate, because all the processes included result in the elimination from the flux of a neutron that can initiate the $^{16}O(n, p)^{16}N$ reaction. This brings one to the concept of removal cross-section (σ_R, Σ_R) which has been recommended by Nargolwalla *et al.*[3,4] and by Adams *et al.*[8]. This cross-section value takes into account the small change in direction of the neutrons during elastic collisions and is defined by

$$\sigma_R(E) = \sigma_x(E) + \sigma_n(E)[1 - \bar{\mu}(E)] \tag{3}$$

where $\sigma_n(E)$ = the elastic scattering cross-section at energy E;
$\bar{\mu}(E)$ = the average cosine of the angle of elastic scattering in the laboratory system at energy E (here 14.5 MeV).

This removal cross-section should be regarded as a semi-empirical parameter which depends on the absorber, on the neutron energy and on the geometry.

γ-Ray attenuation

The γ-ray attenuation is also described by an exponential law:

$$A/A_0 = \exp(-\mu d) \tag{4}$$

where A = ^{16}N γ-activity recorded in a particular energy range with d cm of an absorber inserted between the ^{16}N source and detector;
A_0 = ^{16}N γ-activity without absorber.

The μ value (cm^{-1}) to be chosen for a given material is obviously correlated to the mass absorption coefficient, μ/ρ, but also depends on such parameters as geometry and the energy range selected by the measuring chain. The problem has been discussed empirically by Nargolwalla *et al.*[4] and theoretically by Nikolaenko and Shtan[6].

EXPERIMENTAL

Neutron attenuation

All experiments were performed with the irradiation facility described by Hoste *et al.*[9]. In this facility, sample and flux monitor are pneumatically conveyed through a pair of rectangular transfer tubes. At the irradiation station these tubes come together so that normally a sample and a flux monitor are irradiated behind each other in front of the 30-mm tritium target. The flux monitor consists of a steel box (diameter 26 mm, thickness 9 mm) filled with a compressed Fe_2O_3–graphite pellet (diameter 22 mm; thickness 7 mm) and has been described in detail elsewhere[1].

The Sames J accelerator was placed so that the distance from the tritium target to the oxygen monitor in the first transfer tube exceeded 7 cm; hence the neutron flux can be regarded as approximately monodirectional. Discs (diameter 60 mm) of different metals with varying thicknesses were inserted between the target and the oxygen flux monitor, and as close to the latter as possible. The measured ^{16}N activity in the monitor was compared to that obtained in the same geometry without a metal disc interposed between the accelerator cap and the monitor. The results are summarized in Fig. 1. Least-squares fitting of these curves allowed the determination of Σ from eqn. (1). The results are given in Table I (column 2).

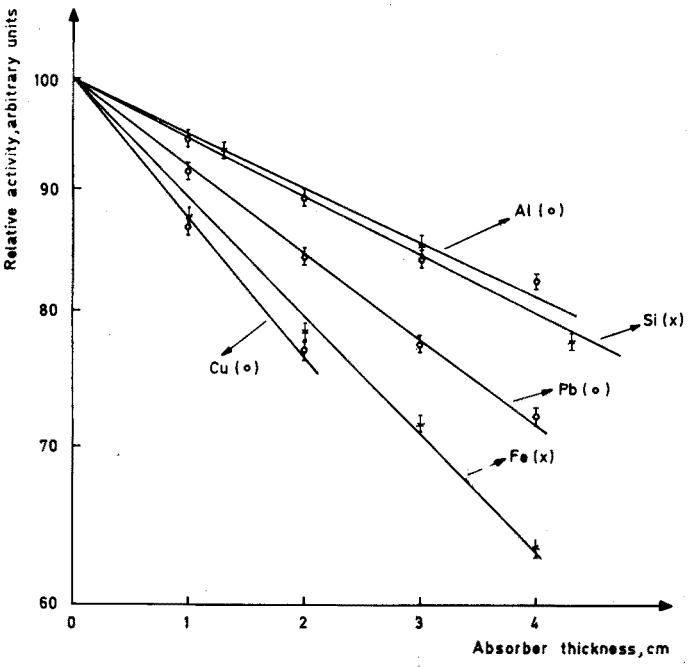

Fig. 1. Determination of Σ for Si, Al, Fe, Cu and Pb.

Owing to better counting statistics, more precise Σ values can be obtained when the accelerator is placed near the first transfer tube, a sample (diameter 26 mm, thickness 9 mm) being positioned in the first tube and the oxygen monitor

TABLE I

Σ VALUES (cm^{-1}) FOR VARIOUS METALS

Metal	Σ *from Fig. 1 and eqn. (1)*	Σ *from eqn. (8)*
Al	0.053 ± 0.013^a	0.056 ± 0.004^b
Si	0.056 ± 0.008^a	—
Fe	0.115 ± 0.010^a	0.111 ± 0.004^b
Ni	—	0.138 ± 0.004^b
Cu	0.134 ± 0.007^a	0.129 ± 0.004^b
Zr	—	0.069 ± 0.005^b
Mo	—	0.095 ± 0.005^b
Ag	—	0.099 ± 0.008^c
Cd	—	0.069 ± 0.007^c
Sn	—	0.058 ± 0.006^c
Sb	—	0.064 ± 0.006^c
Ta	—	0.125 ± 0.004^b
W	—	0.138 ± 0.005^b
Pb	0.084 ± 0.006^a	—
Bi	—	0.075 ± 0.008^c

[a] Standard deviation from least-squares fitting.
[b] Standard error on the mean of 25 determinations.
[c] Standard error on the mean of 10 determinations.

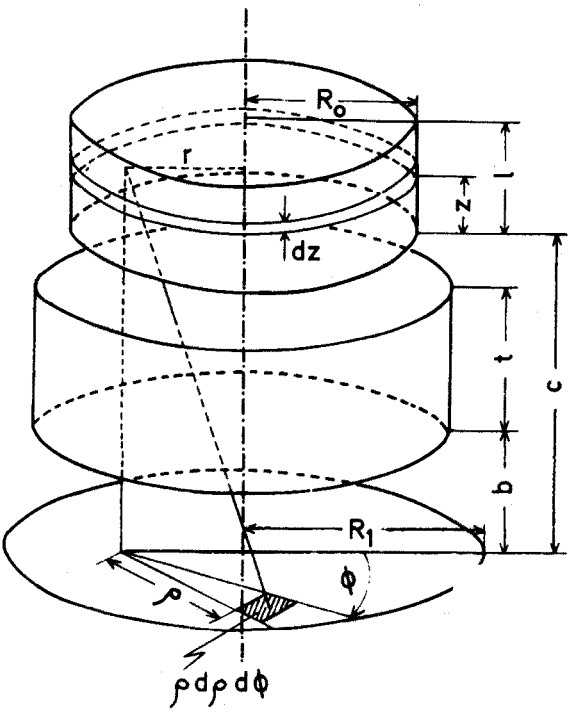

Fig. 2. Shielding of flux monitor (top) by sample (middle).

in the second one. The distance from the target to the monitor amounts then to 18.8 mm. However, because the neutron flux no longer approximates a mono-directional beam, a correction has to be applied, taking into account that, on average, the obliquely impinging neutrons travel a longer path within the sample. This correction can be computed if one assumes that the target is radiating homogeneously and side effects (caused by the fact that the target is a little larger than the sample) can be neglected (see Fig. 2). The average number of neutrons crossing a certain infinitesimal disc of the monitor, when a sample with a cross-section Σ is placed in front of it, can be calculated from:

$$\phi_\Sigma = \frac{2\pi\omega}{l}\int_0^l \mathrm{d}z \int_0^{R_0} r\,\mathrm{d}r \int_0^{2\pi} \mathrm{d}\phi \int_0^{\rho_{\max}} \frac{\rho\, \mathrm{e}^{\frac{-\Sigma t\,[\rho^2+(c+z)^2]^{\frac{1}{2}}}{c+z}}\,\mathrm{d}\rho}{\rho^2+(c+z)^2} \tag{5}$$

$$\rho_{\max} = r\cos\phi + (R_1^2 - r^2\sin^2\phi)^{\frac{1}{2}} \tag{6}$$

where ω = neutron flux (n cm^{-2} s^{-1}) at the surface of the target;
l = inner thickness of the standard capsule (0.7 cm);
R_0 = inner radius of the standard capsule (1.1 cm);
t = thickness of the sample (0.9 cm);
c = distance from the target to the front side of the standard (1.98 cm);
R_1 = radius of the target (1.5 cm).

If no sample is placed in front of the monitor, the above expression reduces to:

$$\phi_0 = \frac{2\pi\omega}{l}\int_0^l \mathrm{d}z \int_0^{R_0} r\,\mathrm{d}r \int_0^{2\pi} \mathrm{d}\phi \int_0^{\rho_{\max}} \frac{\rho\,\mathrm{d}\rho}{\rho^2+(c+z)^2} \tag{7}$$

By means of a Fortran-programmed PDP-9 computer, values of ϕ_Σ and ϕ_0 were calculated after series expansion of the last integral for Σ ranging from 0.05 to 0.20 cm^{-1}. It was found that

$$\phi_\Sigma/\phi_0 = \exp(-\Sigma f t) \tag{8}$$

with $f = 1.13$ for the whole Σ-range. The fact that the f-factor is independent of Σ can easily be seen in the limiting case that $\Sigma t \ll 1$; indeed division of eqn. (5) by eqn. (7) yields:

$$\phi_\Sigma/\phi_0 = 1 - \Sigma t \frac{\int_0^l \mathrm{d}z \int_0^{R_0} r\,\mathrm{d}r \int_0^{2\pi} \mathrm{d}\phi \int_0^{\rho_{\max}} \frac{\rho[\rho^2+(c+z)^2]^{\frac{1}{2}}\,\mathrm{d}\rho}{[\rho^2+(c+z)^2](c+z)}}{\int_0^l \mathrm{d}z \int_0^{R_0} r\,\mathrm{d}r \int_0^{2\pi} \mathrm{d}\phi \int_0^{\rho_{\max}} \frac{\rho\,\mathrm{d}\rho}{\rho^2+(c+z)^2}}$$

$$= 1 - \Sigma f t \approx \mathrm{e}^{-\Sigma f t} \tag{9}$$

where f depends only on the geometry, and not on Σ *i.e.* on the nature of the sample.

When eqn. (8) is used, the second experimental approach yielded the Σ values which are listed in Table I, column 3.

TABLE II

γ-RAY ATTENUATION COEFFICIENTS (cm^{-1}) FOR ^{16}N γ-RAYS

Metal	μ^a	μ^b	μ^c
Al	0.072 ± 0.006^d	0.071	0.057
Fe	0.222 ± 0.007	0.239	0.199
Cu	0.266 ± 0.008	0.276	0.232
Cd	0.305 ± 0.007	0.309	0.266
Pb	0.480 ± 0.010	0.493	0.444

[a] Experimental (4.5–6.5 MeV).
[b] "Total narrow beam γ-ray attenuation coefficients" from ref. 10 (6 MeV).
[c] Calculated from eqn. (10) and data from ref. 10.
[d] Standard error on the mean of 30 determinations.

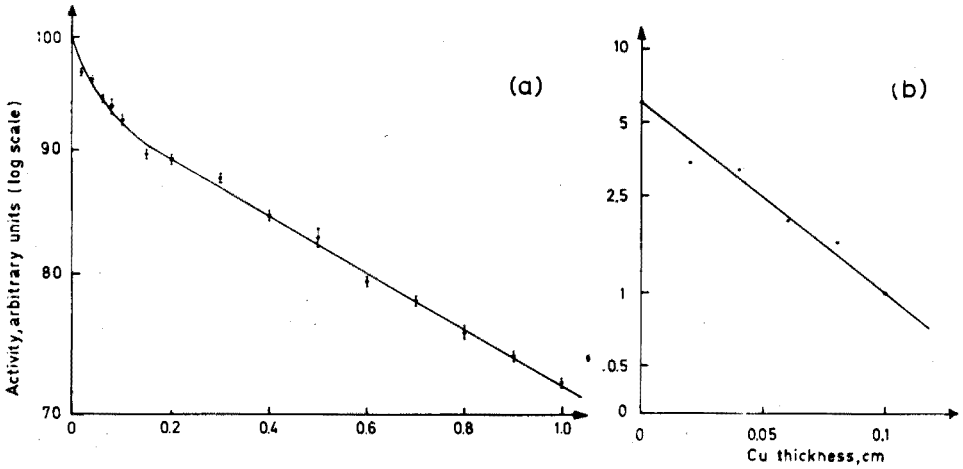

Fig. 3. β- and γ-ray attenuation in copper. (a) $\beta + \gamma$-ray absorption: $\mu_\gamma = 0.257\ cm^{-1}$; (b) β-ray absorption: $\mu_\beta = 17.9\ cm^{-1}$.

γ-Ray attenuation

Experiments were carried out to check whether the γ-ray attenuation coefficients listed in the literature[10] for 6-MeV γ-rays are adequate in the case of ^{16}N γ-rays counted in a window of 4.5 to 6.5 MeV. The oxygen monitor described above was irradiated and counted in the aluminium transfer tube by means of a 7.5 cm × 7.5 cm NaI(Tl) crystal, shielded from the 10.4-MeV β-rays by 2 cm of aluminium. To approximate a monodirectional γ-beam the detector was placed 6 cm away from the monitor. Between monitor and detector, plates of different metals (1 cm thick) were inserted as close to the detector as possible. Normalization of the irradiation conditions was done by means of the oxygen flux monitor. Linear γ-ray attenuation coefficients were calculated from eqn. (4). Experimentally determined μ values and literature values for 6-MeV γ-rays ("narrow beam total γ-ray attenuation coefficients") are listed in Table II. If one wants merely to apply the simple corrections for γ-ray attenuation effects, one should pay particular attention to prevent the detection of the ^{16}N β-rays ($E_\beta = 10.4$ MeV (26%) and 4.3 MeV (68%)). Figures 3 and 4 show absorption curves in copper and aluminium,

measured in the same way as for the determination of γ-ray attenuation coefficients, but without β-ray shielding and with varying metal thicknesses inserted between the activated oxygen standard and the detector.

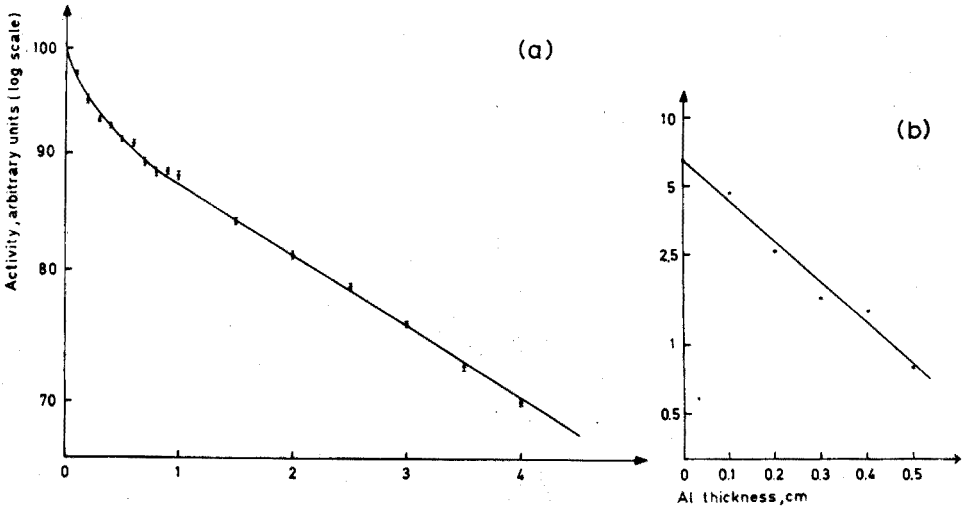

Fig. 4. β- and γ-ray attenuation in aluminium. (a) $\beta+\gamma$-ray absorption: $\mu_\gamma = 0.071$ cm^{-1}; (b) β-ray absorption: $\mu_\beta = 4.09$ cm^{-1}.

TABLE III

EXPERIMENTAL AND LITERATURE σ VALUES (barn)

Metal	σ^a	σ^b	σ_R *(14.5 MeV)*^{c}	σ_x *(14.0–14.5 MeV)*^{d}	$\sigma_x{}^e$	σ_T *(14 MeV)*^{f}
Al	0.88±0.21	0.93±0.07	1.03	1.00	—	1.75
Si	1.12±0.16	—	1.05	1.02	—	1.9
Fe	1.35±0.12	1.31±0.05	1.39	1.36	1.58	2.55
Ni	—	1.51±0.05	1.52	1.40	—	2.7
Cu	1.58±0.08	1.52±0.05	1.55	1.47	1.42	2.9
Zr	—	1.61±0.12	1.86	1.72	—	4.0
Mo	—	1.48±0.08	1.91	—	1.59	4.1
Ag	—	1.69±0.14	2.05	1.83	—	4.15
Cd	—	1.49±0.15	2.08	1.90	—	4.2
Sn	—	1.74±0.18	2.12	1.90	—	4.5
Sb	—	1.94±0.18	2.15	1.96	—	4.6
Ta	—	2.26±0.07	2.63	2.0	—	5.3
W	—	2.17±0.08	2.65	2.41	2.59	5.3
Pb	2.55±0.18	—	2.82	2.50	—	5.35
Bi	—	2.65±0.28	2.82	2.55	—	5.5

[a] From Table I, column 2.
[b] From Table I, column 3.
[c] From Nargolwalla *et al.*[4], interpolated from Avery *et al.*[11].
[d] Mean value of experimental data, from Allen *et al.*[12].
[e] Data mentioned by Nikolaenko and Shtan[6].
[f] Experimental data from Hughes and Schwartz[13].

DISCUSSION

Table III lists σ values calculated from eqn. (2) and from the Σ values of Table I, together with literature data. Apparently the present experimental values agree best with the σ_x values compiled by Allen *et al.*[12]. This is not in disagreement with the removal concept proposed by Nargolwalla *et al.*[4] and Gijbels *et al.*[1]. The small deviations from σ_R can probably be attributed to a difference in relative detector size between a finite flux monitor and the point detector used in the determination of σ_R by Avery *et al.*[11]. The large size of the former detector, which covers nearly the entire shadow of the sample, lowers the probability that small-angle elastic scattering removes a neutron from the effective flux (*i.e.* the flux that can induce the reaction $^{16}O(n, p)^{16}N$ within the monitor). Hence one should expect a "removal cross-section" lying between the σ_x values compiled by Allen *et al.*[12] and the σ_R values tabulated by Nargolwalla *et al.*[4].

Table II indicates that the measured μ values are on average slightly smaller than the theoretical ones. This can be attributed to the counting above 4.5 MeV of some secondary γ-rays from Compton scattering. It can be shown that secondary photons of energy between 4.5 and 6.1 MeV are contained in à cone, with an angle of approximately 14°.

The differential cross-section for (Compton) scattering of a photon of initial energy $h\nu$ into the energy range from $h\nu'$ to $h\nu' + dh\nu'$ is given by Siegbahn[14]

$$\frac{d\sigma}{dh\nu'} = \frac{\pi r_0^2 mc^2}{(h\nu)^2}\left[\frac{h\nu'}{h\nu} + \frac{h\nu}{h\nu'} - 2\left(\frac{mc^2}{h\nu'} - \frac{mc^2}{h\nu}\right) + \left(\frac{mc^2}{h\nu'} - \frac{mc^2}{h\nu}\right)^2\right] \qquad (10)$$

where r_0 = the classical radius of the electron;
mc^2 = rest energy of the electron.

If one makes the extreme assumption that all the secondary photons having an energy in the range 4.5–6.1 MeV are counted, the Compton contribution to the total linear absorption coefficient can be obtained by integrating eqn. (10) between 0 and 4.5 MeV. This contribution turns out to be 0.75 times the normal Compton contribution (assuming that no secondary photons are counted). The total attenuation coefficient is then found by adding this value to the linear attenuation coefficients for pair production and photoelectric effect from Storm and Israel[10]. The μ values calculated by this method are listed in Table II, column 4. Obviously the measured values lie between the two theoretical values. This is to be expected since in our geometry only part of the secondary photons between 4.5 and 6.1 MeV resulting from Compton interaction are counted. However, in view of the close agreement between the theoretical attenuation coefficients (columns 3 and 4) and the experimental coefficient (column 2), the literature values for 6 MeV[10] seem to be well suited, when γ-rays are counted with a window from 4.5 to 6.5 MeV. This is in disagreement with the results of Nargolwalla *et al.*[4] who state that the attenuation coefficients decrease by a factor 2 when γ-rays are counted between 4.8 and 8 MeV.

It can be seen from Figs. 3 and 4 that, without β-shielding, about 6% of the recorded counts in the 4.5–6.5 MeV energy interval are due to β-radiation. This value can be reduced to less than 0.5% by inserting a 2-mm copper shield

between monitor and detector. Obviously, the β-ray contribution can be reduced to a negligible level in the same manner when ^{16}N is counted in other matrices.

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SUMMARY

A detailed account is given of neutron and γ-ray attenuation effects in 14-MeV neutron activation analysis of oxygen. Appropriate neutron cross-section values have been determined in two different ways and compared with literature values. It appears that the attenuation process is best described in terms of non-elastic scattering cross-sections. It is also shown that the narrow beam total γ-ray attenuation coefficients at 6 MeV, given in the literature are suitable for correction purposes if ^{16}N γ-rays are counted with a window of 4.5–6.5 MeV. Attention was paid to the contribution of β-rays when the ^{16}N activity is counted in this energy interval with a NaI(Tl) detector.

RÉSUMÉ

Une étude d'erreurs systématiques est effectuée pour le dosage de l'oxygène par activation neutronique 14-MeV. Des valeurs de section transversale neutronique ont été déterminées de deux manières différentes et comparées avec les valeurs données dans la littérature. Il apparaît que le processus d'atténuation est mieux décrit par les sections transversales de dispersion élastique. On constate également que les coefficients d'atténuation du faisceau de rayons-γ à 6 MeV, donnés dans la littérature, peuvent servir à des corrections lorsque les rayons-γ ^{16}N sont comptés avec une fenêtre de 4.5 à 6.5 MeV. On tient compte du rôle dans cet intervalle d'énergie, avec un détecteur NaI(Tl).

ZUSAMMENFASSUNG

Eine eingehende Berechnung der Neutronen- und γ-Strahlenverluste bei der Aktivierungsanalyse von Sauerstoff mit 14 MeV Neutronen wird vorgelegt. Auf zwei verschiedenen Wegen wurden Neutronen-Wirkungsquerschnitte ermittelt und mit Literaturwerten verglichen. Es scheint, dass die Verluste am besten mit Wirkungsquerschnitten für nichtelastische Streuung beschrieben werden. Es wird ebenfalls gezeigt, dass die Koeffizienten, die in der Literatur für Gesamt-γ-Strahlverluste bei 6 MeV angegeben werden, sich für Korrektionszwecke eignen, wenn die γ-Strahlen von ^{16}N mit einem Kanal von 4.5–6.5 MeV gemessen werden. Der Beitrag der β-Strahlen wurde berücksichtigt, wenn die ^{16}N-Aktivität in diesem Energieintervall mit einem NaJ(Tl)-Detektor gemessen wurde.

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CONTRIBUTION A LA DETERMINATION POTENTIOMETRIQUE DES CONSTANTES DE STABILITE DES COMPLEXES 1:2 AVEC LES ACIDES AMINOPOLYACETIQUES MeH_nY_2

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(Reçu le 24 octobre 1972)

La potentiométrie est l'une des plus anciennes méthodes de détermination des constantes de stabilité des complexes et celle basée sur la mesure du pH est fréquemment utilisée lorsque le ligand manifeste des propriétés acido–basiques[1–10].

Certains acides aminopolyacétiques tels l'acide iminodiacétique (IDA), l'acide nitrilotriacétique (NTA), l'acide hydroxyéthyléthylènediaminotriacétique (HEDTA) forment, avec les cations à nombre de coordination élevé, à côté des complexes de stoechiométrie 1:1, des entités de stoechiométrie 1:2 et même 1:3[11,12].

Dans deux publications antérieures[13,14], nous avons montré, par la technique de distribution avec un échangeur d'ions, que le HEDTA forme avec les terres rares comme le thulium et l'europium des complexes 1:2 différemment protonés que nous pouvons représenter par l'équilibre suivant

$$MeY + H_nY \rightleftharpoons MeH_nY_2 \qquad (1)$$

n pouvant, en principe, prendre des valeurs entre 3 et 0.

Nous avons également déterminé les constantes de dissociation des différents complexes MeH_nY_2 pour dix éléments de la famille des lanthanides à partir de la courbe de titrage par la soude, d'une solution contenant une certaine concentration C_{H_3Y} de HEDTA en présence d'une quantité équivalente de complexe MeY en précipité. Ces valeurs avaient été obtenues dans un milieu à force ionique variable en admettant, à tout instant, l'équilibre entre la solution et la phase solide MeY.

Nous nous proposons, dans cette publication, de décrire une méthode de détermination de ces constantes qui diffère de la précédente en ce sens que le milieu garde une force ionique constante tout au long du titrage et que le complexe MeY est entièrement en solution.

Principe de la méthode

La méthode de détermination des constantes de stabilité des complexes MeH_nY_2 est basée sur l'exploitation des courbes de titrage potentiométrique, par la soude, d'une solution 10^{-2} M en H_3Y d'une part et d'une solution de H_3Y 10^{-2} M et MeY 10^{-2} M d'autre part, dont la force ionique est maintenue constante et égale à 1 au moyen de perchlorate ($H^+/Na^+ClO_4^-$). Les constantes ainsi obtenues sont conditionnelles et mixtes puisque la force ionique égale l'unité et que les valeurs sont obtenues à partir des concentrations des différentes espèces et de l'activité en ions H^+.

CONDITIONS EXPÉRIMENTALES

Réactifs

HEDTA utilisé (Sigma Chem. Comp., St. Louis, U.S.A.) a été purifié par une triple cristallisation dans l'eau désionisée.

Les complexes MeY sont préparés par réaction directe, à la température d'ébullition, entre les oxydes de lanthanides (Fluka puriss. pureté >99.9%) et une solution aqueuse de HEDTA 10^{-1} *M*. Cette réaction demande environ une heure pour les lanthanides centraux (Gd, Eu, Sm) et plus de douze heures pour les éléments du début et de la fin de la famille. Afin d'éviter toute formation de complexes 1:2, on ajoute un excès d'environ 5% d'oxyde de lanthanide; lorsque l'attaque est terminée, on filtre l'excès d'oxyde et on concentre la solution par ébullition. On laisse enfin cristalliser pendant une nuit, sous agitation constante. Les complexes sont complètement déshydratés par chauffage à 170° pendant une nuit et pesés comme MeY[15,16].

La force ionique est maintenue constante ($\mu = 1$) par $NaClO_4$ (Merck P.A.).

Toutes les solutions sont préparées à partir d'eau désionisée de résistivité supérieure à 10 MΩ.

Appareillage

Le pH est mesuré au moyen d'une électrode de verre Metrohm. L'électrode de référence Ag/AgCl préparée selon Bates[17] est reliée à la cellule de mesure par l'intermédiaire d'un pont "Wilhelm"[18]. Afin de minimiser les potentiels de jonction, la partie du pont en contact avec l'électrode de référence est remplie d'une solution 0.990 *M* en $NaClO_4$/$1.000 \cdot 10^{-2}$ *M* en NaCl tandis que l'autre partie, en contact avec la solution à titrer, est remplie par une solution 1.000 *M* en $NaClO_4$.

La température de l'électrode de référence et de la cellule de titrage est maintenue constante à $25.00 \pm 0.05°$ au moyen d'un thermostat Haake R 20. Les trois embouchures rôdées de la cellule de titrage sont occupées respectivement par l'électrode de verre, le pont de jonction et la burette; les titrages se font donc à l'abri total de l'atmosphère.

La f.e.m. est mesurée au moyen d'un potentiomètre Leeds et Northrup type K2; un électromètre Vibron 33 B est utilisé comme instrument de zero. Cet assemblage permet une mesure de différence de potentiel avec une erreur de 30 μV.

Etalonnage de l'électrode de verre

Il y a peu d'informations dans la littérature concernant l'étalonnage des électrodes de verre dans des solutions de force ionique élevée. Nous avons utilisé, pour étalonner l'électrode de verre, des solutions résultant du mélange de volumes déterminés d'une solution de Na_2HPO_4 $5.00 \cdot 10^{-2}$ *M* et d'une solution de NaH_2PO_4 $5.00 \cdot 10^{-2}$ *M* en maintenant la force ionique constante et égale à 1 par $NaClO_4$. Le pH de ces solutions est déterminé par extrapolation des valeurs de Maronny[19] qui a cependant travaillé en milieu KCl. Nous avons été amenés à travailler en milieu $NaClO_4$, les chlorures donnant des complexes chlorés avec les lanthanides; nous avons admis que le passage du chlorure au perchlorate ne modifiait pas l'étalonnage.

MÉTHODE ET RÉSULTATS

Nous allons rapidement établir les différentes équations qui vont nous permettre de calculer les constantes d'équilibre de complexation à partir des deux courbes potentiométriques de titrage.

Détermination des pK de HEDTA dans nos conditions expérimentales

La détermination des constantes de stabilité des complexes 1:2 nécessite, en premier lieu, la connaissance des constantes d'acidité de H_3Y dans le même milieu. La courbe de titrage de HEDTA (Fig. 1, courbe a) peut s'exprimer mathématiquement de la façon suivante: selon Rossotti et Rossotti[20], le nombre de protonation $\bar{n}$ d'un acide tribasique H_3Y, que l'on peut définir comme le nombre moyen de protons liés à l'anion Y^{3-} est donné par la relation

$$\bar{n} = 3 - a - \frac{[H^+] - [OH^-]}{f C_{H_3Y}} \tag{2}$$

où

a = degré de neutralisation = nombre d'équivalent-grammes de base ajoutés divisé par le nombre de moles d'acide: $a = C_{OH}\, v / C_{H_3Y}\, v_0$.

C_{H_3Y} = concentration totale du triacide dans le volume v_0.

C_{OH} = concentration de la base servant au titrage.

v = volume de base ajouté.

v_0 = volume initial de la solution à titrer.

D'autre part, $\bar{n}$ est égal à

$$\bar{n} = \frac{\sum_{n=1}^{n=3} n \beta_{Hn} [H^+]^n}{1 + \sum_{n=1}^{n=3} \beta_{Hn} [H^+]^n} \tag{3}$$

où β_{Hn} sont les constantes globales de formation $\beta_{Hn} = [HnY]/([Y][H^+]^n)$, $\beta_{H3} = 1/(K_{H1}\, K_{H2}\, K_{H3})$, $\beta_{H2} = 1/(K_{H2}\, K_{H3})$, $\beta_{H1} = 1/K_{H3}$, K_{H1}, K_{H2} et K_{H3} étant les trois constantes d'acidité de H_3Y.

En combinant les équations (2) et (3) et en tenant compte de la dilution pendant le titrage, on obtient l'équation (4):

$$v = \left[3 - \frac{[H^+] - [OH^-]}{f C_{H_3Y}} - \frac{\sum_{n=1}^{n=3} n \beta_{Hn} [H^+]^n}{1 + \sum_{n=1}^{n=3} \beta_{Hn} [H^+]^n} \right] \frac{v_0 C_{H_3Y}}{C_{OH}} \tag{4}$$

$f = v_0/(v_0 + v)$ étant le facteur de dilution.

Tous les termes de l'éqn. (4) sont connus ou mesurés à l'exception des trois constantes β_{Hn}; on peut donc les déterminer à partir de la courbe de titrage en résolvant l'éqn. (4) sur ordinateur par un programme d'approximations successives.

Les résultats sont donnés dans le Tableau I.

Rappelons que nous introduisons les activités en ions hydrogène au lieu de leur concentration et que, par conséquent, les constantes obtenues sont des constantes mixtes.

TABLEAU I

CONSTANTES D'ACIDITÉ DE L'ACIDE TRIBASIQUE HEDTA

($\mu = 1.000$ ($NaClO_4$); $t = (25.00 \pm 0.05)°$)

$K_{H1} = (1.41 \pm 0.05)\ 10^{-3}$ equiv.-gramme l^{-1}
$K_{H2} = (1.52 \pm 0.05)\ 10^{-6}$ equiv.-gramme l^{-1}
$K_{H3} = (4.46 \pm 0.18)\ 10^{-10}$ equiv.-gramme l^{-1}

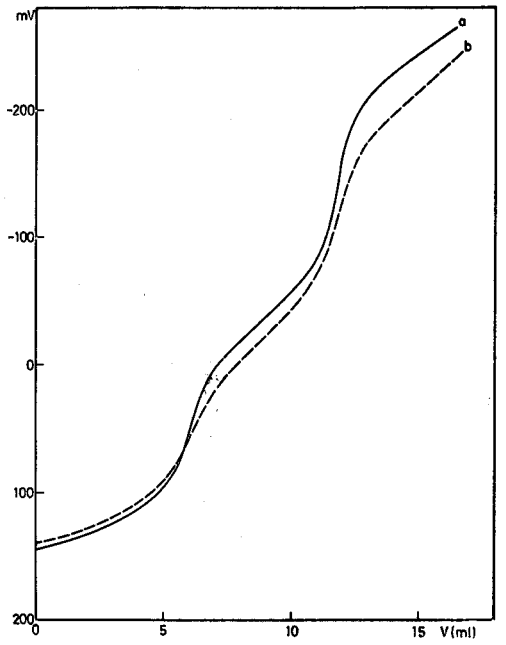

Fig. 1. (a) Courbe de titrage de l'acide tribasique HEDTA (H_3Y) dans les conditions expérimentales suivantes: 60 ml de H_3Y 10^{-2} M titrés par NaOH 0.0998; $\mu = 1$ ($NaClO_4$); $t = 25.00 \pm 0.05°$.
(b) Courbe de titrage du même acide, dans les mêmes conditions, en présence d'une quantité équimolaire du complexe MeY (EuY).

Etude des complexes $MeH_nY_2^{(3-n)-}$

Un exemple de courbe de titrage d'une solution équimoléculaire en H_3Y et MeY est donné sur la Fig. 1, courbe b.

L'évolution du pH, en fonction du nombre d'équivalent-grammes de NaOH ajoutés, peut s'interpréter par les relations suivantes: si T_H représente la concentration analytique en protons non-neutralisés, on peut écrire:

$$T_H = 3C_{H_3Y} f - C_{OH} f' =$$

$$= [H^+] - [OH^-] + \sum_{n=1}^{n=3} n([H_nY] + [MeY_2H_n]) \qquad (5)$$

avec $f' = v/(v_0 + v)$.

De plus, $[H_nY] = C'_{H_3Y}\,\Theta_n$ (6)

où C'_{H_3Y} est la concentration en HEDTA libre dans le volume v_0+v, non liée à MeY et Θ_n les fonctions de distribution des différentes formes de l'acide tribasique avec le pH. Pour simplifier l'écriture, nous avons adopté pour Θ_n l'inverse des conventions habituellement admises, Θ_3 correspondant à H_3Y, Θ_2 à H_2Y^-, Θ_1 à HY^{2-} et Θ_0 à Y^{3-}.

$$\frac{1}{\Theta_3} = 1 + \frac{K_{H1}}{[H^+]} + \frac{K_{H1}K_{H2}}{[H^+]^2} + \frac{K_{H1}K_{H2}K_{H3}}{[H^+]^3}$$

$$\frac{1}{\Theta_2} = \frac{[H^+]}{K_{H1}} + 1 + \frac{K_{H2}}{[H^+]^2} + \frac{K_{H2}K_{H3}}{[H^+]^2}$$

$$\frac{1}{\Theta_1} = \frac{[H^+]^2}{K_{H1}K_{H2}} + \frac{[H^+]}{K_{H2}} + 1 + \frac{K_{H3}}{[H^+]}$$

$$\frac{1}{\Theta_0} = \frac{[H^+]^3}{K_{H1}K_{H2}K_{H3}} + \frac{[H^+]^2}{K_{H3}K_{H2}} + \frac{[H^+]}{K_{H3}} + 1$$

En considérant que dans notre travail $C_{MeY} = C_{H_3Y}$

$$C'_{H_3Y} = C_{H_3Y}f - \sum_{n=0}^{n=3} [MeH_nY_2] = [MeY] \tag{7}$$

La substitution des relations (6) et (7) dans l'équation (5) conduit à

$$-C_{OH}f' = -3C_{H_3Y}f + [H^+] - [OH^-] + \sum_{n=1}^{n=3} n([MeY]\Theta_n + [MeY]^2 K_n \Theta_n) \tag{8}$$

K_n étant les constantes de stabilité des complexes $MeH_nY_2^{(3-n)-}$

$$K_n = \frac{[MeH_nY_2^{(3-n)-}]}{[MeY][H_nY^{(3-n)-}]}$$

La seule inconnue est la concentration MeY non complexé que l'on détermine de la manière suivante:

$$fC_{MeY} = [MeY] + \sum_{n=0}^{n=3} [MeY_2H_n] \tag{9}$$

En tenant compte des relations (6) et (7) et de l'équilibre de complexation de MeH_nY_2, cette relation (9) devient:

$$fC_{MeY} = [MeY] + [MeY]^2 \sum_{n=0}^{n=3} K_n\Theta_n \tag{10}$$

La combinaison des expressions (8) et (10) conduit à une relation qui, appliquée aux différents points de la courbe de titrage, nous fournit un système d'équations qui permet, en principe, la détermination des différentes constantes K_n; nous avons utilisé, à cet effet, un programme par ordinateur basé sur la méthode des moindres carrés dont voici le principe.

En choisissant quatre valeurs raisonnables mais arbitraires de K_n, on peut calculer le second membre de l'équation (8), soit y cette valeur, et comparer les

valeurs calculées et expérimentales de y pour les différents points de la courbe en déterminant

$$U = \sum_i (y_i \text{ calc.} - y_i \text{ mes.})^2$$

Les valeurs les plus probables pour K_n sont celles qui rendent U minimum. Si on développe cette équation en série de Taylor[21], on obtient

$$U = U_0 + \sum_n \frac{\delta U}{\delta K_n} \delta K_n \tag{11}$$

TABLEAU II

CONSTANTES DE STABILITÉ DES COMPLEXES DOUBLES FORMÉS ENTRE LES LANTHANIDES TRIVALENTS ET LE HEDTA (H_3Y)

($K_n = [MeY_2H_n]/[MeY][H_nY]$

Complexes	K_3 *(l mole^{-1})*	K_2 *(l mole^{-1})*	K_1 *(l mole^{-1})*	K_0 *(l mole^{-1})*
PrY_2H_n	$(5.50\pm0.98)\cdot10^1$	$(3.70\pm0.84)\cdot10^1$	$(8.56\pm0.62)\cdot10^1$	$(6.27\pm0.81)\cdot10^2$
NdY_2H_n	$(4.22\pm0.63)\cdot10^1$	$(1.11\pm0.29)\cdot10^1$	$(6.03\pm0.64)\cdot10^1$	$(5.32\pm0.37)\cdot10^2$
SmY_2H_n	$(4.80\pm0.57)\cdot10^1$	$(4.53\pm0.93)\cdot10^0$	$(8.75\pm0.70)\cdot10^1$	$(7.73\pm0.47)\cdot10^2$
EuY_2H_n	$(3.30\pm0.68)\cdot10^1$	$(4.21\pm1.01)\cdot10^0$	$(1.67\pm0.50)\cdot10^2$	$(1.33\pm0.31)\cdot10^3$
GdY_2H_n	$(3.60\pm0.54)\cdot10^1$	0	$(2.37\pm0.28)\cdot10^2$	$(1.62\pm0.20)\cdot10^3$
TbY_2H_n	$(8.7\ \pm0.9\ )\cdot10^0$	0	$(2.72\pm0.45)\cdot10^2$	$(1.850\pm0.087)\cdot10^3$
DyY_2H_n	$(2.06\pm0.16)\cdot10^0$	0	$(2.78\pm0.12)\cdot10^2$	$(1.933\pm0.095)\cdot10^3$
HoY_2H_n	$(2.02\pm0.22)\cdot10^0$	0	$(2.93\pm0.14)\cdot10^2$	$(2.45\pm0.12)\cdot10^3$
ErY_2H_n	$(1.90\pm0.39)\cdot10^1$	0	$(1.94\pm0.12)\cdot10^2$	$(1.70\pm0.10)\cdot10^3$
TmY_2H_n	$(3.48\pm0.40)\cdot10^1$	$(1.22\pm0.31)\cdot10^1$	$(1.55\pm0.11)\cdot10^2$	$(1.458\pm0.097)\cdot10^3$
YbY_2H_n	$(7.2\ \pm1.2\ )\cdot10^1$	$(4.06\pm0.69)\cdot10^1$	$(1.10\pm0.10)\cdot10^2$	$(1.282\pm0.092)\cdot10^3$
LuY_2H_n	$(8.8\ \pm1.5\ )\cdot10^1$	$(1.07\pm0.32)\cdot10^2$	$(1.08\pm0.26)\cdot10^2$	$(1.29\pm0.11)\cdot10^3$

TABLEAU III

COMPARAISON DES CONSTANTES D'ACIDITÉ DE L'ACIDE HEDTA (H_3Y) ET DES DIFFÉRENTS COMPLEXES MeY_2H_3

Acides	pK_1	pK_2	pK_3
H_3Y	2.85	5.82	9.35
PrY_2H_3	3.02	5.45	8.49
NdY_2H_3	3.43	5.08	8.40
SmY_2H_3	3.88	4.53	8.40
EuY_2H_3	3.74	4.22	8.45
GdY_2H_3	3.93[a]		8.52
TbY_2H_3	3.59[a]		8.52
DyY_2H_3	3.27[a]		8.51
HoY_2H_3	3.25[a]		8.43
ErY_2H_3	3.83[a]		8.41
TmY_2H_3	3.30	4.71	8.38
YbY_2H_3	3.10	5.39	8.28
LuY_2H_3	2.77	5.81	8.27

[a] Ces valeurs correspondent à l'équilibre $[MeY_2H^{2-}][H^+]^2/[MeY_2H_3]$, elles sont égales à $-\log\sqrt{(K_{H2,1})}$.

$\delta U/\delta K_n$ sont les dérivées partielles de U et δK_n l'incrément de modification des constantes K_n.

En admettant, en première approximation, que les dérivées partielles $\delta U/\delta K_n$ peuvent être exprimées par une fonction parabolique, le programme, par des variations appropriées des constantes K_n tend à minimiser les 4 membres de la somme dans la relation (11). Les constantes sont ainsi modifiées jusqu'à ce que U arrive à sa valeur minimum; à ce moment, les 4 termes de la somme sont nuls et les 4 constantes K_n sont les "meilleures constantes". U est égal à U_0, qui nous donne l'écart-type des constantes calculées.

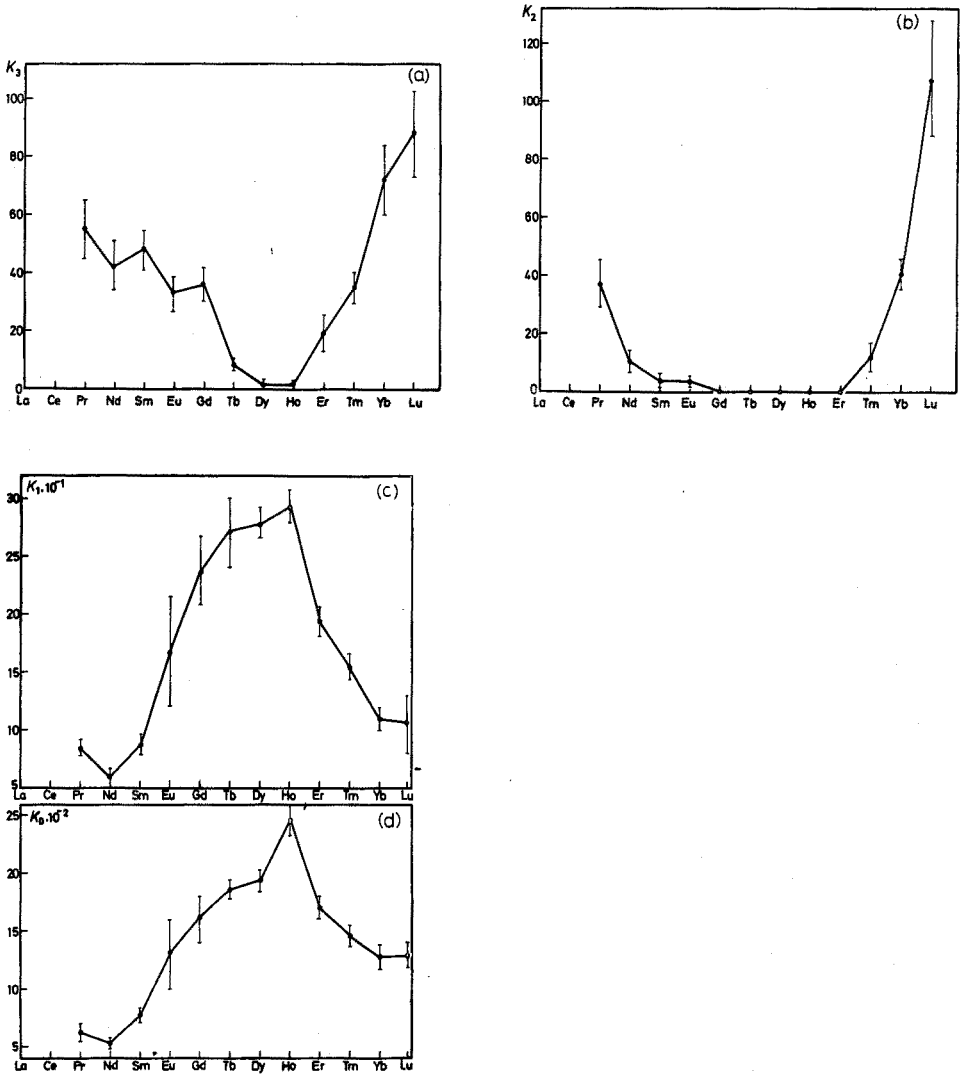

Fig. 2. Comparaison des valeurs des constantes de formation K_0, K_1, K_2 et K_3 pour différents lanthanides. (a) K_3; (b) K_2; (c) K_1; (d) K_0.

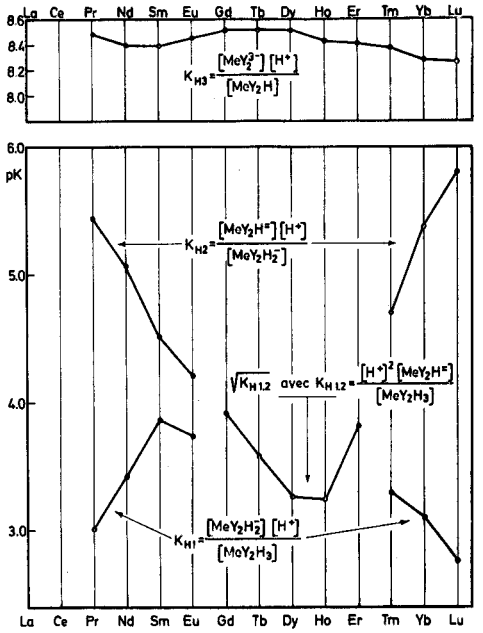

Fig. 3. Evolution des constantes d'acidité des complexes MeY_2H_n en fonction du nombre atomique des lanthanides. pK_1; pK_2; pK_3.

Les résultats obtenus sont présentés dans le Tableau II. Le Tableau III compare les valeurs des constantes d'acidité de HEDTA et des complexes MeY_2H_n. Les Figs. 2 et 3 montrent l'évolution des constantes de stabilité et d'acidité en fonction du nombre atomique des lanthanides.

DISCUSSION

L'interprétation des données thermodynamiques relatives aux complexes des lanthanides avec les acides aminopolyacétiques a donné lieu à des discussions très nombreuses et les discontinuités observées dans ΔS et $(-\Delta H)$ en fonction de Z, pour EDTA et HEDTA sont attribuées par les uns à des différences d'hydratation des ions lanthanides et par les autres à des différences d'hydratation des complexes MeY, les complexes des lanthanides légers contenant une molécule d'eau de plus que les lanthanides lourds[22,23]. Les arguments en faveur de cette dernière interprétation ont été avancés par Geier et Karlen[24] sur la base de l'étude des complexes mixtes des lanthanides avec EDTA d'une part et IDA ou NTA ou OXS (l'acide 8-hydroxyquinoléine-5 sulfonique) d'autre part.

Les grandeurs thermodynamiques, pour le complexe mixte Me(EDTA)(IDA) tout particulièrement, semblent montrer que la formation de ce complexe peut s'écrire comme suit:

$$Me(EDTA)(H_2O)_x + IDA \rightleftharpoons Me(EDTA)(IDA) + x\,H_2O \qquad (I)$$

avec $x=3$ pour les lanthanides légers
$x=2$ pour les lanthanides lourds
$x=2$ et 3 pour les lanthanides intermédiaires

Ceci suffit à montrer que l'interprétation des variations de constantes de stabilité des complexes des lanthanides avec le nombre atomique n'est pas simple et soulève pas mal de problèmes, même lorsqu'on connaît les trois grandeurs $-\Delta G$, $-\Delta H$ et ΔS; il serait donc vain de vouloir interpréter complètement nos résultats sur la seule base de la connaissance de $-\Delta G$.

Notons cependant que, en ce qui concerne les constantes d'acidité des complexes MeH_nY_2 (Fig. 3), la constante K_{H3} ne varie que très peu avec Z, contrairement aux deux autres constantes K_{H2} et K_{H1}; ce fait semble indiquer que ce dernier groupement ligand ne se fixe pas sur l'ion central ou encore, que le second ligand Y^{3-} fixé sur MeY n'est coordonné que par l'intermédiaire de quatre groupements. Cela voudrait dire que, en reprenant l'hypothèse avancée ci-dessus pour le complexe MeY_2^{3-}, la réaction peut s'écrire:

$$Me(H_2O)_9 + 2\,(HEDTA) \rightleftharpoons Me(HEDTA)_2 + 9\,H_2O \qquad (II)$$

situation assez analogue à celle de la réaction (I).

Or, il se fait que la variation de ΔG correspondant à la formation du complexe mixte (I) a la même allure que celle de K_0 dans notre cas.

La protonation de $MeHY_2^{2-}$ sera d'autant plus difficile, c'est-à-dire le pK du couple acido-basique considéré sera d'autant plus faible que le groupement considéré sera plus fortement fixé sur l'ion central. Les graphiques de la Fig. 3 montrent donc que l'influence du Z sur la stabilité des liaisons entre l'ion central et les différents groupements coordonnés n'est pas la même; en considérant le couple $MeH_2Y_2^-/MeHY^{2-}$, on voit que la complexation diminue le pK_2 de l'acide considéré à cause de l'interaction entre l'ion central et le groupe basique formé et l'on voit aussi que cette interaction paraît maximale pour les éléments centraux.

Dans le cas du couple $MeH_3Y_2/MeH_2Y_2^-$, on observe la variation inverse; la complexation augmente le pK_1 de l'acide H_3Y et cette augmentation paraît la plus importante pour les éléments centraux; ceci est peut-être la résultante de différentes interactions telles que liaisons hydrogène intramoléculaires, interactions entre l'ion central et le proton, d'une part, et le groupement basique, d'autre part.

CONCLUSIONS

Comme le montrent les résultats rassemblés dans le Tableau III, les deux acides tribasiques H_3Y et MeY_2H_3 sont de force très semblable; la présence de MeY n'affecte que très peu la courbe de titrage de H_3Y et la détermination potentiométrique de constantes de stabilité dans de telles conditions est très difficile. Les résultats obtenus (Tableau II) montrent qu'une application soigneuse de la méthode décrite permet cependant la détermination de valeurs significatives des constantes de stabilité des complexes doubles.

La variation de $-\Delta G$ en fonction de Z permet d'émettre certaines hypothèses sur le comportement des complexes des lanthanides avec les acides aminopolyacétiques en solution aqueuse mais l'interprétation complète de nos résultats ne pourra être envisagée qu'après la détermination des deux autres grandeurs thermodynamiques $-\Delta H$ et ΔS.

RÉSUMÉ

La méthode potentiométrique avec mesure du pH a été étendue au cas de la détermination des constantes de stabilité des complexes de stoechiométrie 1:2 protonés; à cet effet, on titre successivement, par une base, une solution contenant l'acide chélatant polybasique H_nY et une autre contenant en plus une quantité stoechiométrique d'une entité qu'il peut complexer par réaction d'addition; la force ionique est maintenue constante. L'interprétation des courbes de titrage rend possible le calcul des constantes de formation de ces complexes d'addition. Les calculs sont effectués, sur ordinateur, au moyen d'une méthode par approximations successives. Le cas des complexes 1:2 formés entre les lanthanides trivalents et l'acide hydroxyéthyléthylènediaminetriacétique (HEDTA) est spécialement étudié.

SUMMARY

An extension of the potentiometric method based on pH measurement is applied to the determination of the stability constants of addition complexes. On the one hand, the polybasic chelating agent H_nY, and on the other hand the same acid in presence of an equimolar concentration of the entity to be complexed, are titrated with standard sodium hydroxide in a medium of constant ionic strength. By means of a least-square computer program, the stepwise stability contants of the addition complexes are obtained. This method is applied to the 1:2 complexes formed between several trivalent lanthanides and hydroxyethylethylenediaminotriacetic acid (HEDTA).

ZUSAMMENFASSUNG

Die auf einer pH-Messung beruhende potentiometrische Methode wurde auf die Bestimmung der Stabilitätskonstanten von Anlagerungskomplexen angewendet. Einerseits wurde die mehrbasige komplexbildende Säure H_nY und andererseits dieselbe Säure in Gegenwart einer äquimolaren Konzentration der zu komplexierenden Metallverbindung mit Natronlauge titriert. Die Ionenstärke wurde mit $NaClO_4$ auf 1 gehalten. Mit Hilfe eines Computerprogramms, das auf der Berechnung der kleinsten Fehlerquadrate beruht, wurden die schrittweisen Bildungskonstanten der Anlagerungskomplexe erhalten. Die Methode wurde auf die 1:2-Komplexe angewendet, die zwischen einigen dreiwertigen Lanthaniden und Hydroxyäthyläthylendiamintriessigsäure (HEDTA) gebildet werden.

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A SIMULTANEOUS DETERMINATION OF LEAD-210 AND POLONIUM-210 IN SEA WATER

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In sea water, especially in the surface water, lead-210 (half-life, 21.4 yr) and polonium-210 (half-life, 138 d), both of which are radon daughter nuclides, come mainly from the atmosphere. Therefore, these nuclides may afford valuable information about problems at the air–sea interface. Furthermore, their concentrations, or the concentration ratios of the two nuclides, may be used as a tracer of insoluble material in the ocean, because these nuclides are heavy metals. However, little work has been done on these subjects; this might be, at least in part, due to the lack of simple and precise analytical procedures for the determination of these nuclides in sea water.

Rama, Koide and Goldberg[1] first determined lead-210 in sea water by using a method involving coprecipitation with lead chromate, separation by ion exchange and counting the β-activity of bismuth-210 produced from lead-210. In this method, however, polonium-210 could not be determined and a low-background β-counting system was required. Recently, Shannon and Orren[2] determined lead-210 and polonium-210 in sea water off South Africa; they used a direct solvent extraction technique to concentrate the nuclides from a 1.5-l sea water sample and subsequently determined each of the nuclides by counting the α-activity of polonium-210. However, as they pointed out, the amount of sample was too small to determine accurately the concentration of lead-210.

This paper describes a simpler and more precise method for the determination of lead-210 and polonium-210 in sea water. In the proposed method, these nuclides are coprecipitated with calcium carbonate and then polonium is selectively separated from other nuclides by spontaneous deposition onto a silver disc. The content of lead-210 is measured by the activity of its granddaughter, polonium-210, produced during the storage of the sample containing lead-210 for more than 3 months. In the development of this method, particular caution was taken to avoid the adsorption loss of polonium-210 onto the wall of container used during analysis and storage.

EXPERIMENTAL

Apparatus

A 2π-gas flow counter (Aloka, Model JDC-11) was used for α-counting and a 400-channel pulse-height analyzer (Hitachi, Model RAH 403) coupled with a

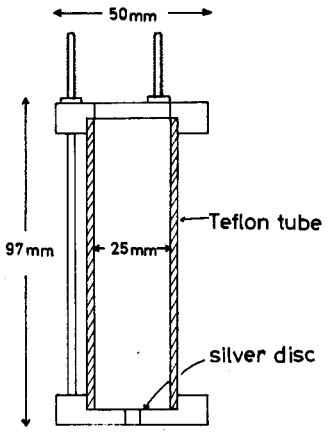

Fig. 1. Electrodeposition cell for the spontaneous deposition of polonium-210.

surface-barrier silicon detector (Ortec, active area, 950 mm^2) was used for α-spectrometry.

An atomic absorption spectrophotometer (Hitachi, Model 207) was used for the determination of lead, and a spectrophotometer (Hitachi, Model 101) for the determination of bismuth.

The deposition cell used for spontaneous electrodeposition of polonium-210 is illustrated in Fig. 1; this consists of a Teflon tube and a disc of silver (30 mm in diameter, 0.05 mm thick).

Reagents

All reagents used were of analytical grade except for sodium carbonate which was of the cheaper food-additive quality.

Polonium-210 standard solution. A solution of polonium-210 in equilibrium with lead-210 (Japan Radioisotope Association, Tokyo) was diluted with 3 *M* nitric acid solution to make a solution of about 300 d.p.m. ml^{-1} for polonium-210. An aliquot of the diluted solution was evaporated to dryness on a silver disc at about 100° and standardized against a standard α-ray uranium source (Japan Radioisotope Association).

Lead and bismuth carrier solution. In 1 l of 20% nitric acid solution, dissolve 7.99 g of lead nitrate and 11.56 g of bismuth nitrate pentahydrate. This solution contained 5 mg each of lead and bismuth per ml.

Procedure

In Fig. 2, the recommended scheme for determinations of polonium-210 and lead-210 is shown. Immediately after sampling, transfer a 30–50 l aliquot of sea water into a container of which the inside has been covered with a thin polyethylene bag (0.05 mm thick). Acidify with 100 ml of 15 *M* nitric acid. Add 5 ml of the carrier solution and stir vigorously. After about 1 h, neutralize with 125 ml of 15 *M* ammonia solution and then add slowly 120 g of sodium carbonate dissolved in 1 l of deionized water, stirring the sea water vigorously.

After allowing it to stand for more than one day, carefully siphon off

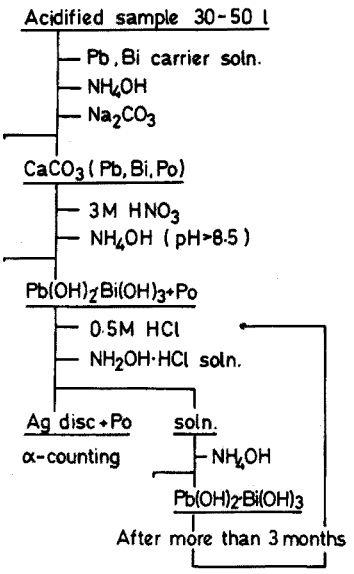

Fig. 2. Schematic procedure for determination of lead-210 and polonium-210 in sea water.

most of the supernatant solution, and remove the remaining solution from the precipitate of carbonates by decantation or by using a syringe. Reserve the precipitate in the polyethylene bag as such.

In the laboratory, wash the bag with 3 *M* nitric acid solution, transfer and dissolve the precipitate in about 350 ml of 3 *M* nitric acid solution. After removing carbon dioxide completely by heating the solution on a hot plate, add about 70 ml of 6 *M* ammonia solution. Filter off the precipitate of lead and bismuth as well as polonium, and dissolve in about 40 ml of 0.5 *M* hydrochloric acid solution.

Transfer the solution to the electrodeposition cell and add 1 ml of 20% (w/v) hydroxyammonium chloride solution. Allow to stand on a hot plate for more than 3 h at 70–90°. Then, reserving the solution, rinse the cell with 0.5 *M* hydrochloric acid and water and take off the silver disc. Count the α-activity of polonium-210 on the silver disc by using the 2π-gas flow counter and/or the α-spectrometer.

To the reserved solution, add 10 ml of 6 *M* nitric acid and filter the solution. Adjust the volume to 100 ml with water and take a 1-ml portion of the solution to determine the chemical yields of lead and bismuth.

To the remaining portion of the solution add 25 ml of 6 *M* ammonia solution. Filter the precipitate of hydroxides containing lead and store for more than 3 months. Again determine the activity of polonium-210 produced from the lead-210 during the storage in the way described above, and calculate the activity of lead-210 from the activity of polonium-210.

RESULTS AND DISCUSSION

Coprecipitation and separation of polonium-210 and lead-210 from sea water sample of large volume

Because of high adsorbability of polonium in neutral or alkaline solution on

the container wall, rapid treatment of sea water samples is required to minimize the loss of polonium-210. A coprecipitation method is applied for the rapid separation of polonium-210 and lead-210 from sea water, because the direct solvent extraction method is laborious for numerous samples of 30–50 l. The precipitation of lead chromate or iron(III) hydroxide has been used by previous workers[1,3] to coprecipitate lead-210 and polonium-210 from large volumes of sea water. However, these methods were followed by a rather complicated procedure including ion-exchange separation. The calcium carbonate method used here seems to be the best, because the precipitate is easily decomposed in acidic solution and these nuclides can be simply separated from the solution. The recovery of these nuclides collected in the calcium carbonate is about 85% for each nuclide and substantially equal to those for the overall procedure. These recoveries are discussed in detail later, together with the overall chemical yields.

Separation of the nuclides from calcium-rich solution was tested at various pH values by addition of various amounts of ammonia solution. Lead in the precipitate was determined by the atomic absorption method, and bismuth by a colorimetric method[4]. Polonium-210 in the precipitate was also determined by counting the α-activity on a silver disc after the electrodeposition. As shown in Fig. 3, hydroxides of these elements precipitated quantitatively above pH 8.5.

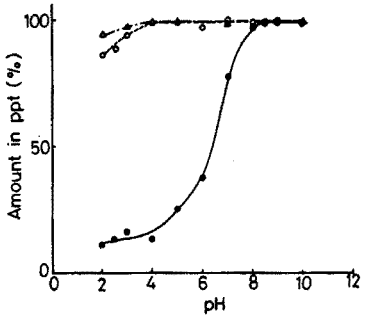

Fig. 3. Separation of lead, bismuth and polonium-210 from calcium-rich solution at various pH values. The solution contained 20 g of calcium as $CaCl_2$, 5 ml of the carrier solution and 1 ml of the polonium-210 standard solution in 400 ml of the total volume. (●) Pb; (○) Bi; (△) Po.

Electrodeposition of polonium-210 onto a silver disc

In order to separate polonium-210 from the other cations and radioactive nuclides, and to prepare a thin-layer source for α-counting, it is most convenient to deposit the polonium onto a silver disc spontaneously. Flynn[5] examined the method and described the optimal conditions, where polonium was quantitatively deposited onto a silver disc by allowing the solution to stand for 75 min at 85–90° on a hot plate with stirring. However, in the present experiments, the deposition was not complete until after a period of 2.5 h; mechanical stirring was unnecessary because the solution mixed well on heating the bottom of the cell.

Bismuth as a carrier of polonium-210

As polonium in water is highly insoluble and absorbable, it probably exists in the solid phase in sea water. Since bismuth is also insoluble in alkaline solution, and

its chemical properties are similar to those of polonium, bismuth was used as a carrier of polonium. The validity of the usage was examined as follows. To about 40 l of synthesized sea water, 1 ml of the standard solution of polonium-210 was added with stirring. Then the polonium-210 was determined by the procedure described above. The recovery of bismuth was also determined. The results of the 4 repeated measurements are shown in Table I. The recovery of polonium-210 agreed well with that of bismuth within ±4%.

TABLE I

THE RECOVERIES OF BISMUTH AND POLONIUM-210 IN THE SYNTHESIZED SEA WATER

	Recovery of bismuth (%)	*Polonium-210 (d.p.m.)*		*Polonium-210 (d.p.m.) corrected*[a]	*Polonium-210 corrected*
		Added	*Found*		*Polonium-210 added (%)*
1	67	360	258	385	107
2	81	360	293	362	101
3	70	360	254	363	101
4	87	360	305	351	98
				Average	102±4[b]

[a] Polonium-210 found/recovery of bismuth.
[b] Standard deviation.

Chemical yields of lead-210 and polonium-210

The chemical yield of lead-210 throughout the procedure was obtained by measuring the concentration of lead in the solution remaining after the electrodeposition. A hundredth portion of the solution was placed in a 25-ml volumetric flask and diluted to the mark with 0.1 *M* nitric acid solution. Standard solutions for the determination of lead were prepared in the same manner with the carrier solution. The concentration of lead was measured by the atomic absorption method. The variation of the recoveries of lead from 127 sea water samples is shown as a histogram in Fig. 4, where about 85% of the samples fall in the recovery range of 75–95%.

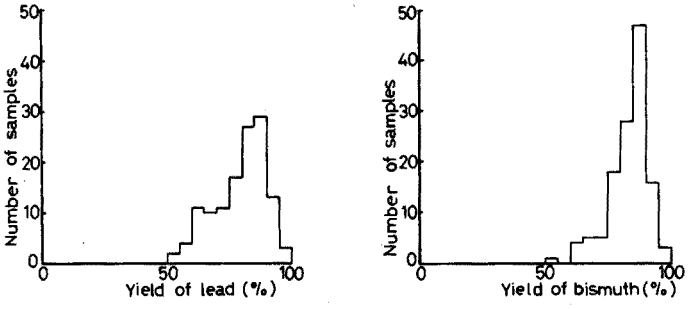

Fig. 4. Chemical yields of lead-210 in the analyses of 127 sea water samples.

Fig. 5. Chemical yields of polonium-210 assumed to be equal to the recovery of bismuth for the same samples as those in Fig. 4.

It was assumed that the yield of polonium-210 would equal that of bismuth, and the latter was determined as follows. A 5-ml portion of the solution remaining after the determination of lead was taken and the content of bismuth was determined by the spectrophotometric method with thiourea in nitric acid solution[4]. The recovery of bismuth, as shown in Fig. 5, correlates well with that of lead, although the former is slightly higher. The recovery is lowered mainly by loss of the precipitate of calcium carbonate or by the incomplete coprecipitation of these nuclides from sea water.

Reagent blank

Contamination of lead-210 from reagents was examined by using 1 l of solutions containing the same amounts of all reagents as the samples. The duplicate results were 0.03 ± 0.03 and -0.01 ± 0.03 d.p.m. per sample. The contamination of lead-210 was negligibly small compared with the amount contained in more than 30 l of sea water samples.

Calculations

Concentrations of lead-210 and polonium-210 at the time of sampling were calculated from the equations of radioactive decay or growth. In the calculation, it was assumed that bismuth-210 was in equilibrium with lead-210 at the time of sampling and at the end of electrodeposition. Error derived from this assumption does not exceed 3% for 5 months of storage. The half-life of lead-210 of 21.4 yr[6] was used in the calculation. The error caused by the uncertainty of the half-life (from 19.4 to 22.3 yr) does not exceed 1% for a sample stored for less than three years.

Precision

Some examples of replicate analyses are shown in Table II. These determinations were done on about 45-l samples of sea water collected from the surface of the ocean.

The counting error was about $\pm 10\%$, being calculated as one standard deviation, for polonium-210, when it was counted for 2 h; the error was about 7%

TABLE II

REPLICATE ANALYSES OF SURFACE SEA WATER

Location	*Date of sampling*	*Lead-210 (d.p.m. per 100 kg)*	s_r	*Polonium-210 (d.p.m. per 100 kg)*	s_r
28°29'N, 145°02'E	23 June 1971	23.2 ± 1.5[a]		5.7 ± 0.6[a]	
28°29'N, 145°02'E	23 June 1971	26.7 ± 1.9	1.5	6.6 ± 0.6	0.4
28°29'N, 145°02'E	23 June 1971	26.1 ± 1.6		6.2 ± 0.5	
28°29'N, 145°02'E	23 June 1971	23.6 ± 1.5		5.8 ± 0.5	
28°23'N, 144°59'E	30 June 1971	18.7 ± 1.4	0.4	3.3 ± 0.4	0.8
28°23'N, 144°59'E	30 June 1971	17.9 ± 1.3		4.9 ± 0.5	
32°40'N, 145°31'E	3 July 1971	13.8 ± 1.2	0.2	5.7 ± 0.6	0.3
32°40'N, 145°31'E	3 July 1971	14.2 ± 1.2		5.1 ± 0.5	
44°05'N, 154°02'E	21 July 1971	11.1 ± 0.9		4.7 ± 0.5	
44°05'N, 154°02'E	21 July 1971	10.6 ± 0.9	0.8	3.0 ± 0.4	0.8
44°05'N, 154°02'E	21 July 1971	12.5 ± 0.9		4.4 ± 0.4	

[a] 1 σ of counting error.

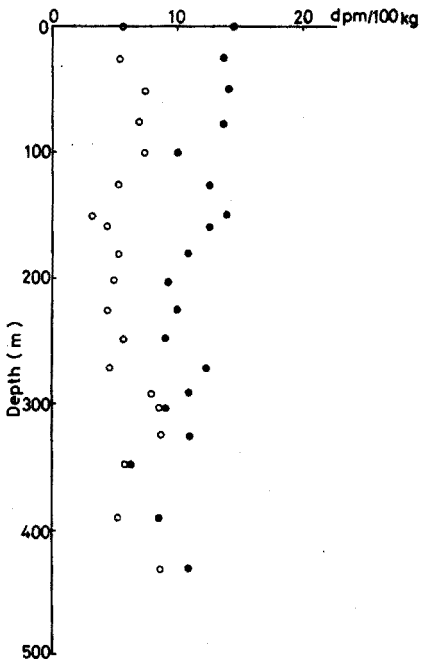

Fig. 6. Vertical distributions of lead-210 and polonium-210 in the Pacific Ocean at 44°09'N, 154°00'E. Solid and open circles represent the activities of lead-210 and polonium-210, respectively.

for lead-210 stored for about 5 months. As shown in Table II, the standard deviations in replicate determinations for lead-210 were within the counting error, while those for polonium exceeded it in two cases, probably owing to the error derived from the sampling and the concentration process. This shows that the precision of the determination of lead-210 is somewhat improved by longer counting time. It may be better to use a radioactive yield tracer of polonium-208 or polonium-209 and an α-spectrometric technique, if available, for the accurate determination of polonium-210 with a better accuracy than 10%.

Distribution of lead-210 and polonium-210 in the surface layer of the Pacific Ocean

Sea water samples were collected from the North Pacific Ocean during the cruise of R/V Hakuho-maru, KH-71-3 in 1971, and were analysed by the procedure described above. The vertical distributions of lead-210 and polonium-210 in the surface water down to a depth of 500 m are illustrated in Fig. 6. The ratios of polonium-210 to lead-210 were smaller than unity in the surface layer of the ocean. The full data as well as the oceanographic implications of the results will be published elsewhere, although some results have already been published[7].

We are deeply grateful to Prof. M. Nishimura for valuable discussions and critical reading of the manuscript.

SUMMARY

A new radiochemical procedure is described for the determination of lead-210

and polonium-210 in sea water. These nuclides are concentrated by coprecipitation with calcium carbonate from a sea water sample of large volume and then separated from calcium by formation of the hydroxides. Polonium-210 is deposited spontaneously onto a silver disc and determined by an α-counting technique. Lead-210 is also determined by counting the activity of polonium-210 produced during storage for 3 months.

RÉSUMÉ

Une nouvelle méthode radiochimique est proposée pour le dosage du plomb-210 et du polonium-210 dans l'eau de mer. Ces nucléides sont concentrés par coprécipitation avec carbonate de calcium, et ensuite séparés d'avec le calcium par formation des hydroxydes. Le polonium-210 est déposé spontanément sur un disque d'argent et dosé par comptage-α. Le plomb-210 est également dosé par comptage de l'activité du polonium-210 produit après 3 mois.

ZUSAMMENFASSUNG

Ein neues radiochemisches Verfahren für die Bestimmung von Blei-210 und Polonium-210 in Meerwasser wird beschrieben. Diese Nuklide werden durch Mitfällung mit Calciumcarbonat in einer grossvolumigen Meerwasserprobe angereichert und dann vom Calcium durch Bildung der Hydroxide abgetrennt. Polonium-210 wird auf einer Silberscheibe spontan abgeschieden und durch ein α-Messverfahren bestimmt. Blei-210 wird ebenvalls durch Messung der Aktivität von Polonium-210 bestimmt, das in einem Zeitraum von 3 Monaten nachgebildet wird.

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THE NEW METHOD FOR THE DETERMINATION OF COBALT IN SEA WATER BY SOLVENT EXTRACTION WITH 2-NITROSO-5-DIETHYLAMINOPHENOL

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o-Nitrosophenol and naphthol derivatives are widely used in the spectrophotometric determination of cobalt; among the various nitroso compounds, 2-nitroso-5-dimethylaminophenol[1] and 2-nitroso-5-diethylaminophenol[2] are useful reagents for the solvent extraction and spectrophotometric determination of trace amounts of cobalt. Cobalt has been determined in nickel salts[3], iron and steel[4] and pure reagent chemicals[5] with 2-nitroso-5-dimethylaminophenol. The dimethyl and diethyl reagents are similar in pK_a, absorption spectra, molar absorptivities, etc. However, the partition coefficients of the diethyl reagent and its cobalt complex between aqueous solution and 1,2-dichloroethane are much larger than those of the dimethyl reagent. The logarithm of the partition coefficient of the cobalt–2-nitroso-5 diethylaminophenol complex is about 7.

Cobalt is one of the most interesting trace ions in sea water and is very difficult to determine. Most of the methods which have been used to determine cobalt in sea water necessitate a preconcentration, *e.g.* by cocrystallization[6,7], coprecipitation[8–11], solvent extraction[12], chelating resins[13,14]. In general, these procedures require large volumes of sample solution and are time-consuming. A few workers[15,16] have determined cobalt in sea water spectrophotometrically after solvent extraction, but the procedures are not very satisfactory for the concentrations of cobalt normally found in sea water (*ca.* 0.1 $\mu g\ l^{-1}$).

In the work described here, cobalt in sea water was determined spectrophotometrically by means of solvent extraction with 2-nitroso-5-diethylaminophenol. The proposed method eliminates preconcentration, requires relatively small volumes of sample solution, and provides simplicity and precision.

EXPERIMENTAL

Reagents

2-Nitroso-5-diethylaminophenol solution (0.2%). The reagent was obtained by nitrosation of *m*-diethylaminophenol in hydrochloric acid solution with sodium nitrite. This crude reagent was recrystallized three times from hydrochloric acid solution. The recrystallized reagent was dissolved in 0.01 *M* hydrochloric acid solution.

Standard cobalt solution. Prepare a stock solution (0.3 mg Co ml^{-1}) from cobalt chloride hexahydrate, and standardize by EDTA titration. Before use, dilute

this solution accurately with 0.1 *M* hydrochloric acid solution to 0.06 μg Co ml^{-1}.

Sodium citrate solution. Dissolve 200 g of trisodium citrate dihydrate in water. To this solution, add 20 ml of 0.2% reagent solution. Dilute to 500 ml with water. After 1 h, remove the cobalt complex and the excess of reagent by extracting with 20 ml of 1,2-dichloroethane, until the aqueous phase is colourless. Filter the aqueous phase through dry filter paper. This filtrate contains no detectable cobalt.

Sodium chloride solution. Dissolve 313 g of sodium chloride in water and dilute with water to 970 ml. To this solution, add 20 ml of 0.2% reagent solution and 10 ml of sodium citrate solution. After 1 h, extract the cobalt complex and the excess of reagent with 20 ml of 1,2-dichloroethane, until the aqueous phase is colourless. Then shake the aqueous phase twice with 20-ml portions of benzene. Warm the aqueous phase to about 90° to remove the dissolved benzene.

All the reagents used were of analytical grade.

Apparatus

A Hitachi-Perkin Elmer model 139 spectrophotometer equipped with a microcell holder, and a Hitachi model EPS-3T recording spectrophotometer were used for measuring absorbances in small cells (0.7-ml and 1.5-ml capacity) of 50 mm path length. An Iwaki model KM shaker was used for extracting the cobalt complex from sea water.

Procedure

Filter the sea water samples through a 0.45-μm membrane filter and put 2-l (or 1-l) aliquots into a separatory funnel (about 2.3 l). To this, add 10 ml (or 5 ml) of sodium citrate solution and 20 ml (or 10 ml) of reagent solution. Mix thoroughly and allow to stand for 30 min. Add 10 ml (or 5 ml) of aqueous 10% EDTA solution and 20 ml (or 10 ml) of 1,2-dichloroethane. Shake the separatory funnel for 10 min with a shaker. Remove the organic phase into a stoppered test tube, and back-extract the excess of the reagent and other metal chelates successively with three 5-ml portions of (1+2) hydrochloric acid, a 5-ml portion of 1 *M* potassium hydroxide and a 5-ml portion of (1+2) hydrochloric acid. Filter the organic phase through dry filter paper and measure the absorbance at 462 nm in a cell of 50-mm path length. Obtain the reagent blank by adding EDTA solution before sodium citrate solution.

In the case of sea water samples, to which concentrated hydrochloric acid had been added (1 ml per 1 l sea water), wash the membrane filter by filtering 500 ml of 0.01 *M* hydrochloric acid solution before filtering sea water, and neutralize the sea water sample with about 4 ml (or 2 ml) of (1+2) ammonia water before adding the reagent. The optimal pH is 5.5–7.5. When unfiltered sea water samples are used, the organic phase withdrawn after shaking should be centrifuged at about 2000 r.p.m. for 5 min and put into the stoppered test tube.

RESULTS AND DISCUSSION

Effect of pH, shaking time, standing time and amount of reagent

The above-mentioned procedure was used in studying the effects of pH, shaking time, standing time and amounts of the reagent added on the extraction

of cobalt; distilled water and sea water samples were used. The pH values were adjusted with hydrochloric acid or potassium hydroxide solution. The optimal pH range was 4.5–8.5 for distilled water containing 0.24 μg of cobalt, but 5.5–7.5 for sea waters. The minimal shaking time was 6 min in both cases, and a time of 10 min was selected for certainty. The standing time necessary for development of the complex was only 10 min, but a period of 30 min was considered advisable. When distilled water containing 0.24 μg of cobalt was used, maximal absorbance was obtained with a reagent addition of 10 ml of 0.2% solution per litre.

Absorbance spectra

In Fig. 1 are shown the absorbance spectra of the cobalt complex and the reagent blank in the organic phase. The maximal absorption of cobalt complex occurs at 462 nm, where the absorbance of the reagent blank is very low.

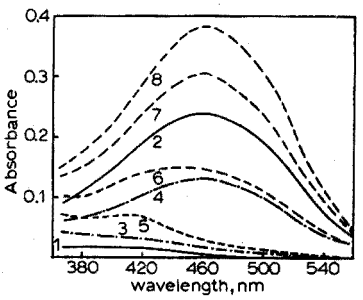


Fig. 1. Absorbance spectra. (1) Distilled water + EDTA + citrate + reagent. (2) Distilled water + 0.12 μg Co l^{-1} treated with citrate, reagent and EDTA in that order. (3) Filtered sea water treated with EDTA, citrate, ammonia solution and reagent in that order. (4) Filtered sea water treated with citrate, ammonia solution, reagent and EDTA in that order. (5) Unfiltered sea water treated as for (3). (6) Unfiltered sea water treated as for (4). (7) Unfiltered sea water with 0.12 μg Co l^{-1} added treated as for (4). (8) Unfiltered sea water with 0.18 μg Co l^{-1} added treated as for (4).

Removal of the excess of reagent extracted into the organic phase

The large excess of reagent in the organic phase causes errors in determining the absorbance of the cobalt complex, hence its removal is necessary. This can be done with either alkaline or acidic solution. In the recommended procedure, the reagent was extracted into the organic phase from 2 l of distilled water with 20 ml of 1,2-dichloroethane. When the reagent in the organic phase was stripped as follows: (1) four times with (1+2) hydrochloric acid solution, (2) four times with (1+2) hydrochloric acid solution and once with 1 *M* potassium hydroxide solution, and (3) three times with (1+2) hydrochloric acid solution, once with 1 *M* potassium hydroxide solution and once with (1+2) hydrochloric acid solution, the absorbances were 0.063, 0.016 and 0.014, respectively. Accordingly, the last stripping procedure was preferred.

Filtration of sample solution

In this method for determining cobalt in sea water, cobalt can be determined without filtration, if the samples are allowed to stand for about one day after sampling. The reagent blank, however, is then higher than when the sample is filtered.

TABLE I

REAGENT BLANK

Sea water sample[a], *1 l*	*Standing time after sampling*	*Absorbance*
Unfiltered	5 h	0.162
Unfiltered	1 d	0.131
Unfiltered + HCl[b]	5 h	0.098
Unfiltered + HCl	1 d	0.064
Filtered	5 h	0.016
Filtered	1 d	0.033
Filtered + HCl	5 h	0.027
Filtered + HCl	1 d	0.034

[a] This was sampled offshore at Shibukawa, Okayama Prefecture, Japan.
[b] Sample contains 1 ml of concentrated hydrochloric acid per liter.

From Table I, filtered sea water samples are preferable. Filtration of distilled water through filter paper or membrane filters was examined. When the water contained 1 ml of concentrated hydrochloric acid per liter, trace amounts of cobalt (*ca.* 0.01 $\mu g\ l^{-1}$) were dissolved out from the filter paper (30 cm diam.) or membrane filter (47 mm diam.). The variation in the results for filtered water containing cobalt was greater than in the case of no filtration or filtration after washing the filter paper or membrane filter with acidic solution. Accordingly, it is essential to wash the filter paper or membrane filter with acidic solution before use. Membrane filters are best washed by filtering about 0.5 l of 0.01 *M* hydrochloric acid solution through them.

Effect of co-existing ions

Some metal ions, such as iron(III), iron(II), copper and nickel ions, react with the reagent and to some extent, are extracted into the organic phase with the cobalt complex. These complexes, except cobalt, can, however, be stripped completely with hydrochloric acid. The other metal ions and anions existing commonly in sea water do not interfere.

Addition of EDTA

As mentioned previously[3], the cobalt–2-nitroso-5-dimethylaminophenol complex is not decomposed by addition of EDTA, but the complex is not formed if EDTA is added before the reagent. These phenomena are also found with the analogous diethyl reagent. Table II shows that when EDTA is added before the addition of the diethyl reagent, cobalt does not react, and the absorbances of distilled water and sea water filtered through a membrane filter are almost equal. However, when the reagent is added before the addition of EDTA, the cobalt complex does not decompose. Accordingly, the reagent blank can be obtained by changing the order of addition of EDTA.

Calibration curve

The solubility of 1,2-dichloroethane depends on the amounts of salts dissolved in the water. In general, sea water samples are found to contain about 35 g of

TABLE II

EFFECT OF EDTA

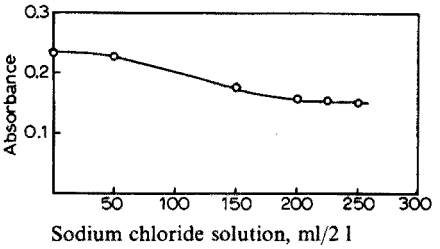
Sample (2 l)	*Order of addition*[a]	*Absorbance*
Distilled water	E+C+R	0.012
	C+R+E	0.026
	C+R	0.030
Distilled water containing 0.12 μg Co l^{-1}	E+C+R	0.012
	C+R+E	0.245
	C+R	0.242
Filtered sea water[b]	E+C+NH_3+R	0.014
	C+NH_3+R+E	0.135
Unfiltered sea water	E+C+NH_3+R	0.051
	C+NH_3+R+E	0.164

[a] E = EDTA solution; C = sodium citrate solution; R = reagent solution; NH_3 = (1+2) NH_3.
[b] Sea water containing 2 ml of conc. HCl filtered through membrane filter.

various salts per liter. Therefore, the volumes of organic phase withdrawn after shaking sea water with 1,2-dichloroethane differ from those in distilled water. When 2-l samples of distilled water and sea water were shaken with 20 ml of 1,2-dichloroethane, the volumes of the organic phase withdrawn were about 4.5 ml and 8 ml, respectively. Sodium chloride solution was therefore used for the calibration curves. Figure 2 shows that the absorbances at amounts of sodium chloride almost equal to the total amount of salts in sea water, *i.e.*, at 225 ml of sodium chloride solution (35.2 g of sodium chloride per l), were almost constant. When the calibration curve was prepared by using a solution containing 35.2 g of sodium chloride per l, its slope was almost equal to the slope of the curve prepared by adding appropriate amounts of cobalt to 2-l aliquots of sea water (Fig. 3). The slopes of the curves prepared by adding appropriate amounts of cobalt (0–0.24 μg l^{-1}) to 2-l samples of distilled water, sodium chloride solution (35.2 g l^{-1}) and sea water (filtered or unfiltered) were 0.201, 0.120 and 0.128 absorbance unit per 0.1 μg of cobalt, respectively. When 1 l of sea water was shaken with 10 ml of 1,2-dichloroethane, the slope was 0.166 absorbance unit per 0.1 μg of cobalt. All the curves, if the appropriate blank was subtracted from each of the absorbances, were straight lines passing through the origin.

Stability of cobalt in sea water samples

The cobalt content in sea water stored in ordinary containers decreases gradually. As shown in Fig. 4, constant absorbances were obtained only for about one day after sampling. If sea water samples are acidified by adding 1 ml of concentrated hydrochloric acid per liter of sea water, constant absorbance can be obtained during at least 10 days. The absorbance of the latter samples is, however, a little higher than the natural sample. It seems, therefore, that cobalt in sea water samples should be determined at the earliest opportunity after sampling, or samples should be acidified.



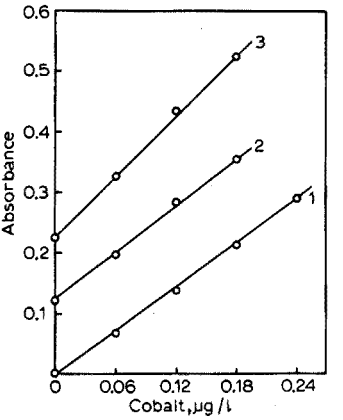


Fig. 2. Effect of sodium chloride. Sodium chloride solution, 313 g l^{-1}. Cobalt concentration, 0.12 μg l^{-1}, measured against reagent blank.

Fig. 3. Calibration curves. (1) Sodium chloride solution (35.2 g l^{-1}), 2 l taken. (2) Unfiltered sea water, 2-l sample. (3) Unfiltered sea water, 1-l sample. Reference, reagent blank.

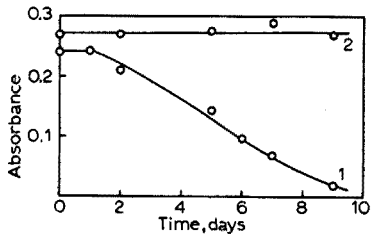


Fig. 4. Stability of cobalt in sea water. (1) No HCl added. (2) Containing 1 ml of conc. HCl per l.

Analysis of sea waters

The results obtained by the proposed method for trace amounts of cobalt in sea water samples are given in Table III. The amounts of cobalt in 1 ml of concentrated hydrochloric acid and 2 ml of (1+2) ammonia water correspond to 0.009 μg Co l^{-1}. So, when sea water samples containing 1 ml of hydrochloric acid were used, 0.009 μg was subtracted from the value obtained.

Table IV indicates that the reproducibility was sufficient for the determination of trace amounts of cobalt in sea water samples, when 1-l or 2-l samples were used. The sensitivity is also good enough to determine the cobalt in sea water (0.12–0.17 absorbance unit per 0.1 μg of cobalt). The amounts of cobalt in sea water determined by this method agree with the amounts reported in recent papers, *i.e.*, 0.13 and 0.24 μg l^{-1} [12] and 0.12 μg l^{-1} [13].

The author is greatly indebted to Professor Kyoji Tôei of Okayama University for his valuable advice and discussion.

TABLE III

COBALT CONTENT IN SEA WATER

Sample[a]	*Sample taken (l)*	*Absorbance*	*Cobalt ($\mu g\ l^{-1}$)*
Seashore, May 26th, 1972[b,c]	2	0.130	0.099
		0.136	0.104
Seashore, June 14th, 1972[b,c]	2	0.092	0.068
		0.093	0.068
		0.094	0.069
		0.094	0.069
		0.095	0.070
		0.101	0.075
		0.101	0.075
Seashore, June 26th, 1972[b]	2	0.121	0.092
		0.113	0.085
Same sample[b,c]	2	0.121	0.092
Offshore, July 19th, 1972[b,c]	1	0.266	0.151
		0.266	0.151
		0.269	0.153
		0.274	0.156
		0.286	0.163
Same sample, untreated	1	0.230	0.139
Same sample, filtered only[c]	1	0.240	0.144

[a] These were sampled at Shibukawa, Okayama Prefecture, Japan.
[b] Samples contain 1 ml of concentrated hydrochloric acid per l, and 0.009 μg Co ml^{-1} was subtracted from the value obtained from the calibration curve.
[c] Samples were filtered through a membrane filter.

TABLE IV

REPRODUCIBILITY OF ABSORBANCE OF COBALT IN SEA WATER

(10 determinations).

	2-l sample	*1-l sample*
Mean of absorbance	0.096	0.272
Standard deviation	0.004	0.008
Relative std. dev.	4%	3%

SUMMARY

Cobalt in sea waters can be determined spectrophotometrically by means of 2-nitroso-5-diethylaminophenol after extraction of the complex into 1,2-dichloroethane. No preliminary concentration is needed. Interferences are prevented by masking or by stripping from the organic phase. The method is applicable over the range 0–0.24 μg Co l^{-1} when 1-l or 2-l samples are taken. The relative standard deviation is 4% for 0.15 μg Co l^{-1}. The stability of cobalt in sea water samples is discussed.

RÉSUMÉ

Le cobalt dans les eaux de mer peut être dosé par spectrophotométrie au moyen de nitroso-2-diéthylamino-5-phénol, après extraction du complexe dans le dichloro-1,2-éthane. Aucune concentration préliminaire n'est nécessaire. Les interférences sont évitées par traitement de la phase organique. Cette méthode est applicable de 0 à 0.24 μg Co l^{-1} pour des échantillons de 1 ou 2 litres. La déviation standard relative est de 4% pour 0.15 μg Co l^{-1}. On examine la stabilité du cobalt dans les échantillons d'eau de mer.

ZUSAMMENFASSUNG

Kobalt in Meerwasser kann nach Extraktion als 2-Nitroso-5-diäthylaminophenol-Komplex mit 1,2-Dichloräthan spektrophotometrisch bestimmt werden. Eine vorhergehende Anreicherung ist nicht erforderlich. Störungen können durch Maskierung oder durch Waschen der organischen Phase vermieden werden. Die Methode ist auf den Bereich 0–0.24 μg Co l^{-1} anwendbar, wenn Proben von 1 l oder 2 l eingesetzt werden. Die relative Standardabweichung ist 4% für 0.15 μg Co l^{-1}. Die Stabilität von Kobalt in Meerwasserproben wird diskutiert.

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CONDUCTANCE OF TRIS(1,10-PHENANTHROLINE)IRON(II) AND TRIS(2,2'-BIPYRIDINE)IRON(II) HALIDES AND PERCHLORATES IN WATER AND NITROBENZENE IN RELATION TO SOLVENT EXTRACTION OF THE ION PAIRS

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Tris(1,10-phenanthroline)iron(II) and tris(2,2'-bipyridine)iron(II) chelate cations (abbreviated to $Fe(phen)_3^{2+}$ and $Fe(bip)_3^{2+}$, respectively) are well extracted into nitrobenzene with certain anions, such as perchlorate, thiocyanate and iodide, which are large in size and are known as strong structure-breaking ions[1] in aqueous solutions. This phenomenon has been extensively utilized for the spectrophotometric determination of the anions[2–6]. Studies[7] on this system have shown that the distribution ratios of these ion pairs for nitrobenzene increase in the order $Cl^- < Br^- < I^- < ClO_4^-$, *i.e.* the order of increasing ionic radius of the anions. Furthermore, it has been found that the number of the water molecules transferred with the ion pair in question into nitrobenzene increases in the order $ClO_4^- < I^- < Br^- < Cl^-$, *i.e.* the reverse order of the distribution ratio; a mechanism for the water participation in this system has been suggested[8]. However, it is essential to know how the ion pairs exist in both phases, and especially to what extent ion association occurs in the organic phase, in order to analyze the mechanism of the solvent extraction more quantitatively.

Conductance measurements provide the ion-association constants, K_A, and the hydrodynamic radii (Stokes radii) of the ion pairs in solution. The extent of ion association is expected to have an important influence on the extraction behavior of the ion pairs.

EXPERIMENTAL

Materials

$Fe(phen)_3^{2+}$ and $Fe(bip)_3^{2+}$ chelate salts. Crystals of $Fe(phen)_3^{2+}$ chloride, bromide, iodide and perchlorate were prepared from Mohr's salt, 1,10-phenanthroline and sodium salts of the corresponding anions in a water–ethanol mixture. Crystals thus obtained were recrystallized twice from conductivity water and air-dried. The corresponding salts of $Fe(bip)_3^{2+}$ were prepared by a similar method. The purity was checked by analysis (Table I). The stability of these chelate salts in solutions was confirmed by spectrophotometry; the chloride salts in nitrobenzene were found to decompose slightly, so that measurements on chloride salts in nitrobenzene were not made.

TABLE I

ANALYTICAL DATA

		% H	% C	% N	% Fe (ash)
$Fe(phen)_3Cl_2 \cdot 7H_2O$	Found	4.82	55.31	10.66	7.04
	Calcd.	4.82	54.49	10.59	7.04
$Fe(phen)_3Br_2 \cdot 6H_2O$	Found	4.16	49.55	9.70	6.44
	Calcd.	4.21	50.02	9.73	6.46
$Fe(phen)_3I_2 \cdot 3H_2O$	Found	3.62	47.85	9.29	6.38
	Calcd.	3.35	47.81	9.30	6.18
$Fe(phen)_3(ClO_4)_2 \cdot H_2O$	Found	3.26	53.94	10.59	
	Calcd.	3.23	53.16	10.33	
$Fe(bip)_3Cl_2 \cdot 5H_2O$	Found	4.88	52.56	12.17	
	Calcd.	5.00	52.57	12.26	
$Fe(bip)_3Br_2 \cdot 5H_2O$	Found	4.56	46.22	10.85	7.21
	Calcd.	4.43	46.54	10.85	7.21
$Fe(bip)_3I_2 \cdot 5H_2O$	Found	3.95	41.70	9.64	6.46
	Calcd.	3.95	41.50	9.68	6.43
$Fe(bip)_3 \cdot (ClO_4)_2$	Found	3.38	49.69	11.63	
	Calcd.	3.34	49.82	11.62	

Potassium chloride. Potassium chloride for calibrating the conductance cells was of analytical-reagent grade, which was recrystallized three times from conductivity water and was dried at 500°.

Nitrobenzene. Commercial nitrobenzene was washed with acid, base and conductivity water, successively. It was then dried over anhydrous calcium chloride for a week. The filtrate was passed through molecular sieve and fractionated at reduced pressure (below 10 mm Hg). The product was still slightly yellow, its density being 1.1986, which was the same as the value reported by Taylor and Kraus[9] but was slightly higher than the 1.1977 reported by Hirsch and Fuoss[10]. Therefore other physical constants used for analysis are quoted from Taylor and Kraus[9]. The specific conductance of the nitrobenzene was negligible for the present purpose.

Conductivity water. Conductivity water was prepared by passing distilled water through a mixed-bed ion-exchange column just before each run. Its specific conductance was usually less than $8 \cdot 10^{-7}$ mho cm^{-1}. The values of 78.54 and 0.008949 were used for the dielectric constant and viscosity in poise, respectively[11].

Apparatus and procedure

Conductances were measured by a conductometer (Model MY-7, Yanagimoto Mfg. Co.) with the Wheatstone bridge (800 Hz), which was calibrated with a standard resistance box (Shimazu Seisakusho Co.). Flask-type cells with lightly platinized electrodes were used. Cell constants were determined with standard aqueous solutions of potassium chloride, with the constants of Lind *et al.*[12]. All measurements were carried out in a water bath thermostated to $25 \pm 0.01°$ with a mercury-in-glass thermoregulator.

It was necessary to know the solvent conductance precisely in water, especially at low salt concentration. Therefore the following procedure was applied.

Stock solutions, about 0.01 *M*, were prepared accurately by weight. About 80 g of conductivity water was accurately weighed into the cell and its resistance was measured after temperature equilibration. Then about 1 g of the stock solution weighed accurately in a glass cup was added to the cell, and the resistance was measured after temperature equilibration. This procedure was continued to an appropriate concentration. Measurements in nitrobenzene and in less dilute aqueous solutions were carried out by the dilution technique[13]. At least two runs were made on each salt and their agreement was good to 0.2%. Measurements on $Fe(phen)_3(ClO_4)_2$, $Fe(phen)_3I_2$ and $Fe(bip)_3(ClO_4)_2$ in water, and $Fe(phen)_3Cl_2$ and $Fe(bip)_3Cl_2$ in nitrobenzene, were impossible owing to their low solubilities in the respective solvent and to slight decomposition of the chloride salts in nitrobenzene.

RESULTS AND DISCUSSION

Experimental data are given in Table II, where Λ is the equivalent conductance and C is the concentration in equivalent per liter. Figure 1 shows some of the representative Λ–$\sqrt{C}$ plots, which have an approximately linear slope over the working range of concentration (10^{-3}–10^{-5} eq.). These data were analyzed by the Jenkins–Monk[14] and the Fuoss–Edelson[15] methods in which two assumptions were made. For 2:1 electrolytes, the association equilibria are represented as follows,

$$M^{2+} + X^- \overset{K_{A1}}{\rightleftharpoons} MX^+ \qquad [M^{2+} = Fe(phen)_3^{2+} \text{ or } Fe(bip)_3^{2+}]$$

$$MX^+ + X^- \overset{K_{A2}}{\rightleftharpoons} MX_2 \qquad [X^- = Cl^-, Br^-, I^- \text{ or } ClO_4^-]$$

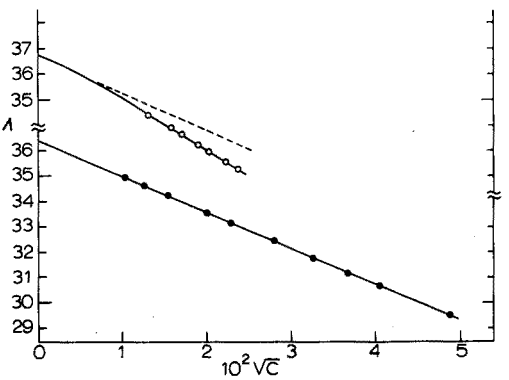

Fig. 1. Equivalent conductances of $Fe(phen)_3Br_2$ (○) and $Fe(phen)_3(ClO_4)_2$ (●) in nitrobenzene as a function of $\sqrt{C}$. Broken line is a theoretical Onsager slope. In the case of $Fe(phen)_3(ClO_4)_2$, the experimental line coincides with an Onsager slope.

where K_{A1} and K_{A2} refer to the first and second association constants, respectively. The validity of the first assumption that K_{A2} is zero, is justified by the fact that bis(2,9-dimethyl-1,10-phenanthroline)copper(I) perchlorate has been found to be completely dissociated[16] even in nitrobenzene with a dielectric constant of 34.82. The second assumption, that the mobility of M^{2+} is twice that of MX^+, is also justified by the fact that the chelate cations are considerably larger than the anions,

TABLE II

EQUIVALENT CONDUCTANCES

$10^4\,C$	Λ	$10^4\,C$	Λ	$10^4\,C$	Λ	$10^4\,C$	Λ
Fe(phen)$_3$Br$_2$		*Fe(phen)$_3$Br$_2$*		*Fe(bip)$_3$Cl$_2$*		*Fe(bip)$_3$I$_2$*	
in water		*in nitrobenzene*		*in water*		*in nitrobenzene*	
24.011	104.64	5.672	32.30	44.095	99.66	12.458	31.08
22.440	104.85	4.990	32.66	40.071	100.3	11.255	31.44
18.889	105.55	4.121	32.99	39.784	100.3	10.250	31.65
16.358	106.08	3.597	33.31	35.832	100.9	8.8290	32.05
13.930	106.59	2.939	33.62	35.028	101.0	8.4909	32.12
13.563	106.71	2.507	33.92	30.614	101.9	7.3261	32.52
10.328	107.54	1.730	34.41	29.118	102.1	6.7636	32.68
8.3758	108.08	*Fe(bip)$_3$I$_2$*		25.943	102.7	6.1013	32.96
6.5307	108.69	*in water*		24.448	102.9	6.0987	32.96
Fe(phen)$_3$Cl$_2$		13.149	106.6	23.646	103.1	5.3132	33.18
in water		10.758	107.1	20.405	103.7	4.8521	33.43
11.308	105.37	9.5346	107.4	19.655	103.9	3.9650	33.83
10.566	105.54	9.1015	107.6	16.890	104.6	3.7570	33.89
9.9217	105.71	8.0672	107.9	15.680	104.9	3.2247	34.18
6.9654	106.80	7.8140	108.0	13.709	105.3	3.0441	34.29
6.4759	106.90	6.8313	108.2	11.006	106.0	2.6428	34.47
5.3497	107.30	6.7575	108.2	10.130	106.3	2.3467	34.67
4.2142	107.67	6.2360	108.5	8.6897	106.9	2.1248	34.82
3.9711	107.78	5.7170	108.7	2.5405	109.3	1.6601	35.18
3.3798	108.14	4.9907	108.9	*Fe(bip)$_3$(ClO$_4$)$_2$*		1.3365	35.28
2.2905	108.69	4.9665	108.9	*in nitrobenzene*		1.0649	35.61
Fe(phen)$_3$(ClO$_4$)$_2$		4.4761	109.0	10.211	32.39	0.95363	35.69
in nitrobenzene		3.1927	109.6	8.5449	32.84	0.82633	35.83
23.788	29.52	2.3595	110.0	7.0925	33.29	0.66763	36.00
16.354	30.61	2.1236	110.1	5.9913	33.69	0.52082	36.09
13.410	31.15	*Fe(bip)$_3$Br$_2$*		5.0447	34.01	0.39274	36.27
10.637	31.72	*in water*		4.1233	34.39	*Fe(bip)$_3$Br$_2$*	
7.8569	32.41	39.690	103.5	3.5145	34.64	*in nitrobenzene*	
5.1678	33.15	39.483	103.5	3.2834	34.70	10.3237	31.52
4.0112	33.52	35.451	104.1	2.5437	35.09	8.7075	32.10
2.3558	34.24	33.062	104.4	1.9394	35.46	8.0178	32.38
1.5841	34.62	33.056	104.4	1.4794	35.72	7.0990	32.73
1.0571	34.93	32.209	104.5	1.3799	35.86	6.6466	32.96
Fe(phen)$_3$I$_2$		31.865	104.6	1.0906	36.04	6.1794	33.17
in nitrobenzene		31.448	104.6	0.98462	36.11	5.9432	33.26
13.266	30.43	28.749	105.1	0.56214	36.56	5.2204	33.63
10.412	31.11	28.336	105.1	0.41270	36.74	4.5554	34.00
7.6047	31.90	28.047	105.2	0.28104	36.93	4.1419	34.19
5.6012	32.55	25.533	105.6	0.20711	37.05	3.5920	34.49
4.6456	32.88	23.124	106.0	0.11991	37.28	3.3858	34.63
2.8989	33.56	22.545	106.1			2.7091	35.07
2.3340	33.91	17.407	107.1			2.6997	35.05
1.7777	34.21	8.5793	109.0			2.0956	35.52
1.4026	34.43	6.8019	109.6			1.9777	35.63
		4.5843	110.3			1.3992	36.12
		3.1365	111.0				

so that M^{2+} is thought to be hydrodynamically equivalent to MX^+ except in charge type. When the first association constant, K_{A1}, is small and the salt concentration is relatively low, *i.e.*, the degree of dissociation is near to unity, the latter assumption does not affect the final results appreciably, because MX^+ is not a predominant species present in solutions. The Fuoss–Edelson method gave self-consistent limiting equivalent conductance and the first association constant, K_{A1}, with the aid of successive approximation by the least-squares method, whereas the Jenkins–Monk method gave the degree of dissociation, α_c, at each concentration which reproduced the observed conductance best. The K_{A1} value at each concentration was calculated by means of the mass action law and the averaged value gave the final K_{A1} value. The activity coefficient was estimated from the Debye–Hückel limiting law. Anion mobilities in nitrobenzene and water used for analysis were taken from the literature[17,18]. All the calculations were carried out by an electronic computer, TOSBAC 3400.

Some representative Λ'–x plots from the Fuoss–Edelson method are shown in Fig. 2, where $\Lambda' = \Lambda F$, $x = C'\Lambda'(\Lambda' - \Lambda_0/2)$, $C' = Cf$, and $\Lambda' = \Lambda_0 - K_{A1}x/\Lambda_0$. F is a function of C and Λ_0, which corrects the conductance ratio for the effect of interionic forces on mobility. Parameters thus obtained are listed in Table III. K_{A1}

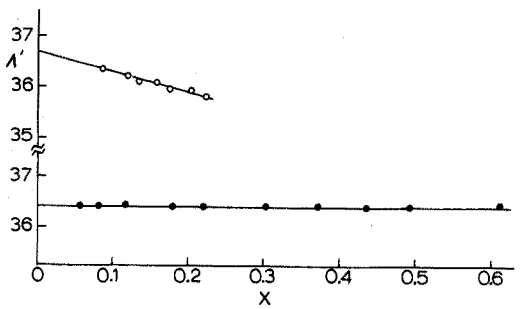

Fig. 2. Plots of Λ' *vs.* x in nitrobenzene. (○) [Fe(phen)$_3$]Br$_2$; (●) [Fe(phen)$_3$](ClO$_4$)$_2$.

TABLE III

DERIVED PARAMETERS

	Λ_0	λ_0^-	λ_0^+	K_{A1}(F-E)	K_{A1}(J-M)
In water					
Fe(phen)$_3$Br$_2$	112.95±0.03	78.14[a]	34.8	3.4 ±0.4	3.5 ±0.5
Fe(phen)$_3$Cl$_2$	111.24±0.04	76.35[a]	34.9	7.3 ±1.2	6.5 ±2.6
Fe(bip)$_3$I$_2$	112.53±0.04	76.84[a]	35.7	0	0
Fe(bip)$_3$Br$_2$	113.91±0.03	78.14[a]	35.8	0.50±0.20	0.49±0.30
Fe(bip)$_3$Cl$_2$	112.01±0.03	76.35[a]	35.7	9.1 ±0.3	9.4 ±1.5
In nitrobenzene					
Fe(phen)$_3$(ClO$_4$)$_2$	36.39±0.01	20.9[b]	15.5	0	0
Fe(phen)$_3$I$_2$	36.26±0.01	20.4[b]	15.9	51.5 ±2.4	55.2 ±5.5
Fe(phen)$_3$Br$_2$	36.69±0.04	21.6[b]	15.1	145 ±10	154 ±7
Fe(bip)$_3$(ClO$_4$)$_2$	37.71±0.01	20.9[b]	16.8	66.1 ±2.4	72 ±11
Fe(bip)$_3$I$_2$	37.24±0.01	20.4[b]	16.8	94.9 ±2.5	94 ±5
Fe(bip)$_3$Br$_2$	38.38±0.01	21.6[b]	16.8	221.4 ±2.4	236 ±6

[a] Ref. 18. [b] Ref. 17.

values clearly indicate that all the salts investigated are almost completely dissociated in water and that hydrodynamically $Fe(phen)_3^{2+}$ is slightly larger than $Fe(bip)_3^{2+}$; the Stokes radius of the former is 5.27 Å and that of the latter is 5.13 Å. These values are smaller than those estimated from their bond lengths (6.2 Å and 5.9 Å, respectively, which are in good agreement with those obtained from viscosity *B*-coefficients by means of the Einstein equation[19], *i.e.* 6.5 Å and 5.9 Å, respectively). The corresponding radii in nitrobenzene are 5.84 Å and 5.39 Å, respectively, which are also smaller than the values from the bond lengths. As pointed out by Fuoss[20] and Zwanzig[21], there is a contribution from the dipole relaxation of the solvent molecules to the Stokes radius, which decreases the ionic mobility and therefore increases the estimates of the Stokes radius. Moreover, when an ion moves through the solvent molecules, "slippage" may occur, especially when the ion is smaller than the solvent molecule in size. Thus the Stokes radius does not directly correspond to the crystallographic radius except when the radius of the ions is considerably larger than the dimension of the solvent molecules[22].

From Table III, it is clear that in nitrobenzene, the K_{A1} values of $Fe(phen)_3^{2+}$ salts are smaller than those of the corresponding $Fe(bip)_3^{2+}$ salts, and that the order of K_{A1} values is that expected from the electrostatic theory, *i.e.*, the larger the ion pair, the greater the dissociation. This order agrees with that of the distribution ratios of these salts between water and nitrobenzene[7]. Though measurements with chloride salts in nitrobenzene were impossible, K_{A1} values of these salts in this solvent are expected to be larger than those of the corresponding bromide salts, according to the electrostatic theory. It is quite surprising for $Fe(phen)_3(ClO_4)_2$ to dissociate completely even in nitrobenzene with relatively low dielectric constant, for the Bjerrum critical distance[23] in this solvent amounts to 16 Å. If 8.6 Å is chosen as a contact distance (the ionic radius of perchlorate is 2.36 Å), the K_{A1} value is estimated to be 126 from the Bjerrum theory and 68 from the Fuoss theory[24]. Halide salts also seem to have smaller association constants than those expected from the simple electrostatic theory in nitrobenzene. These facts may be interpreted in terms of the stabilization of the chelate cations; iron(II) is surrounded by bulky aromatic ligands so that its charge is screened considerably. In addition, though nitrobenzene has a much lower dielectric constant (34.82) than water (78.54) at 25°, its dipole moment is appreciably larger (3.99 *D*). Thus it is plausible that the chelate cation may be stabilized in nitrobenzene by ion–dipole interaction.

If the results are examined from the point of view of solvent extraction, it can be said that the more the ion pair dissociates in nitrobenzene, the better it is extracted. If this principle holds for this system, it is natural that $Fe(phen)_3X_2$ can distribute into nitrobenzene better than $Fe(bip)_3X_2$ for a given counter anion[7]. Of course, it is the difference between the solubilities of these salts in water and nitrobenzene that determines the distribution ratio. But the extent of association in organic phase is expected to play an important role in the extraction behavior of the ion pair in question, because it is one of the factors that determine the solubility of electrolytes. In Fig. 3, the log *D*, *D* being the distribution ratio which was determined by the procedure described previously[7], is plotted against log K_{A1} in nitrobenzene. There seems to be an approximately linear relationship between log *D* and log K_{A1}.

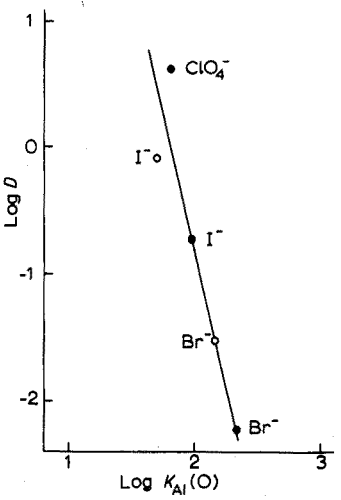

Fig. 3. Relationship between association constants in nitrobenzene and distribution ratios. (●) $[Fe(phen)_3]X_2$; (○) $[Fe(bip)_3]X_2$.

Now the extraction equilibria are expressed as follows,

$$M^{2+}_{(w)} + X^-_{(w)} \overset{K_{A1(w)}}{\rightleftharpoons} MX^+_{(w)}$$

$$MX^+_{(w)} + X^-_{(w)} \overset{K_{A2(w)}}{\rightleftharpoons} MX_{2(w)}$$

$$MX_{2(w)} \overset{K_D}{\rightleftharpoons} MX_{2(o)}$$

$$M^{2+}_{(o)} + X^-_{(o)} \overset{K_{A1(o)}}{\rightleftharpoons} MX^+_{(o)}$$

$$MX^+_{(o)} + X^-_{(o)} \overset{K_{A2(o)}}{\rightleftharpoons} MX_{2(o)}$$

where subscripts (w) and (o) refer to aqueous and organic phases, respectively. Then the distribution ratio of the chelate cation is expressed as,

$$D = K_{ex} \frac{[X^-]^2_{(w)}\{1 + K_{A1(o)}[X^-]_{(o)} f_{(o)} + K_{A1(o)}K_{A2(o)}[X^-]^2_{(o)} f^3_{(o)}\} f^3_{(w)}}{[X^-]^2_{(o)}\{1 + K_{A1(w)}[X^-]_{(w)} f_{(w)} + K_{A1(w)}K_{A2(w)}[X^-]^2_{(w)} f^3_{(w)}\} f^3_{(o)}} \tag{1}$$

where the extraction constant $K_{ex} = K_D K_{A1(w)} K_{A2(w)} / K_{A1(o)} K_{A2(o)}$, and [ ] refers to concentration; f represents the activity coefficient in the respective phase. In practice, $K_{A2(w)}$ and $K_{A2(o)}$ are zero, which was assumed in the treatment of experimental data and was justified. Then eqn. (1) is reduced to eqn. (2):

$$D = K_{ex} \frac{[X^-]^2_{(w)}\{1 + [X^-]_{(o)} K_{A1(o)} f_{(o)}\} f^3_{(w)}}{[X^-]^2_{(o)}\{1 + [X^-]_{(w)} K_{A1(w)} f_{(w)}\} f^3_{(o)}} = \frac{[M^{2+}]_{(o)} + [MX^+]_{(o)}}{[M^{2+}]_{(w)} + [MX^+]_{(w)}} \tag{2}$$

If the ion pair in question is completely dissociated in both aqueous and nitrobenzene phases, K_{ex} is given by a simple equation:

$$K_{ex} = \frac{[M^{2+}]_{(o)}[X^-]^2_{(o)} f^3_{(o)}}{[M^{2+}]_{(w)}[X^-]^2_{(w)} f^3_{(w)}} \tag{3}$$

Equation (3) holds for $Fe(phen)_3(ClO_4)_2$ and is approximately valid for $Fe(phen)_3I_2$ and $Fe(bip)_3(ClO_4)_2$, because the concentration of X^- in both phases was kept low (about less than 10^{-4} mole l^{-1}) in the solvent extraction experiments, so that $[X^-]_{(o)}K_{A1(o)}$ and $[X^-]_{(w)}K_{A1(w)}$ are negligible compared to unity in eqn. (2). The validity of eqn. (3) for $Fe(phen)_3I_2$ was confirmed by the study of salt effect on this system[25]. On the other hand, in the cases of chloride salts, $[X^-]_{(w)}K_{A1(w)}$ is negligible but $[X^-]_{(o)}K_{A1(o)}$ cannot be neglected in eqn. (2). Then the extraction constant in these cases is represented as

$$K_{ex} = \frac{\{[M^{2+}]_{(o)} + [MX^+]_{(o)}\}[X^-]^2_{(o)}f^3_{(o)}}{[M^{2+}]_{(w)}[X^-]^2_{(w)}\{1+[X^-]_{(o)}K_{A1(o)}f_{(o)}\}f^3_{(w)}} \quad (4)$$

Equation (4) is a general expression of the extraction constant for this system.

Though it was made clear that the more the ion pair dissociates in organic phase, the better it distributes, and therefore $Fe(phen)_3X_2$ is more extracted than $Fe(bip)_3X_2$ for a given anion, the water molecules transferred in the extraction experiments are expected to have an interesting influence on the distribution ratio of the ion pair, and also on the association constant in organic phase. It is impossible to take this influence into account in eqn. (4), and no simple explanation can be afforded for the role of water at present time. However, the water molecules attached to the ion pair in nitrobenzene are predicted to promote the dissociation according to the electrostatic theory.

SUMMARY

Conductances of tris(1,10-phenanthroline)iron(II) and tris(2,2'-bipyridine)-iron(II) chlorides, bromides, iodides, and perchlorates were measured in water and nitrobenzene at 25°. Experimental data were analyzed by the Fuoss–Edelson and Jenkins–Monk methods for 2:1 electrolytes. The derived parameters indicated that all the salts studied are almost completely dissociated in water, whereas in nitrobenzene, the association constants were of the order expected from electrostatic theory, which was in turn the order of distribution ratios of the ion pairs between water-and nitrobenzene. An approximately linear relationship was obtained between $\log K_{A1(o)}$ and $\log D$. It is suggested that for the analysis of the mechanism of the extraction of these ion pairs into nitrobenzene, ionic association in the nitrobenzene phase must be considered an important factor.

RÉSUMÉ

Les conductances des chlorures, bromures, iodures et perchlorates de tris-(1,10-phénanthroline)fer(II) et tris(2,2'-bipyridine)fer(II) ont été mesurées dans l'eau et dans le nitrobenzène à 25°. Les paramètres dérivés indiquent que tous les sels étudiés sont presque totalement dissociés dans l'eau; tandis que dans le nitrobenzène, les constantes d'association sont de l'ordre prévu par la théorie électrostatique. Il correspond à l'ordre des rapports de distribution des paires ioniques, entre l'eau et le nitrobenzène. Pour l'analyse du mécanisme d'extraction de ces paires ioniques dans le nitrobenzène, l'association ionique dans la phase nitrobenzène doit être considérée comme un facteur important.

ZUSAMMENFASSUNG

Die Leitfähigkeiten der Chloride, Bromide, Jodide und Perchlorate von Tris(1,10-phenanthrolin)eisen(II) und Tris(2,2′-bipyridin)eisen(II) wurden in Wasser und Nitrobenzol bei 25° gemessen. Die experimentellen Werte wurden nach den Methoden von Fuoss–Edelson und Jenkins–Monk für 2:1-Elektrolyte analysiert. Die abgeleiteten Parameter zeigten, dass alle untersuchten Salze nahezu vollständig in Wasser dissoziiert sind. Die Assoziationskonstanten in Nitrobenzol waren in der aufgrund der elektrostatischen Theorie erwarteten Reihenfolge, welche mit der Reihenfolge der Verteilungsverhältnisse der Ionenpaare zwischen Wasser und Nitrobenzol in Beziehung stand. Eine nahezu lineare Beziehung wurde zwischen $\log K_{A1(o)}$ und $\log D$ erhalten. Es wird angenommen, dass für die Analyse des Mechanismus der Extraktion dieser Ionenpaare mit Nitrobenzol Ionenassoziation in der Nitrobenzolphase als ein wichtiger Faktor angesehen werden muss.

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EXTRACTION AND GAS CHROMATOGRAPHIC DETERMINATION OF METHYL-, ETHYL-, AND METHOXYETHYLMERCURY(II) HALIDES*

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The last two decades have seen an increased appreciation of the toxicological significance of mercury pollution of the environment. An important consideration in the transformation of mercury is the biological methylation of mercury, as demonstrated by Jernelöv[1] with aquaria sludge and by Wood[2] in natural systems. Organo-mercury compounds are many times more toxic than inorganic mercury. More importantly, these compounds exist within the human food chain. The organomercurial species in the environment are more abundant in biological material than in air or natural water. Extraction methods are necessary to remove an organomercurial from undesirable matrices and to concentrate it within detectable limits. Therefore, it was decided to study the extraction, and subsequent gas chromatographic determination, of some typical organo-mercury(II) halides.

An early extraction procedure, developed by Gage[3], involved the preparation of a 5% homogenate of the biological material, addition of concentrated hydrochloric acid, and extraction with benzene. His recoveries ranged from 85 to 95%. To avoid or minimize interference from thiols and other sulfur compounds within the tissue homogenate, Westöö[4] added mercury(II) ions which bound the thiol groups and freed the organo-mercurials.

EXPERIMENTAL

Preparation of organo-^{203}Hg halides

Methyl-, ethyl-, and methoxyethylmercury(II) chlorides (Alfa Inorganics, Beverly, Mass.) were tagged according to the reaction[5]:

$$RHgR + {}^*HgCl_2 \rightarrow 2R{}^*HgCl \qquad (1)$$

Mercury II chloride (New England Nuclear Corporation, Boston, Mass.) and high-purity dimethylmercury and diethylmercury (Alfa Inorganics) were used. Reaction (1) yielded a white crystalline solid from hot ethanol and dilute hydrochloric acid. Crystallization occurred after chilling in an ice bath. The crystals were recrystallized three times from boiling ethanol. Melting points of the tagged compounds were found to be:

* Research sponsored by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.

	Found	*Literature*[6]
CH_3HgCl	170°	170°
C_2H_5HgCl	192.5/193	193°

The bromide and iodide compounds were obtained by shaking the organic benzene solution of RHgCl with a ten-fold amount of the respective aqueous halide solution.

*Inoculation of samples with CH_3*HgCl*

Two soil samples were selected to represent the most diversified types found in aquatic systems. One was a typical sand sediment which had been screened and washed of all organic debris; this will be called the inorganic sediment. The second type, an organic sediment, was taken downstream from a sewage plant. Both sediments were dried and inoculated with CH_3*HgCl by covering the sediment with 0.1 *M* hydrochloric acid solutions saturated with the tagged material. The uptake of the tagged material reached equilibrium within 48 h.

Rye seeds were inoculated similarly. Seeds coated commercially with fungicides manufactured from organo-mercurials are one of the primary sources for wildlife ingestion.

The fish samples, obtained from the Ecological Sciences Division, Oak Ridge National Laboratory, were *Gambusia*, a small bottom dweller, approximately 3 cm in length. The fishes were introduced into an aquarium which contained dissolved CH_3^*HgOH. In a 48-h period the fish concentrated the activity from the water by a factor of *ca.* 5000[7]. Fish samples tagged in this way represent the sample tissue as it actually occurs in the aquatic environment. Thus, through natural body processes, the tagged compound was introduced and allowed to bind within the fish tissue. Whole fish tissue samples were taken and homogenized in a ground-glass tissue homogenizer.

Extraction of organo-mercury(II) halides

Extraction studies were conducted in 1-dram, screw-cap vials. The aqueous and organic phases were each 1 ml in volume. Equilibration was complete within a 2-min shaking period. A 500-μl aliquot of each phase was then counted in the γ-scintillation counter.

Rye seeds, humic and inorganic sediments were wet screened (40 mesh) and extracted without further preparation. The extraction of fish tissue was done in 10×75 mm test tubes to avoid sample loss and to facilitate counting. The fish tissue itself was also counted independently from the aqueous and organic extraction phases to ascertain mercury retention by the tissue.

Radioactivity counting

The scintillation counter was a NaI "well-type" counter designed and fabricated at the Oak Ridge National Laboratory. A high-voltage power supply (model 312, Atomic Instrument Co., Cambridge, Mass.) was used and the scintillation detector comprised a RCA 5819 photomultiplier tube with preamplifier model 810A (Atomic Instrument Co.). The absorber was a $\frac{3}{16}$-in aluminum cylinder machined to accommodate 10×75 mm test tubes.

Gas–liquid chromatography

A Varian Aerograph Model 1520B gas chromatograph equipped with an electron capture (tritium foil) detector was used. The column was glass, 8 ft × 0.25 in, packed with 5% HI-EFF-8BP (cyclohexane-dimethanol succinate) adsorbed on 70–80 mesh Anakrom ABS, and operated isothermally at 200°. Nitrogen was the carrier gas; its flow rate was 56 cm^3 min^{-1}. Non-silanized supports exhibited severe tailing. A volume of between 1 and 8 μl of the benzene solvent-extraction phase was injected via a Teflon-tipped liquid syringe. Glass columns with on-column injection technique minimized the decomposition of the organo-mercury(II) halides[8].

Calibration curves of peak height *versus* concentration were plotted on a calculator-plotter, fitted by regression. The relative standard deviation (1σ) over the concentration range 7–32 ng of the respective compound varied from 8.2% at the lowest concentration to 1.1% at the highest concentration of sample injected.

RESULTS AND DISCUSSION

Benzene was chosen for the organic phase in all the work because of its superior extraction efficiency. The following distribution ratios of ethylmercury(II) chloride between various organic solvents and 0.1 *M* hydrochloric acid were obtained: benzene, 36.8; toluene, 27.4; xylene, 22.6; diethylbenzene, 15.5, and cyclohexane, 1.2. Non-halogenated solvents must be employed for the organic phase in an extraction step when an electron capture detector is to be used in the subsequent gas chromatographic step.

The partition coefficients, K_d, for methyl- and ethylmercury(II) chlorides were 11 and 36; thus, extraction would be 91 and 97% complete in one equilibration, respectively. Improved extractability could be achieved by using either the bromide or iodide analogs; K_d values of 44 and 165 were obtained for methyl- and ethylmercury(II) bromide, respectively, and values of 183 and 256 for the corresponding iodides. The bromide and iodide complexes were extracted with a minimum recovery of 97%. Figure 1 (log *D vs.* log Cl, log *D vs.* log Br, log *D vs.* log I) illustrates the decrease in *D* with increasing amounts of halide.

Excellent separations were obtained on the gas chromatographic column, as shown in Fig. 2. Sharp, quantitative peaks were produced, with a return to the baseline between each peak. The elution order was benzene, methylmercury(II) chloride, ethylmercury(II) chloride, and methoxyethylmercury(II) chloride. Relative retention times of the bromide and iodide compounds were identical with those of the chloride compounds; however, increased detector sensitivity paralleled the increase in atomic weight of the halide.

Evidence exists to support the formation of anionic complexes of the general type $RHgCl_n^{1-n}$ ($n=2,3$)[8–10]. If true, an extraction from aqueous system would be adversely affected by high halide concentration. Thus it was deemed necessary to study the distribution ratio as a function of the several variables: hydrogen ion concentration, halide ion concentration, and total RHgX concentration.

The distribution ratio *D* remained constant for hydrogen ion concentrations 10^{-5} *M* and larger (up to 10 *M*). Likewise, *D* remained essentially constant over the interval from 10^{-5} to 10^{-3} *M* RHgCl ($R=CH_3$ and C_2H_5). However, at halide concentrations greater than about 1 *M*, the distribution ratio decreased

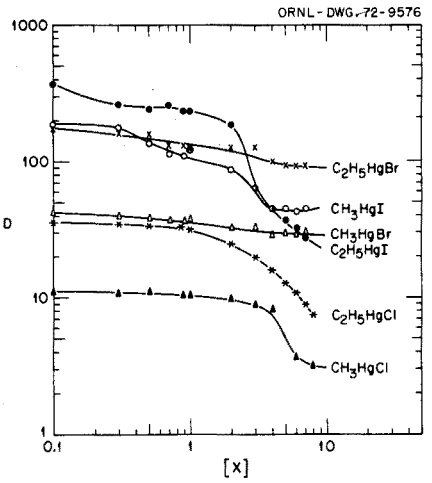

Fig. 1. Log D *vs.* log X curves (where X = Cl, Br, I) for CH_3HgX and C_2H_5HgX.

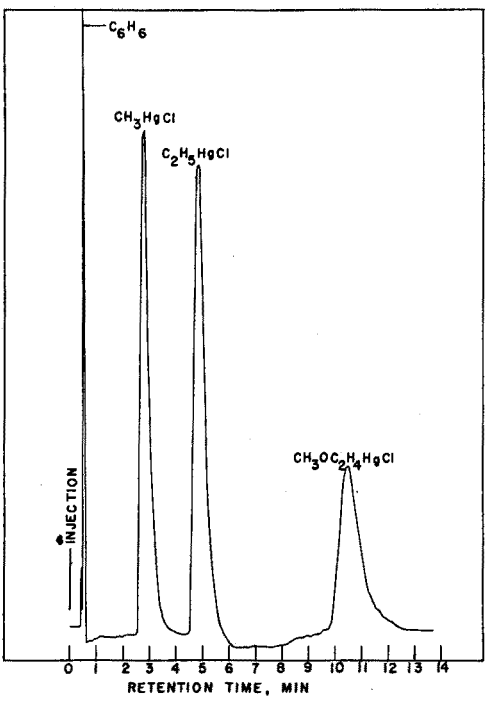

Fig. 2. Chromatogram of CH_3HgCl, C_2H_5HgCl and $CH_3OC_2H_4HgCl$ with an electron capture detector. Conditions: 8 ft × 0.25 in glass column; 5% HI-EFF-8BP on Anakrom ABS; 56 cm^3 N_2 min^{-1}; column temperature, 200° isothermal; detector temperature, 225°; injection block, 200°; recorder, 0.33 in min^{-1}.

significantly in the chloride and iodide systems. For the chloride system, D is given by

$$D = \frac{K_d}{1+K_1[Cl^-]+K_2[Cl^-]^2} \tag{2}$$

where K_d is the partition coefficient and K_1, K_2 are the overall formation constants for $RHgCl_2^-$ and $RHgCl_3^{2-}$, respectively, and the brackets indicate molar concentrations. Upon inverting and rearranging,

$$\frac{K_d - D}{D[Cl^-]} = K_1 + K_2[Cl^-] \tag{3}$$

Plotting the left-hand term of eqn. (3) *versus* the chloride concentration gives a straight line of slope K_2 and intercept K_1 (Fig. 3). For ethylmercury(II) chloride, $K_1 = 0.140$ and $K_2 = 0.0435$. Thus, evidence from this investigation supports the formation of anionic complexes at increased ligand concentrations, albeit quite weak complexes. Only rough estimates were obtained from methylmercury(II) iodide ($K_1 = 0.18$ and $K_2 = 0.16$) and for ethylmercury(II) iodide ($K_1 = 0.6$ and $K_2 = 0.1$).

At high pH values competition by the hydroxide ion opposes the formation of the halide complex. This is illustrated in Table 1, which lists the decrease in

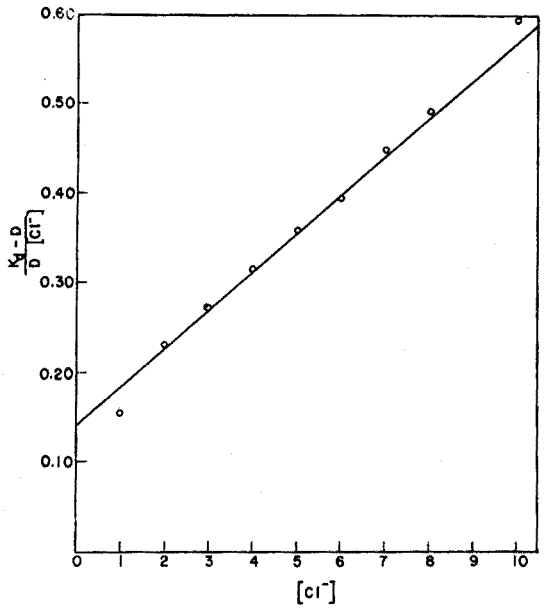

Fig. 3. Plot of $(K_d - D)/D[Cl^-]$ *vs.* $[Cl^-]$ for C_2H_5HgCl.

TABLE I

DISTRIBUTION RATIOS OF C_2H_5HgCl AT DIFFERENT pH VALUES

pH	*D*	*pH*	*D*
7.01	19.0	11.97	0.02
7.95	11.2	13.0	0.01
8.70	7.7	13.0[a]	0.07
10.07	2.8	13.0[b]	3.8
11.14	0.94	13.0[c]	243

[a] Solution contained 1 *M* NaCl.
[b] Solution contained 1 *M* NaBr.
[c] Solution contained 1 *M* NaI.

D with increasing pH for ethylmercury(II) chloride. At high pH values, it is necessary to increase the halide concentration to obtain a favorable distribution ratio for the organomercurials. Conversely, since the hydroxide complex is quite soluble in water, it provides a simple way for back-extraction and concentration of the organo-mercury halides.

The extraction of organo-mercury(II) iodides from biological samples was not as efficient as from aqueous samples. Best recoveries were obtained from a 24-h leach in a 1.0 *M* sodium iodide solution. Quantitative extraction of the organo-mercury(II) iodide required a multiple extraction from the biological matrix and even then complete recoveries were not obtained. However, two extractions brought the recovery within the reproducibility of the subsequent gas chromatographic determinative step. A single 24-h leach of 1-g samples gave the following *D* values:

12.0 ± 0.2 for inorganic sediment; 7.7 ± 0.5 for organic sediment; 5.6 ± 0.8 for fish tissue; and 9.0 ± 0.5 for rye seeds.

SUMMARY

The separation, identification, and determination of methyl-, ethyl-, and methoxyethylmercury(II) halides in biological materials were studied. The procedure developed involved a 24-h leach with 1 *M* sodium iodide, equilibration of the aqueous phase for 2 min with an equal volume of benzene, and then injection of an aliquot of the benzene phase onto a gas chromatographic column consisting of 5% cyclohexanedimethanol succinate held on Anakrom ABS. Excellent baseline separation of the chromatographic peaks was obtained. The extraction steps were monitored with RHgX compounds tagged with ^{203}Hg. Partition coefficients are reported for methyl- and ethylmercury(II) chlorides, bromides, and iodides; several overall formation constants of the anionic complexes $RHgCl_n^{1-n}$ ($n=2, 3$) were determined. Results are reported for the recovery of methyl- and ethylmercury(II) halides from inoculated rye seed, humic and inorganic sediment, and fish grown in an aquarium.

RÉSUMÉ

Une étude est effectuée sur la séparation, l'identification et le dosage des halogénures de méthyl-, éthyl- et méthoxyéthylmercure(II) dans des substances biologiques. On procède par chromatographie gazeuse et extraction. Diverse résultats sont reportés.

ZUSAMMENFASSUNG

Die Trennung, Identifizierung und Bestimmung von Methyl-, Äthyl- und Methoxyäthylquecksilber(II)-halogeniden in biologischen Stoffen wurden untersucht. Bei dem entwickelten Verfahren wird die Probe 24 h lang mit 1 *M* Natriumjodid ausgelaugt, die wässrige Lösung 2 min lang mit dem gleichen Volumen Benzol geschüttelt und ein aliquoter Anteil der Benzolphase in eine gaschromatographische Säule eingespritzt, die mit Anakrom ABS mit 5% Cyclohexandimethanolsuccinat gefüllt ist. Es wurde eine ausgezeichnete Basislinientrennung der chromatographischen Peaks erhalten. Die Extraktionsschritte wurden mit RHgX-Verbindungen kontrolliert, die mit ^{203}Hg markiert waren. Die Verteilungskoeffizienten für Methyl- und Äthylquecksilber(II)-chlorid, -bromid und -jodid werden angegeben; verschiedene Gesamtbildungskonstanten der anionischen Komplexe $RHgCl_n^{1-1}$ ($n=2, 3$) wurden bestimmt. Es werden die Ergebnisse vorlegt, die bei der Bestimmung von Methyl- und Äthylquecksilber(II)-halogeniden in gespritzter Roggensaat, Humus- und anorganischem Sediment und in einem Aquarium gezogenem Fisch erhalten worden sind.

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IDENTIFICATION AND SEMIQUANTITATIVE ESTIMATION OF PHOSPHATE, SILICATE, ARSENATE AND GERMANATE BY THE RING-OVEN METHOD

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In a previous communication[1], ring-oven procedures were described for the detection of phosphate and silicate separately and in presence of each other, based on the well-known molybdate–benzidine test[2] and the use of suitable masking agents. This work has now been extended to cover the detection and semiquantitative estimation of other ions which form heteropoly acids.

The earlier ring-oven procedure allowed the detection of phosphate[1,3] and silicate[1]. This procedure, which it will be shown can be extended for germanate and arsenate, involves the formation of a ring containing any of the four anions in question, by washing the test solution from the centre of the filter paper disc with 0.01 *M* nitric acid; the heteropoly acid is then formed by spraying with ammonium molybdate–nitric acid reagent. Finally benzidine–acetate reagent is applied and the redox reaction yielding molybdenum blue and benzidine blue proceeds after immersion of the paper in saturated borax solution.

The detection of each of the four anions in presence of the other three anions required the use of masking agents, so that molybdenum complexes of various stabilities were formed. For example, a molybdate solution containing 10% sodium citrate allowed detection of phosphate in presence of arsenate, silicate and germanate. Although a similar solution containing only 5% sodium citrate allowed detection of arsenate in presence of silicate and germanate, phosphate interfered owing to preferential complexation. Tartrate had no advantage over citrate in its masking action. Oxalic acid, however, prevented the formation of the polymolybdate from phosphate, silicate and germanate, while phenylfluorone[4] proved highly selective for germanate, allowing its detection in the presence of phosphate, arsenate, and silicate. The use of these masking agents allowed the simultaneous detection of the three anions in presence of each other in mixtures containing either phosphate or arsenate in addition to germanate and silicate.

A semiquantitative estimation of phosphate, germanate, silicate and arsenate was readily developed. However, whereas the ring colours from phosphate were stable enough for measurement, those from the other three anions faded rapidly. This necessitated a study of the various factors affecting the colour stability; of these, exposure to atmosphere proved to be most serious. When the coloured rings developed were coated with pure paraffin wax, other factors such as time, temperature and pH were found to exert only normal controllable effects.

EXPERIMENTAL

Apparatus

Ring oven with accessories. Filter paper discs, 5.5 cm diameter, Whatman No. 1, 41, 42 or Schleicher and Schüll 589[2] white ribbon. pH meter Radelkis Type OP 201/1.

Reagents

Except where otherwise stated, all reagents were of analytical grade.

Ammonium molybdate–nitric acid solution. Dissolve 10 g of ammonium molybdate in 80 ml of cold water. Pour the solution into 20 ml of nitric acid (d = 1.42).

Benzidine acetate solution. Dissolve 50 mg of benzidine in 100 ml of 10% (v/v) acetic acid. The solution is stable in a brown bottle for about one week.

Ammonium molybdate–sodium citrate solution (10% and 5%). Dissolve sodium citrate (10 g-or 5 g) in 100 ml of the above ammonium molybdate–nitric acid solution.

Ammonium molybdate–oxalic acid solution. Dissolve 1 g of oxalic acid in 100 ml of the above ammonium molybdate–nitric acid solution.

Phenylfluorone solution. Dissolve 50 mg of phenylfluorone in 100 ml of 3 *M* hydrochloric acid solution.

Standard solutions. Standard phosphate or arsenate solution (10 mg P_2O_5 or As_2O_5 per ml) were prepared from disodium hydrogen orthophosphate dodecahydrate or disodium hydrogen arsenate. Standard silicate solution (10 mg SiO_2 per ml) was prepared by fusion of 1.003 g of silica (99.7% pure) with 2.5 g of anhydrous sodium carbonate and 3 g of potassium carbonate; after cooling the melt was dissolved in water and diluted to 100 ml. Standard germanate solution (10 mg GeO_2 per ml) was prepared similarly after fusion of 1 g of germanium dioxide with 3.0 g of sodium hydroxide. Solutions containing the required microgram amounts of these anions were prepared by suitable dilution of the stock solutions.

Qualitative identification

General procedure. Apply 5–10 μl of the sample solution (phosphate, germanate, arsenate or silicate) to the centre of a filter paper disc with a capillary pipette. Place the filter on the ring oven and wash to the ring zone with 20 μl of 0.1 *M* nitric acid solution applied in 5-μl portions. Spray the ring with ammonium molybdate–nitric acid solution, and feed about 25 μl of benzidine solution to the centre of the filter by slow suction from a 0.1-ml Ostwald–Folin pipette, without flooding. Immerse the filter paper in a saturated solution of borax. A bright blue-coloured ring is developed. Limits of identification: 0.01 μg P_2O_5, 0.05 μg As_2O_5, 0.02 μg SiO_2, or 0.02 μg GeO_2.

Procedure for phosphate in presence of arsenate, silicate and germanate. Apply and wash out the sample as described above. Spray the ring with ammonium molybdate–sodium citrate solution (10%), and complete as described above. Limit of identification: 0.05 μg P_2O_5 in presence of 100 μg of SiO_2, GeO_2 or As_2O_5 or a total amount of 100 μg of the three anions.

Procedure for silicate in presence of arsenate, phosphate and germanate.

Proceed as above but spray the ring with ammonium molybdate–oxalic acid solution. Limit of identification: 0.08 μg SiO_2 in presence of a total amount of 50 μg of P_2O_5, GeO_2 and As_2O_5 or 50 μg of an individual anion.

Procedure for germanate in presence of arsenate, silicate and phosphate. Apply the sample and wash to the ring zone as above. Spray the paper with the phenylfluorone solution. A pink coloured ring is obtained. Limit of identification: 0.01 μg GeO_2 in presence of a total of 200 μg of SiO_2, P_2O_5 and As_2O_5, or 200 μg of one anion.

Procedure for arsenate in presence of silicate and germanate. Proceed as for phosphate but spray with ammonium molybdate–sodium citrate solution (5%). Limit of identification: 0.1 μg As_2O_5 in presence of a total of 50 μg of SiO_2 and GeO_2 or 50 μg of only one anion.

Simultaneous detection of phosphate, germanate and silicate. Apply the sample and wash to the ring zone as described above. Cut the ring into three sectors. Spray the first sector with ammonium molybdate–sodium citrate solution (10%) and benzidine solution and immerse in the borax solution; a bright blue colour indicates the presence of phosphate. Spray the second sector with ammonium molybdate–oxalic acid solution and benzidine solution and dip in the borax solution; a bright blue colour indicates the presence of silicate. Spray the third sector with phenylfluorone solution; a pink colour indicates the presence of germanate.

Simultaneous detection of germanate, silicate and arsenate. Proceed as described above. Test the first sector for silicate as above. Test the second sector for germanate as above. Spray the third sector with ammonium molybdate–sodium citrate solution (5%) and benzidine solution and then immerse in the borax solution; a blue colour indicates the presence of arsenate.

Semi-quantitative estimation of phosphate, germanate, silicate and arsenate

General procedure. Apply 1 μl of phosphate solution (1 μg P_2O_5) to the centre of a filter paper disc by means of a calibrated capillary pipette. Place the filter on the ring oven and wash to the ring zone with 20 μl of 0.1 *M* nitric acid solution applied in about 5-μl portions. Spray the ring with ammonium molybdate–nitric acid solution and feed about 25 μl of the benzidine acetate solution to the centre of the filter, by slow suction from a 0.1-ml Ostwald–Folin pipette, avoiding any flooding. Dip in an aqueous saturated solution of borax and leave until a bright blue-coloured ring is fully developed (*ca.* 2 min). Repeat the same procedure but applying 2, 4, 6, 8 and 10 μl of the phosphate solution (containing 2, 4, 6, 8 and 10 μg P_2O_5).

Dry the papers in the drying oven and coat with pure melted paraffin wax (B.D.H.) as soon as possible. There is a remarkable difference in the colour intensity of the 6 phosphate rings containing 1–10 μg of P_2O_5.

Procedure for silicate, germanate or arsenate. Proceed as described for phosphate. However, with these three anions the colour fades after a few hours despite the paraffin wax cover. Owing to this colour instability, a scheme of phosphate standards was used. Appropriate microgram amounts of the three anions were measured against phosphate standards to provide a more permanent calibration. The colour equivalence between different amounts of the four anions is shown in Table I.

TABLE I

STANDARD SCHEME FOR PHOSPHATE AND CALIBRATION SCHEME FOR ARSENATE, SILICATE AND GERMANATE

Ring I Ring VI (μg)	*Ring II* (μg)	*Ring III* (μg)	*Ring IV* (μg)	*Ring V* (μg)	(μg)
1 P_2O_5	2 P_2O_5	4 P_2O_5	6 P_2O_5	8 P_2O_5	10 P_2O_5
4 As_2O_5	6 As_2O_5	12 As_2O_5	16 As_2O_5	20 As_2O_5	24 As_2O_5
2 SiO_2	4 SiO_2	6 SiO_2	9 SiO_2	12 SiO_2	15 SiO_2
2 GeO_2	4 GeO_2	6 GeO_2	9 GeO_2	12 GeO_2	15 GeO_2

RESULTS AND DISCUSSION

Stability of colour

Except where otherwise stated all experiments were performed on rings coated with paraffin wax. The effect of atmospheric exposure was tested for a series of uncoated ring colours. Although the colours faded only gradually, after 2 h all the ring colours were faint compared with the originals. On coating the phosphate rings with paraffin wax, the colours remained stable for at least 10 days, after which only a slight fading was observed.

The effect of pH was tested by applying solutions containing 2, 4 and 6 μg of P_2O_5 on the centre of filter paper discs following the usual procedure, the final immersion being done in buffer solutions of pH values ranging between 2 and 9. Below pH 7, very faint colours were obtained; above pH 7, the colours were bright and more stable. A saturated borax solution was simple and also gave the best results.

The effect of temperature was tested on rings containing 2, 4 and 6 μg P_2O_5 and the colour stability was checked after heating at temperatures ranging between 20 and 60° in a drying oven for 2 h. This showed that the optimal temperature for colour stability on wax-coated rings was room temperature, *i.e. ca.* 25°.

Application of the standard scheme for the estimation of phosphate, arsenate, silicate or germanate

Aliquots (1, 2 and 3 μl) of the test solution of phosphate were applied to the centre of three different paper discs and the above procedure was used. These rings were compared visually with those of the standard scheme. The concentration of the unknown test solution was estimated as outlined by Weisz[3].

For example, it was found that the colour developed for 1 μl of phosphate solution had the same intensity as ring II (2.0 μg P_2O_5), the ring from 2 μl of solution gave rise to a colour lying between those of rings III and IV (4 and 6 μg P_2O_5), and the colour of the ring from 3 μl of solution coincided with that of ring V (8 μg P_2O_5) (*cf.* Table I). Thus the unknown solution was calculated to contain 2.5 μg P_2O_5 (2.5 g P_2O_5 l^{-1})r. The test solution actually contained 2.4 g P_2O_5 l^{-1}. Therefore, the absolute error of the analysis was 4%.

In a similar way, the standard scheme shown in Table I was checked for arsenate, silicate and germanate. Table II shows the results obtained. When the

standard scheme was applied in a similar way for triplicate analyses of solutions containing 2–4 μg P_2O_5, the average values found agreed reasonably with the expected amounts. The individual error did not exceed 6.7%, and the average error was ±5.0%. Similarly, the calibration scheme proved efficient for the estimation of arsenate, silicate and germanate; the average errors obtained were ±3.0, ±6.3 and ±2.7%, respectively.

TABLE II

APPLICATION OF THE STANDARD AND CALIBRATED SCHEMES FOR THE SEMI-QUANTITATIVE ESTIMATION OF PHOSPHATE, ARSENATE, SILICATE AND GERMANATE

Anion (as oxide)	*Expected (μg)*	*Found*[a] *(μg)*	*% Error*	*% Average error*[b]
P_2O_5	2.00	2.11	+5.5	±5.0
	2.50	2.40	−4.0	
	3.00	3.20	+6.7	
	3.50	3.33	−4.8	
	4.00	4.17	+4.2	
As_2O_5	3.00	3.00	0.0	±3.0
	4.00	4.15	+3.7	
	5.00	5.25	+5.0	
	6.00	5.83	−2.8	
	7.00	6.75	−3.6	
SiO_2	3.00	3.17	+5.7	±6.3
	4.00	3.83	−4.2	
	5.00	4.50	−10.0	
	6.00	5.67	−5.5	
GeO_2	3.00	3.16	+5.3	±2.7
	4.00	4.02	+0.5	
	5.00	5.25	+5.0	
	6.00	6.00	0.0	

[a] The values recorded are the average of triplicate analyses.
[b] The total average error is ±4.38%.

The individual error as well as the total average error (±4.3%) fall within the acceptable error limits for semiquantitative analysis. Both the qualitative and semiquantitative procedures, whether individual or simultaneous, developed in the present work, offer in general a rapid means for the detection and approximate estimation of any of the four anions considered.

SUMMARY

A general ring-oven procedure was developed for the detection of phosphate, silicate, arsenate or germanate based on the molybdate–benzidine test. Procedures are also described for the simultaneous detection of these anions in mixtures, by means of organic masking agents. A semi-quantitative method is described for the estimation of the four anions; the ring colours of sample solutions are compared with those of standard solutions. Colours obtained from phosphate are stable if the

rings are covered with a film of paraffin wax. A preliminary calibration scheme against phosphate is necessary for the other anions because of colour instability. The maximum error found was $\pm 6.35\%$ while the total average error was $\pm 4.3\%$.

RÉSUMÉ

Une méthode générale, au four à anneau, est mise au point pour l'identification des phosphates, silicates, arsénates ou germanates, basée sur la réaction molybdate–benzidine. On décrit également un procédé pour l'analyse de mélanges de ces quatre anions, à l'aide d'agents de masquage, ainsi qu'une méthode semi-quantitative de dosage.

ZUSAMMENFASSUNG

Für den Nachweis von Phosphat, Silicat, Arsenat oder Germanat wurde ein allgemeines Ringofen-Verfahren entwickelt, das auf dem Molybdat–Benzidin-Test beruht. Es werden auch Verfahren für den Simultannachweis dieser Anionen in Gemischen unter Verwendung organischer Maskierungsmittel beschrieben. Eine halbquantitative Methode zur überschlägigen Abschätzung der vier Anionen wird angegeben; die Ringfärbungen von Probelösungen werden mit jenen von Standardlösungen verglichen. Die mit Phosphat erhaltenen Färbungen sind beständig, wenn die Ringe mit einem Film von Paraffin bedeckt werden. Eine Eichung in bezug auf Phosphat muss für die anderen Anionen wegen der Unbeständigkeit der Färbung vorausgehen. Der maximale Fehler betrug $\pm 6.35\%$, während der mittlere Fehler $\pm 4.3\%$ war.

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THE GRAVIMETRIC DETERMINATION OF URANIUM IN URANYL NITRATE*

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Gravimetric techniques have played a prominent role in the analytical chemistry of uranium, and today they are used extensively throughout the nuclear industry[1]. In the gravimetry of uranium, compounds such as uranyl nitrate are rapidly converted to U_3O_8 (the weighing form) by pyrolysis. Gravimetric data rival that obtained by accurate titrimetric methods when the uranium compounds are extremely pure (>99.9%).

In the gravimetric determination of uranium, the U_3O_8 has usually been assumed not to deviate from stoichiometric composition by more than 0.02%. A second assumption has been that nonvolatile elements which are often present in uranium form predictable oxides within the U_3O_8 matrix, so that the gravimetric results can be corrected for these elements. However, with increasing concentrations of the nonvolatile elements, the accuracy of these corrections becomes very significant. Differences between the gravimetric and titrimetric determinations of uranium in uranyl nitrate have often been observed, these differences being greater when the concentration of nonvolatile elements is high (1000–5000 p.p.m. on a uranium basis). In an attempt to explain these differences, the factors which affect the gravimetric determination of uranium—the effects of ignition conditions and nonvolatile elements in the U_3O_8 composition—were studied.

Investigations reported here show that both the ignition temperature and time affect the stoichiometry of U_3O_8. Brief ignitions of 1–3 h at 850° yield uranium oxides that deviate as much as 0.15% from theoretical U_3O_8. The degree of variance in U_3O_8 stoichiometry is dependent primarily on pyrolysis temperature and time. Brouns and Mills[2] and Petit and Kienberger[3] have reported on the conditions for the preparation of stoichiometric U_3O_8. Their findings and the present data show that a short ignition of uranyl nitrate at 1000° produces a near theoretical U_3O_8 (99.98%); also, this ignition temperature was found to be superior to lower temperatures recommended by many laboratories[1,4]. Ignition at 1000° was shown to result in the formation of predictable compounds while at lower temperatures compound formation was variable.

The present study indicates that many nonvolatile elements present as impurities in uranyl nitrate do not form common oxides, but complex compounds such

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as uranates, phosphates, and double oxides. Hoekstra and Siegal[5] and Spitsyn[6] have reported on the formation of these compounds in the uranium oxide system. Much of the reported work on the formation of impurity compounds in pyrolyzed uranyl nitrates was performed with equimolar ratios of the elements to uranium. With element concentrations similar to those encountered in relatively pure uranyl nitrates (>99.0%), formation of these impurity complexes was found to depend on the pyrolysis conditions as well as on the concentration of the elements in the uranyl nitrate. Of the many impurities studied, calcium and phosphorus were found to have the most effect on the stoichiometry of U_3O_8 in the gravimetric analysis of uranyl nitrate for uranium.

EXPERIMENTAL

Apparatus

A Lindberg Hevi-Duty furnace with a temperature controller to maintain temperatures to $\pm 5°$ was used for the pyrolysis of uranyl nitrate crystals.

Procedures

Uranium oxide (U_3O_8) was dissolved in nitric acid and the uranyl nitrate solutions were spiked with the elements of interest. The solutions were stirred constantly while evaporating at 100° until uranyl nitrate crystals formed. The crystals were desiccated for 24 h and then ignited to U_3O_8 at various temperatures. The uranium content was determined by potentiometric titration with potassium dichromate[7]. The titration results were used to calculate oxide stoichiometries. The oxides were also analyzed for elemental impurities by atomic absorption and colorimetric methods.

An accurately prepared uranyl nitrate solution (U content on a weight basis) was also used to study the effect of elements on oxide stoichiometry. Weighed aliquots were spiked with the elements of interest and the solutions were evaporated to dryness. The nitrate residues were ignited at various temperatures for 1–3 h. The resultant oxides were weighed and stoichiometries calculated from the uranium weight data.

The uranium oxides prepared from pyrolysis of the uranyl nitrate crystals were also analyzed for uranium (IV). The oxides were dissolved in sulfuric acid which contained an accurately measured excess of potassium dichromate. The unreacted dichromate was back-titrated with iron(II) solution. The uranium(IV) was determined from the amount of dichromate used in the dissolution of the oxides.

RESULTS AND DISCUSSION

Effect of ignition conditions

In the conversion of uranyl nitrate to uranium oxide (U_3O_8), many conditions affect the U_3O_8 stoichiometry. Among these are ignition temperature, ignition time, layer thickness, cooling rates, and atmospheric pressure. Of these conditions, ignition temperature and time affect the oxide stoichiometry significantly. Pure uranyl nitrate crystals were ignited at various temperatures ranging from 700° to 1040° for 2 h. The stoichiometries of the oxides were determined by the procedures described; results

TABLE I

EFFECT OF IGNITION TEMPERATURE ON SURFACE AREA OF U_3O_8

Ignition temperature (°)	*Surface area ($m^2\ g^{-1}$)*
700	3.10
800	1.80
1000	0.31
1040	0.27

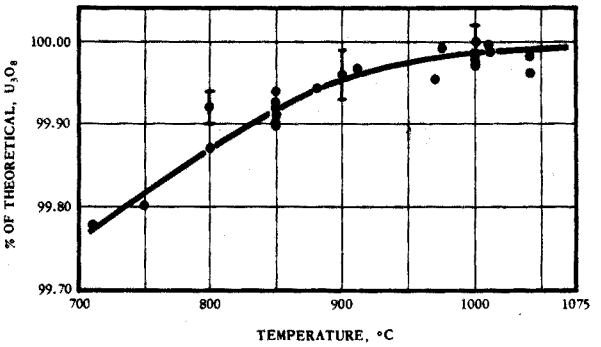

Fig. 1. Effect of ignition temperature on U_3O_8 composition. Starting material, $UO_2(NO_3)_2$; ignition time, 2 h. Brouns and Mills' data are indicated by bars.

are shown in Fig. 1. The pyrolysis of uranyl nitrate at temperatures below 1000° yields a hypostoichiometric U_3O_8. Ignitions at 800° to 900° yield oxides that deviate by 0.05%–0.15% from stoichiometric U_3O_8. This deviation is significant because it affects the accuracy of the uranium analysis. Brouns and Mills[2] data are shown for comparison. Both data sets are in agreement showing that a short ignition of 2 h at 1000° produces a uranium oxide that is nearly stoichiometric.

Surface-area measurements were made on the U_3O_8 generated from uranyl nitrate at the various ignition temperatures; results are shown in Table I. The surface area differences may be used to explain the variations observed in oxide stoichiometry when uranyl nitrate is ignited at different temperatures. The stoichiometry is affected by the back-diffusion of oxygen into the uranium oxide in the cooling cycle. However, ignitions at 1000° or higher produce a sintered crystal which partially prevents the back-diffusion of oxygen.

The behavior of the uranium oxide generated from uranyl nitrate in the ignition cycle was also studied on a thermal balance in the temperature range 25°–1040°. During the heating cycle, the oxide loses weight gradually until 900° when a sharp weight loss occurs. At this temperature the pressure of oxygen in U_3O_8 equals atmospheric pressure[8]. The rate of weight loss decreases considerably above 900° and levels at 1040°. It was found that oxides initially heated at 850° and reheated to 1000° lose weight, but do not return to their starting weight in the cooling cycle. A more stable oxide appears to be formed at 1000° than at 850°. A sintering of the U_3O_8 occurs at the higher temperature; thus, back-diffusion of oxygen is minimized

in the cooling cycle. Consequently, the oxides prepared at 1000° are nearly stoichiometric (99.98% of theoretical U). Thus pyrolysis of the uranyl nitrate at 1000° for 2 h is recommended for accurate uranium determinations.

Effects of impurity elements

The presence of nonvolatile elements affects the accuracy of the gravimetric analysis for uranium because these elements are weighed along with the U_3O_8. Corrections for these impurities are based on element concentration and compound formation within the U_3O_8 matrix. The accuracy of these corrections becomes very significant as the element concentrations increase. Effects of several nonvolatile elements on the stoichiometry of U_3O_8 were evaluated.

Phosphorus, often present in uranyl nitrate, is not volatilized during the ignition. It forms a uranium–phosphorus compound. Uranyl pyrophosphate and uranyl phosphate have been reported to be formed during ignition. To study the effect of phosphorus on U_3O_8 stoichiometry and identify the compounds formed, batches of uranyl nitrate crystals containing varying amounts of phosphorus were ignited. The resultant oxides were analyzed for their total uranium and uranium(IV) contents to determine oxide composition; results are shown in Fig. 2. Calculated stoichiometries based on the formation of uranyl pyrophosphate, uranyl phosphate, and hydrated uranyl phosphate are shown for comparison. These results indicated that a hydrated uranyl phosphate is formed during the conversion of uranyl nitrate to U_3O_8 when phosphorus is present.

Uranium oxides containing phosphorus were observed to gain weight after exposure to air for several hours. This weight gain was attributed to the hygroscopic behavior of uranyl phosphate. To verify this assumption, oxides containing phosphorus were heated in a stream of dry air first at 850° and then at 1050°. After cooling in dry air the oxides were exposed to room air (46% relative humidity and 25°) and weighed at various time intervals. The weight gain is shown in Fig. 3. This behavior precludes the existence of pyrophosphates since pyrophosphates are not hygroscopic.

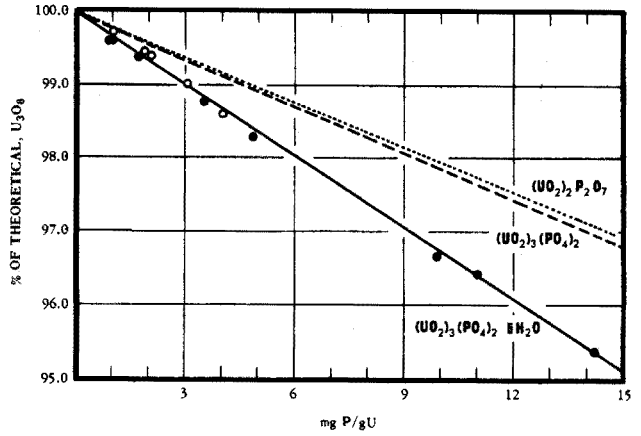

Fig. 2. Effect of phosphorus on the composition of uranium oxide from the ignition of uranyl nitrate. Ignition time, 2 h; ignition temperatures, 850° (○) and 1000° (●). The lines drawn show the calculated values for the different species.

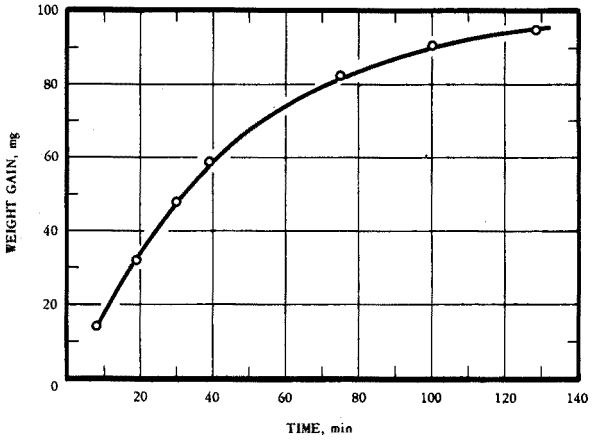

Fig. 3. Weight gain by uranium oxide (5.51 g) containing phosphorus (14 mg g^{-1}).

TABLE II

URANIUM(IV) IN URANIUM OXIDES CONTAINING PHOSPHORUS

mg P/g U added	*Calculated g U(IV)/g*			*Found g U(IV)/g U*
	$(UO_2)_2P_2O_7$	$(UO_2)_3(PO_4)_2$	$(UO_2)_3(PO_4)_2 \cdot 5\,H_2O$	
4.8	0.2696	0.2641	0.2624	0.2618
9.8	0.2563	0.2452	0.2423	0.2422
11.0	0.2529	0.2410	0.2380	0.2406
14.2	0.2444	0.2292	0.2254	0.2270
16.2	0.2394	0.2222	0.2181	0.2184

Uranium oxides containing phosphorus were also analyzed for their uranium-(IV) content. The results are presented in Table II. Calculated contents for these oxides are shown for comparison. These data further verify the formation of the hydrated uranyl phosphate, $(UO_2)_3(PO_4)_2 \cdot 5\,H_2O$.

A detailed study was also made of the behavior of calcium, chromium, copper, magnesium, manganese, nickel, sodium, thorium, zinc, and zirconium in the gravimetric determination for uranium in uranyl nitrate. Many of these elements were found not to form their common oxides within the U_3O_8 matrix and, in combination with phosphorus, to form phosphates.

Calcium has been reported to form calcium triuranate in the presence of U_3O_8[4,5]. However, the present investigations showed that calcium at low concentrations deviates from this behavior. It forms compounds in which there is an excess of oxygen associated with the calcium–uranium oxides[9]. The formation of calcium triuranate is dependent on calcium concentration and ignition temperature. The effect of calcium on oxide stoichiometry is shown in Fig. 4.

Unlike calcium, magnesium forms the triuranate compound at all concentration levels when it is ignited between 850° and 1050°. Copper and zinc nitrates form triuranates when they are ignited with uranyl nitrate at 850°. At higher temperatures, thermal decomposition of the triuranates occurs. Copper forms the diuranate at 1000° while zinc triuranate decomposes to yield ZnO and U_3O_8.

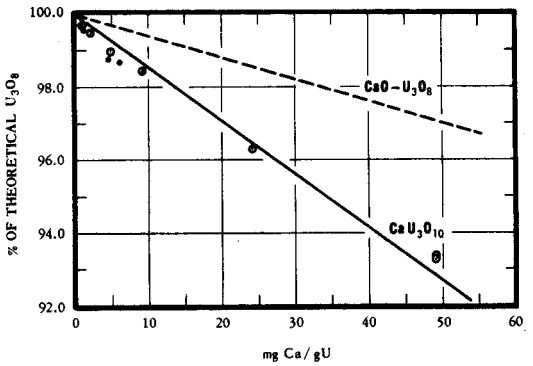

Fig. 4. Effect of calcium on the composition of uranium oxide from the ignition of uranyl nitrate. Ignition time, 2 h; ignition temperatures, 850° (●) and 1000° (○). The lines drawn show the calculated values for the species mentioned.

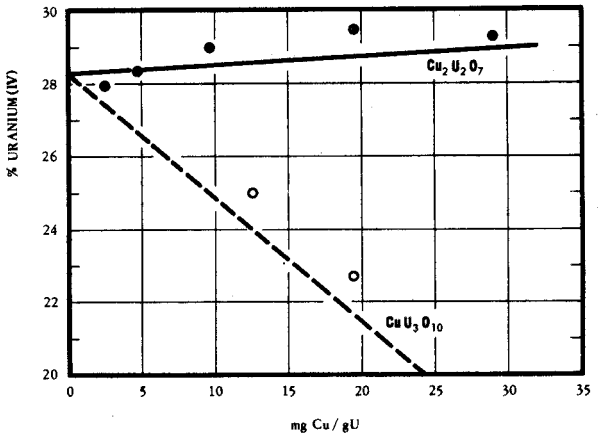

Fig. 5. Effect of copper on the uranium(IV) in uranium oxides from the ignition of uranyl nitrate. Ignition time, 2 h; ignition temperatures, 900° (○) and 1000° (●). The lines drawn indicate the calculated values.

The decomposition of copper triuranate was studied gravimetrically for total uranium content and titrimetrically for uranium(IV) content. It was found that thermal decomposition of copper triuranate occurs after a 1-h ignition at 900°. Ignitions at 1000° for 1 h were found to produce the diuranate. Results for uranium(IV) are shown in Fig. 5.

Chromium and manganese form monouranates in the presence of uranyl nitrate when ignited at 1000°. Manganese triuranate is formed at 850°. Thorium and zirconium in the presence of uranyl nitrate form their common oxides within the U_3O_8 matrix when they are ignited at 1000° for 24–48 h. At lower temperatures both thorium and zirconium form monouranates.

The apparent compounds formed by a number of elements during ignition in the presence of uranyl nitrate are summarized in Table III. The identification of these compounds is based on total uranium and uranium(IV) contents of the oxides

compared to calculated values for uranium in assumed compounds. These compounds, with the exception of thorium and zirconium, formed at levels of 2–5 mg of element per g of uranium after a 2-h ignition period; compounds of thorium and zirconium formed after a 24-h ignition period.

TABLE III

IMPURITY COMPOUNDS FORMED DURING IGNITION OF URANYL NITRATE

Element	*Apparent compound formed by 2 h ignition*	
	850°	*1000°*
Ca	CaU_3O_{14}	CaU_3O_{13}
Cr	$CrUO_4$	$CrUO_4$
Cu	CuU_3O_{10}	$Cu_2U_2O_7$
Mg	MgU_3O_{10}	MgU_3O_{10}
Mn	MnU_3O_{10}	$MnUO_4$
Ni	NiU_3O_{10}	$NiO–U_3O_8$
Na	$Na_2U_2O_7$	$Na_2U_2O_7$
P	$(UO_2)_3(PO_4)_2 \cdot 5\,H_2O$	$(UO_2)_3(PO_4)_2 \cdot 5\,H_2O$
Th	$ThUO_5$	$ThO_2–U_3O_8$[a]
Zn	ZnU_3O_{10}	$ZnO–U_3O_8$
Zr	$ZrUO_5$	$ZrO_2–U_3O_8$[a]

[a] Ignited for 24 h.

Of the elements studied, calcium and phosphorus were found to have the most effect on gravimetric uranium results, especially if their concentrations exceeded 1 mg per g of uranium. Correction of gravimetric results, based on the elements present as impurities in uranyl nitrate, are necessary to obtain accurate results. Ignitions at 1000° are superior to lower ignition temperatures because compound formation is more predictable and any thermal decomposition at this temperature often yields the common oxides within the U_3O_8 matrix.

TABLE IV

EFFECT OF ELEMENTAL IMPURITIES ON URANIUM CONTENT IN OXIDES FORMED FROM URANYL NITRATE IGNITIONS

Impurity (mg/g U)	*% Uranium*			*% Difference grav.-tit.*	
	Prev. grav.	*Improv. grav.*	*Titration*	*Prev. grav.*	*Improv. grav.*
Ca–1.0, P–1.6	84.44	84.33	84.32	+0.14	+0.01
Ca–1.0, P–3.0	84.23	83.93	83.94	+0.34	−0.01
Mg–1.0, P–2.0	84.35	84.25	84.22	+0.15	+0.04
Mg–9.8, P–24.8	79.84	78.54	78.62	+1.55	+0.10
Ca–0.5, Mg–0.5, Zn–0.5, Ni–0.1, P–0.1	84.62	84.56	84.58	+0.05	−0.02
Cu–0.5, Ni–1.0, Th–1.0	84.58	84.53	84.55	+0.04	−0.02

Comparison of gravimetric procedures

Uranyl nitrates containing several impurities were ignited at 1000° for 2 h. The resultant oxides were weighed and then titrated for uranium. Gravimetric results were calculated on the basis of the formation of the common oxides within the U_3O_8 matrix as well as on the basis of the formation of compounds identified in this study. The results are compared in Table IV.

Elemental impurities in uranyl nitrate affect gravimetric uranium results if gravimetric factors are calculated on the basis of common oxide formation. This factor must be corrected on the basis of each element present and the compounds formed during ignition to obtain accurate gravimetric analyses for uranium in uranyl nitrate. Corrections based on the formation of uranates and phosphates yield gravimetric results which agree with the titrimetric determination of uranium.

Analyses of two production samples of uranyl nitrate are shown in Table V. The gravimetric results were corrected for the elemental impurities on the basis of the formation of the common oxides within the U_3O_8 matrix, as well as on the basis of the formation of compounds identified in this study.

TABLE V

DETERMINATION OF URANIUM IN URANYL NITRATE SAMPLES

Impurity (mg/g U)	*% Uranium*			*% Difference grav.-tit.*	
	Prev. grav.	*Improv. grav.*	*Titration*	*Prev. grav.*	*Improv. grav.*
Ni, P, Th 0.4 Total	53.02	52.95	52.95	+0.13	+0.00
Fe, P, Th 3.1 Total	53.64	53.54	53.49	+0.28	+0.09

SUMMARY

A study of the factors which affect the gravimetric determination of uranium in uranyl nitrate is described. In the gravimetry of uranium, the U_3O_8 (weighing form) produced by ignition is usually assumed to deviate <0.02% from theoretical composition; and elemental impurities are assumed to form common oxides within the U_3O_8 matrix. It is shown that these assumptions are incorrect. Ignition temperature and time affect U_3O_8 stoichiometry. Ignitions of uranyl nitrate for 1–3 h at 850° produce U_3O_8 that deviates as much as 0.15% from stoichiometric U_3O_8; deviations are negligible when uranyl nitrate is ignited at 1000° for 2 h. Elemental impurities, particularly calcium and phosphorus, affect the composition of U_3O_8 formed in the ignition of uranyl nitrate. A variety of impurity complexes such as uranates and phosphates are found within the U_3O_8 matrix. Formation of these impurity complexes depends on the elements present, their concentration, and ignition temperature. Therefore, in the gravimetric determination of uranium in uranyl nitrate, the effects of ignition parameters and nonvolatile impurities must be considered in order to obtain accurate uranium determinations.

RÉSUMÉ

Une étude est effectuée sur l'influence des facteurs pouvant influencer le dosage de l'uranium dans le nitrate d'uranyle. La température de calcination, ainsi que sa durée, affectent la stoechiométrie U_3O_8 : écart jusqu'à 0.15% pour une calcination à 850° pendant une à trois heures. La différence est négligeable lorsque le nitrate d'uranyle est calciné à 1000° pendant 2 heures. Des impuretés, en particulier calcium et phosphore, affectent la composition de U_3O_8. Par conséquent, il faudra tenir compte de l'influence des paramètres de calcination et des impuretés non-volatiles, si l'on veut obtenir des résultats précis.

ZUSAMMENFASSUNG

Es wird eine Untersuchung der Faktoren beschrieben, die die gravimetrische Bestimmung von Uran in Uranylnitrat beeinflussen. Bei der Gravimetrie von Uran wird im allgemeinen vorausgesetzt, dass das beim Verglühen entstehende U_3O_8 (Wägeform) weniger als 0.02% von der theoretischen Zusammensetzung abweicht und dass Verunreinigungen durch andere Elemente innerhalb der U_3O_8-Matrix gewöhnliche Oxide bilden. Es wird gezeigt, dass diese Annahmen nicht korrekt sind. Glühtemperatur und Glühzeit beeinflussen die U_3O_8-Stöchiometrie. Wird Uranylnitrat 1–3 h lang bei 850° geglüht, entsteht U_3O_8, das 0.15% von stöchiometrischem U_3O_8 abweicht. Die Abweichungen sind jedoch vernachlässigbar, wenn Uranylnitrat 2 h lang bei 1000° geglüht wird. Verunreinigungen durch andere Elemente, insbesondere Calcium und Phosphor, beeinflussen die Zusammensetzung des beim Verglühen von Uranylnitrat gebildeten U_3O_8. Verschiedene Verunreinigungskomplexe wie Uranate und Phosphate werden innerhalb der U_3O_8-Matrix gefunden. Die Bildung dieser Komplexe hängt von den vorliegenden Elementen, deren Konzentration und von der Glühtemperatur ab. Deshalb müssen bei der gravimetrischen Bestimmung von Uran in Uranylnitrat die Einflüsse der Glühbedingungen und der nichtflüchtigen Verunreinigungen berücksichtigt werden, wenn Uran genau bestimmt werden soll.

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COULOMETRIC TITRATION OF PROTEINS WITH SILVER(I)*

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The sulfhydryl and disulfide groups of proteins are known to be important for protein structure and enzyme function. The total sulfur content (–SH and –SS–) is usually measured when amino-acid analysis is performed. This type of analysis does not distinguish between sulfhydryl and disulfide groups and also gives no information concerning the reactivity of the groups. The lack of reactivity of some sulfhydryl groups is quite common, and usually an excess of reagent or the presence of a protein-denaturing agent is necessary before the sulfhydryl groups will react with appropriate detecting agents.

To obtain information on the type of group and its reactivity, many different reagents have been employed. Because of varying selectivity, reactivity, and molecular size, results may differ, depending upon which reagent is used. Details on the various methods employed for sulfhydryl group analysis can be found in a number of reviews[1–4].

The amperometric titration of sulfhydryl groups with silver(I) in ammoniacal and TRIS media has been extensively used for measuring protein sulfhydryl groups. High results have been reported for some compounds (cysteine, thioglycolic acid, and hemoglobin)[5,6] but with many proteins good agreement with other methods has been reported[1]. In order to titrate the relatively unreactive sulfhydryl groups, the titration has been performed with an excess of silver(I), the unreacted titrant being back-titrated with reduced glutathione (GSH)[7]. Another approach has been to add a known amount of sample to a known but excessive amount of $Ag(TRIS)_2^+$. The decrease in current is noted and the amount of sample to bring the current to zero is calculated[8].

The present paper describes a coulometric titration utilizing electrogenerated silver(I) for the determination of sulfhydryl and disulfide groups in small amounts of native or denatured protein. The titration can be performed in the presence of an excess of silver(I) and it is possible to follow continuously the course of the reaction with time and therefore compare the reactivities of the sulfhydryl and disulfide groups. Unlike the amperometric method, no standard solutions are necessary because an electrical standard is employed. The titrations were investigated in three different electrolytes, each at a different pH.

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EXPERIMENTAL

Chemicals

Reagent-grade chemicals and deionized water were used wherever possible.

Urea was purified by the method of Benesch *et al.*[9]. It was found that purified urea and deionized water were essential for good results.

Proteins were obtained from the following sources: yeast glutathione reductase, human hemoglobin, and bovine pancreas insulin, from Calbiochem; and bovine serum albumin, bovine α-chymotrypsin, bovine β-lactoglobulin, and ovalbumin from Nutritional Biochemical Co.

Apparatus

All coulometric measurements were made with the circuit previously described[10] at a generation current of 0.0157 mA and an indicator potential of 200 mV.

The titration vessel was similar to those used in clinical chloride titrators. The two silver indicator electrodes and the working silver anode were 16-gauge silver wire. The cathode was a 22-gauge platinum wire isolated from the bulk of the cell. The three silver wires and the isolation tube were located in a #2 rubber stopper into which a piece of 2-mm glass tubing was inserted. This enabled the sample to be added by syringe without lifting the stopper. The titration vessel and the excess silver(I) method have been described previously[11].

Reagents

Imidazole electrolyte. The imidazole was weighed and appropriate amounts of 1.0 *M* KCl and 1% (w/v) EDTA (disodium salt) were added to give a solution 0.15 *M* in imidazole, 0.01 *M* in KCl, and 0.01% in EDTA. Nitric acid (1.0 *M*) was then added until the pH was 6.2 at 25°.

TRIS electrolyte. 0.15 *M* 2-Amino-2-hydroxymethyl-1,3-propanediol (TRIS), 0.01 *M* KCl, 0.01% EDTA, pH 7.5. The solution was prepared from stock solutions of the reagents (1.0 *M* TRIS, 1.0 *M* KCl, and 1% EDTA) and 1.0 *M* nitric acid was added until a pH of 7.5 at 25° was obtained.

Ammoniacal electrolyte. 0.05 *M* ammonia, 0.1 *M* ammonium nitrate, 0.01% EDTA. This solution was prepared from stock solutions of the reagents (2.0 *M* ammonia, 1.0 *M* ammonium nitrate, and 1% EDTA).

Imidazole (urea), TRIS (urea), and ammoniacal (urea). The three electrolytes were made 8 *M* in urea and the pH was brought back to the original value with 1.0 *M* nitric acid. The urea was used within a day of purification and the electrolyte so prepared was utilized within 3 days.

Saturated sodium sulfite in 0.01% EDTA. EDTA solution was placed in ice until cold and then solid sodium sulfite was added. The saturated solution was used within 1 h of preparation. Attempts to use sulfite solutions prepared more than 1 h in advance resulted in erratic titrations.

Urea. 10 *M* in 0.01% EDTA.

Procedures

Concentration of protein preparations. For each protein at least three different solutions were prepared by weighing 10–100 mg of protein in a 10-ml volumetric

flask; 2–3 ml of 0.01% EDTA was then added and the flasks were allowed to stand for at least 30 min until the contents were dissolved. Approximately 5 ml more of 0.01% EDTA were added and then 1–2 drops of 2-octanol were added to eliminate foaming. EDTA solution was then added until the flask contained 10 ml of protein solution. These solutions were then quantitatively diluted 1:10 or 1:20 and the absorbance of the diluted solutions was measured at the appropriate wavelength. By knowing the absorptivity of the particular protein (Table I), the actual concentration of protein in the original solution was calculated. The purity of the commercial preparation was obtained by comparing the concentration (mg ml^{-1}) obtained by the absorbance readings with that obtained on the basis of weight.

TABLE I

PHYSICAL CONSTANTS OF PROTEINS STUDIED

Protein	*M.w. (ref.)*	$\varepsilon^{1\%}_{1\,cm}$ *(ref.)*
Bovine serum albumin	66,000 (2)	6.6 at 280 nm (13)
α-Chymotrypsin	24,500 (2)	—
Glutathione reductase	110,000 (12)	18.6 at 280 nm (12)
Human hemoglobin	64,500 (2)	—
Insulin	5,740 (2)	10.7 at 277 nm (14)
β-Lactoglobulin	36,000 (2)	9.5 at 280 nm (13)
Ovalbumin	46,000 (2)	13.6 at 280 nm (15)

Coulometric titration of sulfhydryl by the direct method. A 5-ml portion of titration electrolyte (either imidazole, TRIS, or ammoniacal) was placed in the titration cell by means of a 0–10 ml Measureomatic dispenser (Sargent-Welch Co.). The electrolyte was pretitrated by generating silver(I) at a current of 0.0157 mA, which corresponds to $1.63 \cdot 10^{-4}$ μmole of sulfhydryl titrated per second. The generation was stopped when the indicator current rose 0.060 mA above the bottom of the "current well" (lowest current point). The timer was reset, sample (10–500 μl) was added, and the sample was titrated to the same indicator current above the "current well". The pretitration step was necessary so that impurities in the electrolyte did not interfere with the results. The μmoles of sulfhydryl titrated were calculated by multiplying the titration time by $1.63 \cdot 10^{-4}$. This value was divided by the μmole of protein titrated and reported as mole –SH/mole protein. The molecular weight used for calculating μmoles of protein is found in Table I.

Coulometric titration of disulfide by the direct method. The titration was identical to that for sulfhydryl groups except that 200 μl of cold saturated sodium sulfite (0°) was added to the titration electrolyte before any generation of silver(I) was begun. When the titration time by this procedure was multiplied by $1.63 \cdot 10^{-4}$, the μmole of sulfhydryl plus disulfide were obtained. For proteins, this value was divided by the μmole of protein in the sample and then the mole –SH/mole protein (determined without sulfite present) was subtracted and the results were reported as mole –SS–/mole protein.

Coulometric titration of sulfhydryl by the excess method. A 5-ml portion of titration electrolyte (either imidazole, TRIS, or ammoniacal) was placed in the

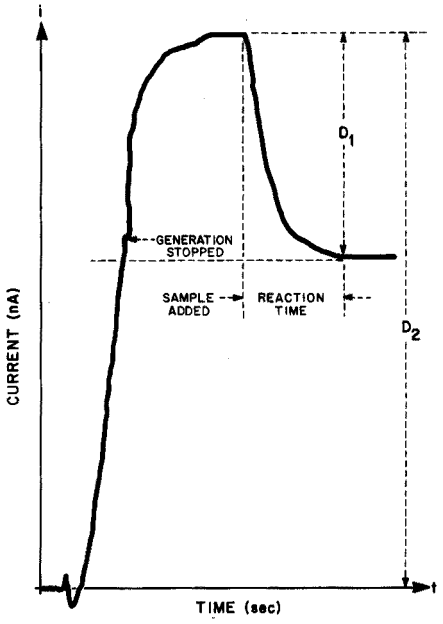


Fig. 1. Typical titration curve for the excess method.

titration cell. Silver(I) was generated at a current of 0.0157 mA for *ca.* 20 s longer than for the pretitration step in the direct method. This ensured that the biamperometric current increase beyond the end-point was linear. The recorder pen was displaced to the bottom of the chart paper and the indicator current was allowed to level off. After the indicator current became constant, the timer was reset and silver(I) was generated until the recorder pen travelled approximately three-fourths of the distance across the chart. The generation was stopped and the indicator current was again allowed to reach a constant value. The sample (5–100 μl) was added by means of an appropriate Hamilton syringe to the titration cell and the indicator current was allowed to reach a constant value once again. A typical titration recording is shown in Fig. 1. It was found that lifting the stopper or disturbing the indicator electrodes caused a change in the current recorder. Also, the recorder had to be damped in order to reach stable current readings.

The titration time of the sample was calculated as follows:

$$\text{titration time (s)} = D_1\ \text{(mm)}/D_2\ \text{(mm)} \cdot \text{generation time}$$

where D_1 is the distance (in mm) on the recorder chart between the current levels of the second generation of silver(I) and the sample addition, and D_2 is the distance (in mm) on the recorder chart between the current levels of the initial and final generation of silver(I). Further calculations using the titration time were identical to those described in the direct method.

In addition to the titration time, the reaction time of the sample could be calculated by measuring the distance between the point of sample addition and the point at which the indicator current became constant. This distance was multiplied by the reciprocal of the chart speed (s mm^{-1}) to give the reaction time. For proteins,

the reaction times were compared to those of GSH under similar conditions and molar concentrations, and reported as the relative reaction times.

Coulometric titration of disulfide by the excess method. The titration was identical to that for sulfhydryl groups except that 200 μl of cold saturated sodium sulfite (0°) was added to the titration electrolyte before any generation of silver(I) was begun. The titration time was calculated in the same manner as the excess method for sulfhydryl groups and the results were calculated in the same manner as for the direct method for disulfide.

RESULTS

Titration of reduced glutathione

As previously reported, GSH in ammoniacal and TRIS media gave theoretical results by both the direct and excess methods[11]. In imidazole medium, only the excess method could be used because of the small rate of change of the indicator current with time. In this medium the excess method gave consistent results, 75% of theoretical. Since Sluyterman[16] had obtained correct results by an amperometric titration with silver nitrate in imidazole medium, this electrolyte was included in these studies.

Titration of oxidized glutathione (GSSG)

The reaction of GSSG and other disulfide groups with sulfite is

$$RSSR + SO_3^{2-} \rightleftharpoons RS^- + RSSO_3^-$$

One mole of sulfhydryl is released for each mole of disulfide. The influence of sulfite ion on the titration of GSH and GSSG is shown in Table II. The results indicate that a minimum of 200 μl of cold saturated sodium sulfite (0.04 *M*) is needed before the theoretical amount of GSSG can be determined. Greater amounts of sulfite give results larger than theoretical. The sulfite was more stable if dissolved in 0.01% EDTA rather than water. The results of titrating GSSG by the direct method in the three media were as follows: ammoniacal electrolyte, 95–101% of theoretical; TRIS electrolyte, 97–103% of theoretical; imidazole electrolyte, 13–25% of theoretical. The reason for the low results in imidazole is not known.

Titration of GSH and GSSG in an electrolyte made 8 M in urea

The results in each electrolyte by both the direct and excess methods were identical with or without urea except for the titration of GSSG in TRIS (urea). When titration by the excess method was used, low (91.3% of theoretical) results were obtained. Direct titration of GSSG in this medium was not possible because of the high pretitration time of the electrolyte (150 s) and the gradual rate of change of indicator current with excess silver(I).

Titration of proteins

The proteins were titrated by both the direct and excess methods; all solutions employed were 0.01% in EDTA. Proteins dissolved in 8 *M* urea were titrated in electrolyte containing 8 *M* urea. A drop or two of 2-octanol did not affect the results

TABLE II

TITRATION OF GSH AND GSSG IN THE PRESENCE OF SULFITE

(Generating solution: 0.05 *M* NH_4OH, 0.10 *M* NH_4NO_3, 0.01% EDTA; amount titrated: 250 μl)

Sample	*Amount of cold sat. sulfite (μl)*	*M* SO_3^{2-}	*μg Taken*	*μg Found*	*% of Theoretical*
GSH	0	0	14.2	14.1	99.1
GSH	50	0.01	14.2	14.3	101.4
GSH	100	0.02	14.2	14.2	100.0
GSH	200	0.04	14.2	14.3	101.4
GSH	250	0.05	14.2	15.3	108.0
GSSG	0	0	14.7	0	0
GSSG	50	0.01	14.7	0	0
GSSG	100	0.02	14.7	9.91	67.4
GSSG	150	0.03	14.7	12.6	85.7
GSSG	200	0.04	14.7	14.6	99.3
GSSG	250	0.05	14.7	15.7	106.6

and was used in all protein solutions to prevent foaming.

It was observed that dissolution was more complete if the proteins were placed in 2 ml of 0.01% EDTA and allowed to stand for 30–60 min before the addition of either the remainder of the 0.01% EDTA or 10 *M* urea. The 2-octanol was added after the 10-ml volumetric flasks were about three-fourths full since it was impossible to dissolve any remaining protein when the alcohol was present. The protein solutions were analyzed within one day of preparation. Multiple determinations of different size samples were made. In only one case was the amount of titrated protein found to influence the final result (μmole –SH/μmole protein). The averaged results for sulfhydryl and disulfide groups are shown in Tables III and IV, respectively. The relative reaction times of the proteins titrated are shown in Table V. There was a slight tendency in some cases for the relative reaction time to decrease as the sample size decreased.

DISCUSSION

Bovine serum albumin

The results for sulfhydryl content of the native protein in TRIS or ammoniacal medium were similar, with slightly higher values observed in the excess method. The results were in reasonable agreement with those found by other workers using amperometric titration with silver(I) in TRIS[9,17] or ammoniacal medium[18]. They also agreed with those using mercury(II) chloride as titrant[18]. In imidazole medium, the results were even lower on a relative basis than those for GSH. No sulfide groups were titrated in the native protein in any medium. The reactivity (see Table V) of the sulfhydryl group was comparable to that of GSH.

The fractional sulfhydryl group content is probably best explained by the observation of King[19] that 70% of bovine serum albumin (BSA) is mercaptalbumin while the other 30% is a mixed disulfide of mercaptalbumin with cysteine and, to a lesser extent, glutathione.

TABLE III TITRATION RESULTS FOR SULFHYDRYL GROUPS (MOLE -SH/MOLE PROTEIN)

Sample	*Imidazole*				*TRIS*				*Ammoniacal*			
	N[a]	*Direct*	*N*	*Excess*	*N*	*Direct*	*N*	*Excess*	*N*	*Direct*	*N*	*Excess*
BSA (native)	1	0.321	2	0.358	2	0.726	1	0.772	3	0.735	1	0.758
BSA (8 *M* urea)	2	0.508	2	0.573	4	1.10	4	1.05	6	0.762	8	0.893
α-Chymotrypsin (8 *M* urea)	1	0	–	—	–	—	1	0.04	–	—	1	0.03
Glutathione reductase (native)	–	—	–	—	–	—	2	4.22	–	—	–	—
Hemoglobin (native)	1	1.50	1	3.79	2	3.30	1	5.53	1	2.44	1	5.90
Hemoglobin (8 *M* urea)	2	4.98	3	5.95	3	5.00	2	5.78	2	3.90	3	4.32
Insulin (8 *M* urea)	1	0.03	–	—	1	0.03	–	—	1	0.05	–	—
β-Lactoglobulin (native)	–	—	1	0.23	1	0.33	2	1.10	1	0.28	1	1.43
β-Lactoglobulin (8 *M* urea)	2	1.51	4	2.37	3	1.64	3	2.04	2	1.69	3	1.83
Ovalbumin (native)	–	—	2	3.96	1	0.74	1	3.49	1	0.60	2	1.82
Ovalbumin (8 *M* urea)	3	2.26	3	4.32	2	3.07	3	3.63	4	2.85	4	3.78

[a] N = number of titrations

TABLE IV TITRATION RESULTS FOR DISULFIDE GROUPS (MOLE -SS-/MOLE PROTEIN)

Sample	*Imidazole*				*TRIS*				*Ammoniacal*			
	N[a]	*Direct*	*N*	*Excess*	*N*	*Direct*	*N*	*Excess*	*N*	*Direct*	*N*	*Excess*
BSA (native)	1	0.00	–	—	1	0.00	1	0.00	–	—	1	0.00
BSA (8 *M* urea)	2	12.1	2	11.8	3	12.2	4	13.1	2	12.2	2	12.8
α-Chymotrypsin	1	3.01	2	2.71	1	5.15	1	4.70	1	3.40	2	3.70
Hemoglobin (native)	–	—	1	0.00	–	—	–	—	–	—	–	—
Hemoglobin (8 *M* urea)	–	—	1	0.00	–	—	1	0.00	–	—	1	0.00
Insulin (8 *M* urea)	2	2.26	3	2.38	–	—	3	2.94	2	2.44	3	2.50
β-Lactoglobulin (native)	1	0.00	–	—	–	—	1	0.00	–	—	1	0.00
β-Lactoglobulin (8 *M* urea)	2	2.80	2	1.95	–	—	3	2.19	2	2.15	3	2.25
Ovalbumin (native)	–	—	1	1.27	–	—	1	0.00	–	—	1	0.00
Ovalbumin (8 *M* urea)	–	—	–	—	1	0.00	1	0.00	1	0.00	1	0.00

[a] N = number of titrations.

TABLE V

RELATIVE REACTION TIMES

Sample	*Imidazole*		*TRIS*		*Ammoniacal*	
	–SH	*–SS–*	*–SH*	*–SS–*	*–SH*	*–SS–*
BSA (native)	1.1	—	1.1	—	1.1	—
BSA (8 *M* urea)	1.0	0.54	1.0	0.73	4.5	3.1
α-Chymotrypsin (8 *M* urea)	—	0.93	—	0.46	—	0.88
Hemoglobin (native)	1.1	—	3.9	—	5.1	—
Hemoglobin (8 *M* urea)	1.0	—	0.88	—	1.3	—
Insulin (8 *M* urea)	—	0.51	—	0.58	—	2.2
β-Lactoglobulin (native)	1.65	—	3.3	—	3.2	—
β-Lactoglobulin (8 *M* urea)	0.71	0.46	0.56	0.57	6.2	1.8
Ovalbumin (native)	1.4	—	3.4	—	2.0	—
Ovalbumin (8 *M* urea)	1.0	—	1.7	—	6.3	—

The denatured BSA showed consistent results in TRIS (urea) medium by either the direct or excess methods with means of 1.10 and 1.05 mole –SH/mole BSA, respectively. These values are in agreement with other workers using amperometric titration in the same medium[9,17]. Kolthoff *et al.*[20], utilizing mercury(II) chloride, and Leach[21], utilizing methylmercury(II) iodide, have reported no increase in sulfhydryl titer after denaturation with 8 *M* urea. The reason for this discrepancy between the results of silver(I) titrations and mercury(II) titrations is not known but it would appear that the albumin involved in mixed disulfide formation can be titrated with silver(I) but not with mercury(II).

The results in ammoniacal (urea) medium were very erratic with a range of 0.678–1.04 mole –SH/mole BSA. The relative reaction time in this medium was also inconsistent and, in most cases, significantly greater than one. The slow decrease of apparent reactivity of the sulfhydryl group on denaturation has been attributed by Kolfhoff *et al.*[18] to a cross-linking reaction the rate of which increases with increasing pH. It is believed that the erratic, low results and the slow reaction times are caused by the cross-linking reaction.

In the presence of sulfite, variable results for disulfide groups were obtained. The most consistent results were in TRIS (urea) by the excess method, where a mean of 13.1 mole –SS–/mole BSA was obtained. These groups reacted at least as rapidly as GSSG. This value is lower than the accepted value of 17 –SS– found by Kolthoff *et al.*[22] using sulfite and mercury(II) chloride. However, values of 13 –SS– have been found with 2-mercaptoethanol[22], thioglycolic acid[23], and borohydride reduction for 15 min (17 –SS– groups for BSA were reported after a 30-min reduction)[24]. It appears that four of the 17 –SS– groups of BSA are more difficult to reduce than are the other 13.

α-Chymotrypsin

The results of titrating solutions of α-chymotrypsin denatured in 8 *M* urea showed a mean of 2.9, 4.9, and 3.6 mole –SS–/mole α-chymotrypsin for imidazole (urea), TRIS (urea), and ammoniacal (urea), respectively. As expected, no –SH groups were found. The disulfide groups in all media reacted rapidly (see Table V).

Some of the variations in the disulfide group content may be due to difficulty in dissolution as four separate purity measurements gave a range of 71.7 to 97.1%. However, the 4.9 value was the same as that found by Cecil and Wake[25] with mercury(II) chloride at a pH of 6.5–7.0 in the presence of sulfite and guanidine.

Glutathione reauctase

Portions (25 μl) of glutathione reductase (experimentally determined concentration $= 4.04 \pm 0.24$ mg ml^{-1}) were titrated in TRIS electrolyte by the excess method. When the molecular weight and $\varepsilon^{1\%}_{1\,cm}$ values of Woodin and Segel[12] for the enzyme from *Penicillum chrysogenum* were used, the yeast enzyme gave results of 4.22 ± 0.26 moles –SH/mole enzyme. In borate buffer at a pH of 9 and in the presence of NADPH, Massey and Williams[26] found 4.05 –SH/molecule. Additional experiments showed that the ability of the enzyme to catalyze the reaction,

$$GSSG + NADPH + H^+ \overset{GSSG\text{-reductase}}{\rightleftharpoons} 2\,GSH + NADP^+$$

was totally lost in the presence of silver(I). This could indicate that the sulfhydryl group is necessary for enzymatic activity.

Human hemoglobin

In all media, the excess method yielded higher results than the direct. This was expected because of the "masked" sulfhydryl groups of hemoglobin. The sulfhydryl content has been shown by Hill *et al.*[27], by converting the sulfhydryl groups to cysteic acid, to be 6 –SH groups per molecule. The excess method with the native protein in TRIS and ammoniacal media gives results approaching 6 –SH groups. The relative reaction time increases as the pH increases (imidazole, TRIS, and ammoniacal medium) and the results increase in the same order. It would appear that pH is a factor in titrating native hemoglobin.

The titration results of Benesch *et al.*[9], using silver(I) in TRIS, at 9.5, seem far too high. Results with organic and inorganic mercury(II) titrants for native hemoglobins have been reported to be between 2.20 and 2.85[28,29], which is lower than those reported here for silver(I). This difference points out the differing reactivity of reagents when titrating "masked" sulfhydryl groups without excess reagent present. No disulfide groups were found.

The excess method in imidazole (urea) and TRIS (urea) gave results in agreement with the accepted value of 6 moles –SH/mole hemoglobin. The reactivity of the denatured groups is similar to GSH. In ammoniacal medium, unexplained low results were obtained.

With neither native nor denatured hemoglobin did silver(I) titrations give high results as has been reported by previous workers[6,9,28]. The rapid titration obtained with the excess method and the small amounts of protein actually titrated, 0.53–3.3 nmoles, may be factors in obtaining correct results.

Insulin

The complete amino-acid sequence of insulin has been elucidated by Sanger *et al.*[30] and Ryle *et al.*[31]. The molecule consists of two peptide chains, the A chain of 21 residues and the B chain of 30 residues. There are three disulfide bonds. Two of these connect the chains at residues 7-7 and 20-19 on the A and B chains,

respectively. The third group forms an intrachain link between residues 6 and 11 on the A chain. There are no free sulfhydryl groups.

Direct and excess methods for denatured insulin gave comparable results. The mean values found for the excess method were 2.38, 2.94, and 2.50 for imidazole (urea), TRIS (urea), and ammoniacal (urea), respectively. Only traces of sulfhydryl groups were found. Cecil and Loening[32,33] have reported 2.5–2.8 groups reacting in urea or guanidine, with all three reacting in the presence of phenylmercury(II) hydroxide. These same authors report a maximum reaction for native insulin at pH values of 6.5–7.0, which would explain TRIS (urea) giving higher results than imidazole (urea) or ammoniacal (urea).

β-Lactoglobulin

The native protein showed no disulfide groups and with the direct method only fractional sulfhydryl values. The excess method, however, showed 1.10 –SH/molecule for TRIS, 1.43 for ammoniacal, and only 0.23 for imidazole. All of these reactions were slower than that for GSH, indicating a "sluggish" reaction.

In 8 *M* urea relatively rapid reactions occurred with and without sulfite in imidazole and TRIS, but a sluggish reaction was observed in ammoniacal medium. The excess method showed higher values than the direct. In imidazole, an interesting effect of sample size was seen. As smaller amounts of sample were titrated, it appeared that more and more silver(I) was bound per mole of protein. This effect was not noticed in the other media. The mean values for sulfhydryl content by the excess method were 2.37, 2.04, and 1.33, for imidazole (urea), TRIS (urea), and ammoniacal (urea). For disulfide group content, the means were 1.95, 2.19, and 2.25. These results are in good agreement with those of many workers by various methods[13,34–36].

Ovalbumin

The sulfhydryl groups of ovalbumin have been investigated with many different reagents. These investigations have shown that ovalbumin is a classic example of the protein which contains, in the native state, "masked" sulfhydryl groups which react with some reagents but not with others. Three sulfhydryl groups in the native state have been reported with *p*-chloromercuribenzoic acid (PCMB)[37] and 4-(*p*-dimethylaminobenzeneazo)-phenylmercury(II)acetate[38], but only two sulfhydryl groups with 2,2′-(2-hydroxy-6-sulfonaphthyl-1-azo)-diphenyl disulfide[39]. With silver(I) in TRIS or ammoniacal medium higher results (4.8–5.6 and 5.0, respectively) have been reported[9,40].

The unreactive nature of the sulfhydryl groups is evident when comparing the direct and excess methods in TRIS medium: 0.74 mole –SH/mole ovalbumin for the direct method and 3.49 for the excess method. Reactions in all media are somewhat slower than that of GSH. In ammoniacal medium, only half the number of sulfhydryl groups were found in comparison to TRIS. Boyer[36] has shown a change in titration values with PCMB depending on pH. He found maximum reaction at a pH of 4.6 with four groups reacting, and that at pH 7.00 only 3.2 –SH groups titrated. His results are similar to ours in that the number of groups reacting increased with decreasing pH.

The direct results in imidazole (urea) were erratic but the excess method gave

values slightly over four. In TRIS (urea) and ammoniacal (urea) media, results approximating three for the direct method and four for the excess method were obtained. These results were interesting because the full number of sulfhydryl groups (4) was found by the excess method, while the three groups found by the direct method have been shown to be the more reactive ones when PCMB[37] or 4-[4-(acetoxymercuri)-phenylazo]phenol[34] were used as titrants.

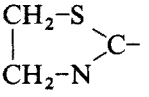
With the native protein, no disulfide groups were found in any medium except imidazole. The value obtained, 1.27, is in agreement with the value of 1 reported by Cecil[2]. The most probable reason for the lack of reaction of the disulfide group is the influence of pH; the influence is probably similar to that noted with the sulfhydryl groups.

CONCLUSIONS

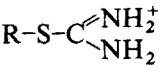
Bovine serum albumin, α-chymotrypsin, glutathione reductase, human hemoglobin, insulin, β-lactoglobulin, and ovalbumin have been titrated with silver(I) in three different media by a direct and excess method. The results show the validity of the excess method for titrating protein sulfhydryl and disulfide groups. A comparison of the direct and excess methods shows the existence of "masked" or sluggishly reacting groups for hemoglobin, β-lactoglobulin, and ovalbumin even when in 8 *M* urea. With the other proteins, the same results were obtained for the direct and excess methods indicating that all the groups are readily accessible.

The difference between the results in the three media are possibly due to their difference in pH. The pH can influence the amount of mercaptide actually present and also the ionization of neighboring groups such as –OH, –COOH, or $-NH_2$. Because of electrostatic forces, these neighboring groups can influence the pK_{SH} of the sulfhydryl groups and also the net charge in the vicinity of the –SH group. Because of the large number of functional groups in proteins and the three-dimensional configurations they adopt, it is very difficult to quantify the influence of pH. Of the proteins studied, ovalbumin, insulin, and hemoglobin best illustrate the influence of pH.

Another possible effect of the medium would be steric hindrance or bonding around the sulfhydryl group. Linderstrøm-Lang and Jacobsen[41] have suggested thiazolidine rings,



and Brush *et al.*[42] have suggested an isothiuronium-type compound,



as possible ways in which the protein –SH is bound. Thioester bonds have also been suggested. The nature of the silver(I) complex (imidazole, TRIS, ammoniacal) reacting with such "bonded –SH" would also influence the reaction rate, especially with isothiuronium compounds. The evidence for the existence of each bond is, at the present time, only indirect.

The excess method was also used to compare the rate of reaction of the proteins relative to GSH. In titrating the sulfhydryl content of the denatured protein, the reaction in ammoniacal medium was always shorter than the reaction in either TRIS or imidazole medium. The higher pH of the ammoniacal medium could have two opposite effects. The higher pH could increase the amount of mercaptide and the net negative charge. Conversely, the increase in net negative charge could increase the pK_{SH} and decrease the rate. The latter effect apparently is the more dominant factor.

Of the three media, TRIS gave results most consistent with other methods. It is recommended that TRIS be the first medium considered for the titration of proteins; however, additional information is possible with the other media.

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SUMMARY

The sulfhydryl groups of some proteins were studied by a coulometric argentometric titration; disulfide groups in the presence of sulfite were titrated by the same technique. Direct procedures and a technique utilizing excess coulometrically generated silver(I) were used. The latter method enabled small samples (20–1000 μg of protein) to be titrated with a precision of less than 4%. The proteins were generally titrated in both the native state and the denatured state (8 *M* urea). Bovine serum albumin, α-chymotrypsin, glutathione reductase, human hemoglobin, insulin, β-lactoglobulin, and ovalbumin were examined and the results compared to those of previous workers.

RÉSUMÉ

Une méthode de titrage par coulométrie argentique est proposée pour le dosage des groupes sulfhydryle de quelques protéïnes. On procède soit par titrage direct, soit avec argent(I) en excès, produit coulométriquement. La méthode indirecte permet l'analyse de très petits échantillons (20 à 1000 μg de protéïne), avec une précision de moins de 4%. Les résultats obtenus pour les substances suivantes: albumine de sérum bovin, α-chymotrypsine, glutathione réductase, hémoglobine humaine, insuline, β-lactoglobuline et ovalbumine, sont comparés avec les valeurs trouvées précédemment.

ZUSAMMENFASSUNG

Die Sulfhydrylgruppen einiger Proteine wurden mittels einer coulometrischen argentometrischen Titration untersucht; Disulfidgruppen in Gegenwart von Sulfit wurden nach demselben Verfahren titriert. Es wurden direkte Titrationen und ein Verfahren erprobt, bei dem ein Überschuss von coulometrisch erzeugtem Silber(I) verwendet wird. Nach der letzteren Methode können kleine Proben (20–1000 μg Protein) mit einer Reproduzierbarkeit besser als 4% titriert werden. Im allgemeinen wurden die Proteine titriert sowohl im natürlichen Zustand als auch im denaturier-

ten Zustand (8 *M* Harnstoff). Serumalbumin aus Rinderblut, α-Chymotrypsin, Glutathion-Reduktase, menschliches Hämoglobin, Insulin, β-Lactoglobulin und Eialbumin wurden untersucht und die Ergebnisse mit denen anderer Autoren verglichen.

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SHORT COMMUNICATION

The synthesis and purification of analytically useful quantities of the monothio derivative of 1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedione

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The fluorinated β-diketones have been investigated extensively as ligands for volatile metal complexes that can be analyzed by gas chromatography[1]. The monothio derivatives of the β-diketones show considerable promise for certain metals, such as nickel, platinum and palladium[2,3].

1,1,1,2,2,3,3-Heptafluoro-7,7-dimethyl-4,6-octanedione (H(fod)) was synthesized by Springer *et al.*[4] specifically for its potential as a reagent for metal chelate gas chromatography. Belcher *et al.*[5] synthesized the monothio derivative of H(fod), *i.e.* T-H(fod), but isolated only small quantities of the pure bis-nickel complex. In the present work, an analytically useful quantity of the pure reagent is prepared and isolated, a test for the purity of the derivative from the parent compound is suggested, and the pure product is characterized.

Synthesis

The synthesis was done as described by Belcher *et al.*[5] with the following changes. A 65-ml volume of H(fod) and 2500 ml of absolute ethanol were used. After the initial treatment with hydrogen chloride gas and hydrogen sulfide and an overnight reaction period, the mixture was treated twice more in the same manner. The reaction mixture darkened more each day from a mild orange color after the first reaction period to a final red orange color. A fourth treatment resulted in essentially no change.

Isolation

The reaction mixture was poured into 3600 ml of distilled water and the resulting mixture was extracted in two batches, each with four 250-ml portions of *n*-hexane. The extracts were combined and washed by extraction with three 100-ml portions of distilled water. The *n*-hexane mixture was then dried over anhydrous magnesium sulfate. After filtering to remove the magnesium sulfate, most of the solvent was removed by distillation at atmospheric pressure with a 60-cm Vigreux column. This method of removing solvent was chosen because a red brown organic phase was always trapped when a rotary evaporator was used. Rather than using the somewhat more tedious sublimation of the bis-nickel chelate for purification, a distillation suggested by Newman[6] for compounds of similar boiling points was

adopted. A 66-cm still packed with 0.25-in diameter glass beads has a sufficient number of plates so that 100% pure H(fod) and 100% pure T-H(fod), as determined by gas chromatography, can be obtained with a middle cut of only 3–4 ml at 3.5–4.5 torr if the reflux ratio is kept at 30:1 or better. This still has significant hold-up volume; hence, when all the H(fod) had been evolved, the pot was allowed to cool and the column was washed down with 150 ml of *n*-hexane. The pot mixture was then transferred to a micro still. The solvent was removed by distillation and the product was distilled from the heavy fractions at 4–5 torr. The resulting product was stored as a solid at dry ice temperatures. No decomposition was detected during a month even though the product was frequently warmed to room temperature for short periods during this time.

Instrumental

IR spectra were recorded on a Perkin-Elmer Model 137 spectrophotometer. Carbon, hydrogen, and sulfur analysis were performed by MHW Laboratories, Garden City, Mich. NMR spectra were recorded with a Varian A-60 for H′ *versus* TMS. The index of refraction was obtained on a Bausch and Lomb Abbe refractometer, Model No. 33-45-02, with standards from R.P. Cargille Labs Inc., Cedar Grove, N. J. Mass spectra were obtained on the AEI-MS-9 by injection of the liquid samples.

Results

A final yield of the pure ruby red product varied between 11 and 15 ml. Elemental analysis resulted in 38.37% C, 3.40% H, 10.69% S; theoretical values are 38.47% C, 3.55% H, and 10.25% S. The refractive index was 1.4226 for the sodium line at 25.0°. With a Gay-Lussac 5-ml pycnometer calibrated with water, the density was established as 1.309 g ml^{-1} at 25.0°.

TABLE I

THE INFRARED SPECTRUM OF T-H(fod)[a]

Band (cm^{-1})	*Shape*
2980	m n
1660	w n
1605	w n
1525	s n
1465	w b
1400–1100	Identical to parent compound
1090	w n
1065	w sh
1040	w sh
960	m n
920	m b
840	m n
815	m b
750	m n
720	w b

[a] Notations: s = strong, m = medium, w = weak, n = narrow, b = broad, sh = shoulder.

IR spectra were obtained on neat samples of T-H(fod) with NaCl discs (Table I). Four peaks occurred within the region 1400–1700 cm^{-1}. A dilute sample was prepared (5% in carbon tetrachloride) to study any change in this region. None was observed in relative intensity, location or shape.

The proton NMR spectrum of T-H(fod) gave three peaks with an area ratio of 0.91:0.93:9.00, indicating that the compound exists *ca.* 100% as the enol or thioenol form, as do other monothio-β-diketones[7,8]. When TMS plus chloroform was used to calibrate the sweep, the peaks occurred at 10.67, 6.72 and 1.32 p.p.m., respectively. With an identical technique, H(fod) was determined to have peaks at 14.91, 6.13 an 1.31 p.p.m. with areas of relative ratios 1.02:1.01:9.00. The results for H(fod) compare favorably with Springer's[9].

The mixture of parent β-diketone and derivative monothio-β-diketone was separated by gas chromatography. The column and conditions described in Table II will produce a symmetrical peak for H(fod) at a retention time of 1.8 min and for T-H(fod) at 5.25 min. When pure β-diketone was used to determine the response of the instrument to H(fod), the detection limit for H(fod) in a mixture of H(fod) and T-H(fod) was found to be *ca.* 0.04% H(fod).

TABLE II

CONDITIONS FOR GAS CHROMATOGRAPHY

(Column temperature, 115°; detector temperature, 180°; injection port temperature, 150°. Carrier gas He, 47 ml min^{-1}. Detector, katharometer; bridge current, 150 mA)

Instrument	F & M Model No. 720.
Column	3.5 ft. × 6 mm o.d. glass.
Packing	20% DC silicone oil 550 on Chromosorb W.

The mass spectrum of T-H(fod), like that of H(fod), is very complex. The most intense peak has an *m/e* value equal to 57 (the *tert*-butyl group). All other peaks are 20% relative intensity or less than the $m/e = 57$ peak. The maximum *m/e* is located at 312, which is the appropriate mass number for T-H(fod). Attempts to confirm the location of the sulfur by the presence of $C_3F_7C{=}O^{\oplus}$ or $(CH_3)_3CC{=}S^{\oplus}$ were unsuccessful.

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SHORT COMMUNICATION

Assay of some physiologically active alkaloids by thermometric titrimetry

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(Received 14th October 1972)

The methods quoted in the British Pharmacopoeia for the determination of milligram amounts of physiologically active bases employ a diversity of techniques, and generally involve lengthy procedures for the extraction of the active ingredients from the inert carriers, before the alkaloid is determined. A general method for the determination of these alkaloids, particularly in the dosage form, without isolation, would have obvious advantages.

The use of thermometric titrimetry for the determination of some compounds in pharmaceutical preparations has been previously reported[1]. Some bases have been assayed, either as their hydrochlorides by titration with aqueous sodium hydroxide, or by titration of the free base with standard hydrochloric acid.

The enthalpimetric determination of nitrogen-containing bases, utilizing their reaction with sodium tetraphenylboron, has also been reported[2,3], but attempts to extend this method to physiologically active alkaloids[3] were not successful, the variations in the results being analytically unacceptable.

The formation of large molecular weight adducts between the bulky anions of the physiologically active bases and appropriate high ionic weight cations, generally enables the gravimetric determination of small amounts of the bases. Preliminary experiments with several such cationic reagents showed that silicotungstic acid is a suitable reagent for the thermometric determination of alkaloids.

In addition to the usual properties required of a substance when it is used as a titrant for normal titrimetry, any substance to be used as a titrant in continuous thermometric titrimetry must have a relatively high solubility in the solvents used, because concentrated titrants are employed. Moreover, the heat of the analytical reaction must be acceptable for thermal methods. In reactions involving precipitation, the formation of the precipitate must be practically instantaneous, and no supersaturation should occur during the titration. Experiments showed that silicotungstic acid fulfils all these requirements when used as a titrant for alkaloids. Other reagents investigated, including sodium tetraphenylboron, potassium tetraiodomercurate(II), and potassium tetraiodoplumbate(II), failed to meet one or more of the above criteria in the determination of alkaloids.

The kinetics of the reactions between silicotungstic acid and all of the alkaloids investigated were such that direct continuous titrations were feasible. Depending upon the pK_b value of the alkaloid, either dilute mineral acid or water was used as

the solvent. The optimal pH for the precipitation of each base was studied. The minimal concentration of precipitant necessary to ensure complete precipitation was also determined.

The standardization of solutions of silicotungstic acid is straightforward and depends upon the acid being tetrabasic towards sodium hydroxide when methyl red or methyl orange is used as indicator[4]. Repeated assays of the control solution showed that no apparent decomposition occurred over one month.

Experimental

Apparatus. The circuit for the basic electrical bridge system, and the details of the titration vessel and insulation have been previously reported[5]. The titrant was delivered at a rate of *ca.* 0.15 cm^3 min^{-1} (the actual rate being determined by gravimetry). The mixtures were stirred magnetically.

Materials. Stock solutions of silicotungstic acid (aqueous 0.3 *M* solutions) were standardized by titration against standard sodium hydroxide solution.

B.P. grade compounds were used for alkaloids contained in pharmaceutical grade preparations; other alkaloids (see Table I) were purified by repeated recrystallization from appropriate solvent mixtures.

TABLE I

DETERMINATION OF VARIOUS ALKALOIDS

Compound	*Solvent*	*No. of results*	*Range of wts. (g)*	*Largest error (%)*
Atropine sulphate[a]	0.1 *M* HCl	5	0.0374–0.0610	−0.86
Brucine[a]	0.1 *M* HCl	5	0.0340–0.0539	+0.6
Cinchonine[b]	0.1 *M* HCl	5	0.0367–0.0524	+0.16
Codeine phosphate[a]	0.1 *M* HCl	8	0.0314–0.0794	+1.3
Codeine phosphate[a] tablets B.P. (nominal 30 mg)	0.2 *M* HCl	10	0.0300	+1.3
Morphine sulphate[a]	Water	10	0.0446–0.0740	+1.3
Morphine sulphate[a] B.P. injection	Water	5	50 mg nominal	−0.25
Pilocarpine nitrate[a]	Water	20	0.0247–0.0864	−0.6
Procaine hydrochloride[b]	0.5 *M* HCl	5	0.0301–0.0640	−1.0
Quinine sulphate[a]	Water	6	0.0283–0.0545	+1.6
Strychnine[a]	0.1 *M* HCl	5	0.0441–0.0673	−1.3
Thiamine hydrochloride (vit. B_1)[b]	0.1 *M* HCl	6	0.0341–0.0539	−0.9

[a] Ratio of silicotungstic acid to alkaloid 1:4.
[b] Ratio of acid to alkaloid 1:2.

Procedure. Dissolve a known weight of the alkaloid contained in the reaction vessel in 10 cm^3 of the appropriate solvent (see Table I). Submerge the tip of the titrant delivery tube and the thermistor beneath the surface of the mixture, and stir for 2–3 min, until the solution reaches thermal equilibrium with its surroundings (indicated by a constant value trace on the recorder). Then titrate with an aqueous (0.3 *M*) solution of silicotungstic acid. Stir vigorously so that efficient heat transfer is achieved through the solution to the thermistor.

The dosage forms of the alkaloids are available either as solutions or as

compacted tablets. Transfer the solutions (as supplied for injection) to a graduated flask, dilute to a known volume and treat aliquots as above. Grind compacted tablets to a fine powder and then treat as a solid alkaloid, without separation of the matrix or filler materials. It is generally necessary to stir for 5–6 min before addition of titrant, to ensure complete dissolution of the basic ingredients of the tablet.

Effect of filler and matrix materials. Various amounts of the inert fillers (magnesium stearate, lactose, sucrose, starch and chalk) generally used in the tablets were added up to the equivalent of a 100% sample content and the mixture was titrated as above. No effects were noticed.

Results and discussion

In thermometric titrimetry, the length of the trace on the recorder between the two points of inflexion, is used as a measure of the volume of titrant added, and is thus one of the primary determinants of the precision of the method. Therefore, a balance must be maintained between the time taken for a titration (and hence the length of recorder trace), the heat losses during the titration and the rate of change of temperature near the equivalence point. These factors are all related to the concentration of the titrant; the smaller the concentration of titrant, the longer the recorder trace, but the more probable are heat losses and curvature of the end-point caused by poor kinetics, both of which contribute to the difficulty of locating the equivalence point with precision.

In many precipitation reactions, the use of a concentrated titrant apparently causes the precipitation to be rapid and hence the heat change to be sensible within the time of addition of titrant. There are various theoretical reasons why this should be so, but general theories of nucleation, precipitation and reaction rates are not, at present, sufficiently precise to allow quantitative calculation of the optimal concentrations; thus empirical methods are necessary to ascertain this particular experimental parameter.

Various concentrations of the silicotungstic acid solution were used as titrants; it was found that a 0.3 *M* solution was necessary to ensure that complete precipitation occurred over the period of a titration and to give an acceptable precision and accuracy. For example, with 0.1 *M* acid only 80–85% of the alkaloid reacted in the time taken for a titration; with 0.2 *M* acid, 90–95% of the alkaloid was determined, but with 0.3 *M* acid and above (up to 0.5 *M*) there was 100% reaction. Since the heat of dilution of aqueous 0.3 *M* silicotungstic acid was practically zero, for the normal sensitivities of the apparatus used, this is the concentration recommended.

The results obtained for the titration of various alkaloids are shown in Table I. The ratio of silicotungstic acid to alkaloid for 100% reaction depends on the number of basic groups present. The pK_b values range from 7.40 (pilocarpine) to 9.85 (atropine)[6]; attempts to determine caffeine ($pK_b = 1.22$) were not successful, for the relatively slow rate of reaction, especially near the equivalence point, caused such curvature of the plot that accurate extrapolation to ascertain the end-point was not feasible.

The only limiting factor related to the amount of filler present is apparently the limit imposed by the efficiency of stirring[2]. If large amounts of starch, magnesium stearate and lactose are used, then as long as the suspension remains mobile and

uniform during the stirring, at least twice the normal amount of filler per tablet can be tolerated.

The overall time of assay of the dosage forms is considerably shorter than that of earlier methods. The accuracy and the precision of the proposed method are generally as good as those for all other recommended methods, and the potential of the method for routine assay of dosage forms is extremely high. If calibrated standards are used, it is simply necessary to compare the length of chart trace obtained in a titration of an unknown sample, with that obtained from a known and acceptable standard, to have an immediate indication as to whether or not the unknown sample is within the commercially required specification. The apparatus needs no modification to allow its routine use, other than to arrange that the titrant is thermostatted to within 2–3° of the sample and sample holder. In practice this has meant simply keeping the apparatus in a draught-free container where ambient fluctuations are negligible during the period between check titrations on known samples.

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SHORT COMMUNICATION

A simple coulometric method for the determination of chloride in natural water

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(Received 14th October 1972)

The determination of trace amounts of chloride in drinking water is usually performed spectrophotometrically[1–3]. One of the most reliable methods is based on the displacement of thiocyanate from mercury(II) thiocyanate by chloride ion and subsequent reaction of the liberated thiocyanate with iron(III) to form the coloured iron thiocyanate complex[4–8]. The method is very sensitive and is widely used in manual as well as automatic analysis of chloride.

Small amounts of chloride can also be determined coulometrically by generating silver ions from a silver electrode and integrating the current passing through the cell[9]. This method is also very sensitive and accurate, but expensive equipment is required. The present work was undertaken in order to construct a more simple instrument for coulometric titration of chloride.

Experimental

All determinations of chloride were performed by coulometric generation of silver ions and biamperometric ("dead-stop") determination of the end-point. Because only small amounts of chloride are present in natural water in Norway, only small generating currents are needed. Consequently, a very simple battery-operated constant-current source can be used for the coulometric titrations. The circuit, consisting of dry cells and standard resistances, is given in Fig. 1. Experiments showed that the current remained constant during the titration at all settings (0.1–4 mA) of switch A. A 1-mm silver wire was used as generating electrode. The cathode (1-mm silver wire) was isolated in a glass tube with a fine-porosity fritted-glass disc. The shield tube was filled with the supporting electrolyte used in the sample solution.

The end-point was determined by applying a constant current of about 1.1 μA between two treated silver wire electrodes and recording the potential–time curve with a strip-chart recorder. (Any kind of recorder with sensitivity in the range 1–10 mV s^{-1} can be used, provided that it is equipped with zero suppression). The silver wires were coated with silver chloride by electrolyzing in potassium chloride for a few minutes. These silver/silver chloride electrodes were stable for several weeks, provided that they were stored in distilled water when not in use.

The electrolysis cell consisted of a 50-ml beaker with a plastic top. During the electrolysis the solution was stirred by means of a synchronous motor. The top

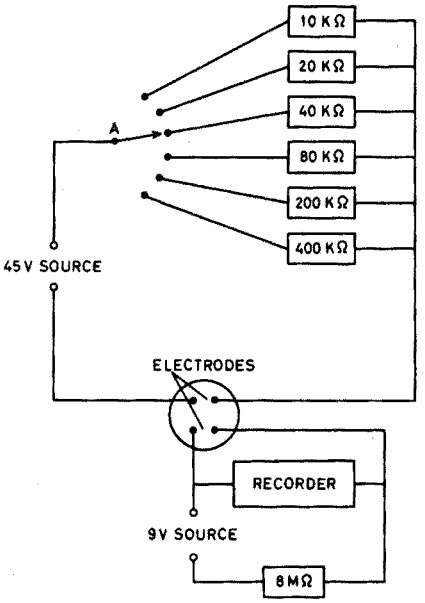

Fig. 1. Schematic diagram of the coulometric titration apparatus.

of the cell was provided with holes for the stirrer, the generating electrode, the shield tube and the two silver/silver chloride indicator electrodes. The generating electrode was placed as close to the stirrer as possible.

In order to decrease the solubility product of silver chloride, 80% methanol–water containing 0.05 *M* nitric acid was used as solvent. It was also found to be advantageous to cool the solution to about 10° in a refrigerator before the titration.

Preliminary experiments, in which known amounts of sodium chloride were titrated, showed that the amount of chloride calculated from the generating current and the time of electrolysis, was a few percent too high at low concentrations of chloride. This is probably because silver ions are generated continuously at a constant rate even close to the end-point. Hence, the solution is easily overtitrated even though it is well stirred. However, the results were perfectly reproducible, and accurate results were obtained if the concentration was determined from a calibration curve (electrolysis time *vs.* concentration) obtained from known amounts of chloride and with the same generating current and recorder speed. A typical titration curve is given in Fig. 2.

From the above experiments the following procedure was outlined.

Procedure. Transfer a 5-ml sample of water (containing 0.1–100 p.p.m. chloride) and 25 ml of 0.05 *M* nitric acid in methanol to the electrolysis cell. (At low concentrations of chloride it is advantageous to cool the solution to about 10° in a refrigerator.) Select a suitable generating current by turning switch A and place the electrodes and stirrer in the solution. Switch on the generating current and the recorder at the same time. Note the time of the electrolysis (the time from start of the titration to a sudden decrease in the potential between the two indicator electrodes, Fig. 2)

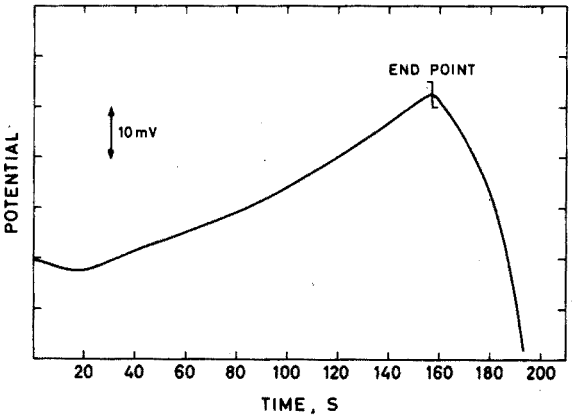

Fig. 2. Coulometric titration curve of 2.5 p.p.m. chloride in 5-ml sample of water. Generating current 0.225 mA, chart speed 0.1 cm s^{-1}.

and read the concentration of chloride from a calibration curve obtained with the same generating current and the same recorder speed.

Results and discussion

Experiments showed that in the concentration range 0.1–1 p.p.m. chloride, the most accurate results were obtained when a known amount of chloride (1 μg ml^{-1} of water sample) was added to the solution before the electrolysis. The results of a few determinations of chloride in water from various Norwegian lakes are given in Table I. The results compare favourably with those obtained by the spectrophotometric mercury(II) thiocyanate method.

The proposed method is simple and rapid and gives satisfactory results in the range 0.1–100 μg of chloride per ml. The method has the advantage that the blank

TABLE I

DETERMINATION OF THE CHLORIDE CONTENT IN VARIOUS WATER SAMPLES

Sample	*Chloride found (μg ml^{-1})*	
	Coulometric titration	*Spectrophotometry*[a]
TH 33	128, 128	126
TF 82	10.0, 10.1	9.8
TL 37	5.9, 5.8	6.2
TK 11	2.6, 2.7	3.0
TH 79	2.2, 2.3	2.4
TL 38	2.0, 2.1	2.0
TF 22	1.5, 1.6	1.6
TF 81	1.2, 1.3	1.4
TC 73	0.8, 0.9	1.0
TH 33	0.7, 0.6	0.5

[a] Results obtained at the Norwegian Institute for Water Research with a Technicon AutoAnalyzer.

is not affected by coloured substances like iron and humus. Of common ions bromide, iodide, thiocyanate and cyanide interfere and must be absent from the solution; these ions interfere also in the spectrophotometric method.

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SHORT COMMUNICATION

Determination of pH in concentrated salt solutions

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(Received 22nd September 1972)

The presence of molar concentrations of dissolved salts causes errors in pH values measured with the glass electrode in a cell with liquid junction that is calibrated with conventional standard buffer solutions. An inherent error exists in the accepted calibration procedure with standard buffers of low ionic strength for measurements in concentrated salt solutions because the liquid junction potential is not constant between the two types of solution. Furthermore, the glass electrode response may be affected by the salt. The "sodium error" of the glass electrode in alkaline solutions is well known[1], and the glass electrode has been shown to be in error when the water activity is different from unity[1].

A procedure that uses salted buffers whose empirical pH values are known has been developed for determining the pH of salt solutions. The procedure has been applied to two concentrated salt solutions of interest in this laboratory, namely, alkaline 4 *M* sodium nitrate and acidic 5.4 *M* lithium nitrate. Two types of glass electrode and several reference electrodes were evaluated.

Experimental

Electrodes. A Beckman 39004 Research glass electrode (E-2 glass bulb), as well as a Beckman 39013 Probe combination electrode (E-2 glass bulb, Ag/AgCl reference with 4 *M* KCl saturated with AgCl), and a Thomas 4094-L15 combination glass electrode (general purpose glass bulb with Ag/AgCl reference with saturated KCl) were used. As reference electrodes were used an Orion Model 90-02 double-junction reference electrode (the "salt bridge" outer chamber was filled with 10% KNO_3, 4 *M* $NaNO_3$, or 5.4 *M* $LiNO_3$), and a Beckman 39170 fiber-junction calomel electrode (saturated KCl). Measurements were made with an Orion Model 801 Digital pH/mV Meter and with a conventional pH meter.

Reagents. 4 *M* Sodium nitrate and 5.4 *M* lithium nitrate were prepared from reagent-grade chemicals and deionized water; the solutions were filtered. A 2.11 *M* sodium hydroxide solution was prepared CO_2-free by the method of Kolthoff *et al.*[2]. The sodium hydroxide and 0.1 *M* hydrochloric acid were standardized by conventional methods.

Standard buffer solutions were prepared as directed from N.B.S. standard reference materials (sodium hydrogencarbonate, sodium carbonate, borax, and potassium tetroxalate) and from Coleman Certified Buffer Tablets dissolved in the

salt solution. Beckman buffer solutions were salted by dissolving salt in the buffer to the appropriate concentration.

Procedure and calculations. Several glass and reference electrodes were paired for measurements in a test solution through a switch box (Beckman Electrode Switch 97200, with batteries removed). Each set of measurements was made during *ca.* 20 min in 50 ml of stirred solution in closed vessels at 25°. The cell used for the measurements was: glass electrode//test solution/salt bridge (optional)/reference electrode, where the double lines represent the glass membrane, and the single lines represent liquid junctions. The measured potential, E (mV), is given by the Nernst equation:

$$E = \varepsilon + 59.16 \log c_H \quad (1a)$$

$$= \varepsilon - 59.16 \text{ pH} \quad (1b)$$

where ε is the sum of the contributions to the potential that are considered constant during the measurements, *i.e.*, the standard potentials of the glass and reference electrodes, the liquid junction potentials, and the activity coefficient of the hydrogen ion at constant ionic strength.

The potential of the cell was determined when known increments of standard 0.1 *M* hydrochloric acid were added to 50 ml of salt solution. The value of ε was calculated by eqn. (1a) from the known hydrogen ion concentration in the solution; ε varied slightly from day to day and was redetermined as needed.

When the pH region of interest was alkaline, a similar calibration was made with standard CO_2-free 2 *M* sodium hydroxide. Then the measured potential was related to the concentration of hydroxyl ion added:

$$E = \varepsilon' - 59.16 \log c_{OH} \quad (2)$$

where

$$\varepsilon' = \varepsilon + 59.16 \log c_H c_{OH} \quad (3)$$

Potentials were measured of standard buffer solutions containing the appropriate salt. From these measured potentials and the previously determined ε, the pH of the buffer was calculated by eqn. (1b).

Results and discussion

The procedures were applied to 4 *M* sodium nitrate at pH 11–13 and to 5.4 *M* lithium nitrate at pH 1–3. Electrodes with general purpose (GP) glass bulbs and "low sodium error" (E-2) glass bulbs were paired with reference electrodes having different liquid junctions.

Calibration curves of hydrochloric acid in the salt solutions are shown in Fig. 1. The GP and E-2 glass electrodes behaved similarly in these acidic solutions. Although the calibration in sodium nitrate obeyed eqn. (1) even at moderate acidities, in lithium nitrate the linear response was attained only below pH 3. The reason for the latter behavior is not known. The slight deviation from linearity in lithium nitrate as the pH approached 1 is probably due to the effect on the liquid junction potential of the change in concentration of the highly mobile hydrogen ions on one side of the boundary. The effect was more pronounced when the salt bridge was 10% potassium nitrate instead of 5.4 *M* lithium nitrate.

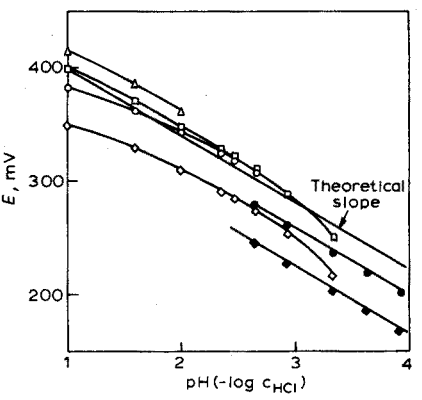

Fig. 1. Calibration curves for hydrochloric acid in salt solutions. Unshaded symbols: 5.4 *M* $LiNO_3$; shaded symbols: 4 *M* $NaNO_3$; (○) E-2, DJ (10% KNO_3); (◇) GP, DJ (10% KNO_3); (□) E-2, DJ (5.4 *M* $LiNO_3$); (△) GP, DJ (5.4 *M* $LiNO_3$). Electrode symbols are defined in Table II footnote.

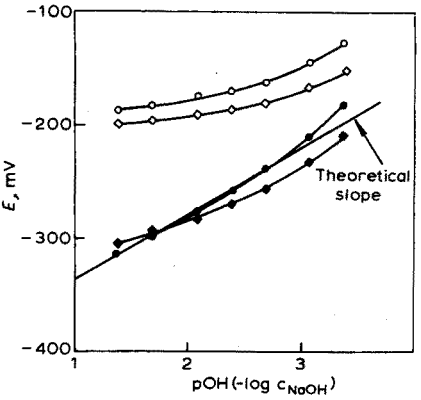

Fig. 2. Calibration curves for sodium hydroxide in salt solutions.

Figure 2 shows calibration curves in salt solutions containing sodium hydroxide. The sodium error of the GP electrode in 4 *M* sodium nitrate was evident below pOH 2.5, but the response of the E-2 electrode was still linear at the last experimental point of pOH 1.4. Response of both electrodes was poor in 5.4 *M* lithium nitrate.

Measurements in alkaline solution not only served to establish satisfactory electrode response, but also enabled estimation of the ion concentration product $c_H c_{OH}$ by eqn. (3). This product can be used to derive pOH values from pH measurements. In concentrated salt solutions the product may differ from the generally used value of $10^{-14.00}$, the thermodynamic dissociation of water at 25°. In 4 *M* sodium nitrate, the calculated value of the ion product was $10^{-14.10}$.

Table I summarizes the pH values of various salted and unsalted buffer solutions. The addition of salt lowered the pH in all cases. Unsalted buffers of different composition with the same pH value did not show the same change in pH on addition of the salt. This difference emphasizes the necessity for redetermining the pH of the buffer when salt is added to stabilize the liquid junction potential.

Table II compares typical results obtained with different pairs of electrodes in salted and unsalted buffer solutions. For the unsalted solutions, the defined pH values (in terms of hydrogen ion activity*) were used to calculate ε from E, the measured potential, by eqn. (1b). The values of ε in salted and unsalted buffers differ by 15–65 mV.

For the salted buffers, the values of pH, determined from ε and E by eqn. (1b), are constant for a given buffer composition, when measured by several different electrode combinations. This imparts a measure of confidence to the salted pH values. The assigned pH values are probably accurate to at least 0.1 pH unit.

* No attempt was made to reconcile hydrogen ion activity (the defined buffer value) with hydrogen ion concentration (the measured values). The ionic strength of the buffer solutions is <0.1. The mean activity coefficient at an ionic strength of 0.1 is typically *ca.* 0.8. Therefore, pH (activity) would be about 0.1 pH unit higher than pH (concentration). This difference is smaller than those of concern here.

TABLE I

pH VALUES OF BUFFER SOLUTIONS

Buffer	*pH at 25°*			*Remarks*
	in H_2O	*in 4 M $NaNO_3$*	*in 5.4 M $LiNO_3$*	
Coleman pH 11 buffer tablet	11.00	10.25	—	NaOH, Na_2HPO_4
0.025 *M* $NaHCO_3$, 0.025 *M* Na_2CO_3[a]	10.01	9.57	—	
Beckman pH 10 buffer solution	10.00	9.75	—	0.01 *M* Borax, adjusted with KOH
Coleman pH 10 buffer tablet	10.00	9.78	—	Borax ground glass, anhydrous Na_2CO_3
0.01 *M* Borax[a]	9.18	8.92	—	$Na_2B_4O_7 \cdot 10\ H_2O$
Coleman pH 3 buffer tablet	3.00	—	2.71	Tartaric acid, potassium hydrogenphthalate
0.010 *M* Potassium tetroxalate[a]	2.15	—	1.90	$KHC_2O_4 \cdot H_2C_2O_4 \cdot 2\ H_2O$
Beckman pH 2 buffer solution	2.00	—	1.96	0.05 *M* potassium tetroxalate, adjusted with KOH
Coleman pH 2 buffer tablet	2.00	—	1.84	Oxalic acid, tartaric acid
0.025 *M* Potassium tetroxalate[a]	1.87	—	1.63	$KHC_2O_4 \cdot H_2C_2O_4 \cdot 2\ H_2O$

[a] Standard reference material, National Bureau of Standards, Washington, D.C.[3,4].

TABLE II

TYPICAL RESULTS IN SALTED AND UNSALTED SYSTEMS WITH VARIOUS ELECTRODE PAIRS

Buffer	*Unsalted*			*Salted*			*Electrodes[b] (glass, reference)*	$pH_{u/s}$[c]
	pH[a]	*E*	*ε*	*pH*	*E*	*ε*		
				(4 *M* $NaNO_3$)				
Beckman pH 10 buffer solution	10.00	−182.7	408.9	9.76	−139.0	438.6	E-2, DJ (4 *M* $NaNO_3$)	9.26
	10.00	−159.7	431.9	9.75	−129.1	447.7	E-2, (C#1)	9.48
	10.00	−158.2	433.4	9.77	−123.9	454.0	E-2, (C#2)	9.42
0.025 *M* $NaHCO_3$, 0.025 *M* Na_2CO_3	10.01	−176.0	415.6	9.58	−127.4	439.5	E-2, DJ (10% KNO_3)	9.18
0.01 *M* Borax	9.17	−126.1	417.0	8.92	−88.0	439.5	E-2, DJ (10% KNO_3)	8.53
				(5.4 *M* $LiNO_3$)				
Beckman pH 2 buffer solution	2.00	358.6	476.9	1.97	398.8	515.6	E-2, GP (C)	1.32
	2.00	281.6	399.9	1.98	349.9	467.0	E-2, DJ (5.4 *M* $LiNO_3$)	0.85
	2.00	290.6	408.9	1.96	352.0	467.9	E-2, Calomel #1	0.96
	2.00	290.3	408.6	1.96	351.8	467.7	E-2, Calomel #2	0.96
	2.00	326.9	445.2	1.96	364.8	480.8	GP (C)	1.36

[a] Defined in terms of hydrogen ion activity.

[b] Electrodes defined as follows: E-2: Beckman Research. E-2: (C #1 and #2) Beckman Probe combination. GP (C): Thomas combination glass. DJ: Orion double-junction reference with composition of solution in outer chamber in parentheses. Calomel #1 and #2: Beckman fiber-junction calomel.

[c] See text.

The last column ($pH_{u/s}$) gives the results that were obtained by the conventional method of calibration with dilute unsalted buffer, followed by measurement of the salted solution. The results are a function not only of the type of buffer used, but also of the type of liquid junction at the reference electrode.

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SHORT COMMUNICATION

An improved synthesis of 2-perimidylammonium ion for use as a sulfate reagent

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(Received 10th October 1972)

Recently, Stephen[1] proposed a nephelometric analysis for sulfate which was based on the use of 2-perimidylammonium chloride as the precipitating agent. The 2-perimidylammonium cation [subsequently abbreviated (PDA^+)] precipitates sulfate ion, forming a white product having the formula $(PDA)_2SO_4$. The preparation of PDA salts was first described by Sachs[2] who also mentioned the extremely low solubility of the compound $(PDA)_2SO_4$. Stephen's preparation of the soluble reagent follows closely the directions given by Sachs; the reaction involves extended heating of 1,8-diaminonaphthalene with thiocyanate ion, and extraction and purification of the product in relatively low yield. This synthetic route to 2-perimidylammonium salts is inconvenient, tedious and unattractive, and is likely to discourage many investigators from experimenting with this potentially valuable reagent.

In research concerning sulfur compounds as air pollutants, the properties of $(PDA)_2SO_4$ have possibilities in several analytical applications. However, it proved necessary to develop a synthetic method for the 2-perimidylammonium cation that did not involve sulfur-containing reagents, because the purification procedure described by Stephen[1] did not easily remove every trace of sulfur-containing impurities from the product. Even trace impurities were found to spoil this reagent for analytical use.

A synthetic method for 2-perimidylammonium bromide, which meets the above criteria for reagent purity, and also simplifies the preparation of this reagent, is described below.

Experimental

Materials. Dimethoxyethane and cyanogen bromide were reagent-grade chemicals. The reagent, 1,8-diaminonaphthalene was recrystallized from hot ethanol by addition of water and refrigeration of the 1:1 ethanol–water mixture for 12–28 h at 10°. The dried recrystallized compound melted at 60–61° (lit. 60.5°).

Preparation. A solution of 4.4 g of cyanogen bromide in 10 ml of dimethoxyethane was added to a solution of 6.0 g of 1,8-diaminonaphthalene dissolved in 10 ml of dimethoxyethane in an Erlenmeyer flask. The reaction temperature was maintained at 20–25° by immersing the reaction vessel in a cold water-bath (15–20°). Within 2 min of mixing the reactants, a light tan product began to crystallize on the walls of the reaction vessel. After 1 h, the reaction mixture had

considerably lightened in color to a pale red. The crystals were collected on a filter with suction and washed with diethyl ether until the filtrate was colorless. The product is 2-perimidylammonium bromide, (PDA)Br, and the yield is 6–8 g (60–80%) when dry. The reagent can be further purified by recrystallization from boiling ethanol (45 ml of ethanol per gram of raw product) by which one obtains white crystalline needles (m.p. 265°). The solid form of the reagent is really the monohydrate, $(PDA)_2Br_2 \cdot H_2O$. [Analysis. Calculated for $C_{22}H_{22}N_6OBr_2$: 48.37% C, 4.06% H, 15.38% N; found: 48.01%C, 4.06%H, 15.37% N.]

The addition of a dilute sulfuric acid solution to a solution of 2-perimidyiammonium bromide prepared by the new synthesis resulted in the precipitation of a white solid, for which elemental analyses fit the formula, $(PDA)_2SO_4$. [Analysis. Calculated for $C_{22}H_{20}N_6O_4S$: 56.94% C, 4.34% H, 18.09% N; found: 56.45% C, 4.20% H, 18.11% N.]

Results and discussion

Cyanogen bromide is a crystalline solid which is easily stored at 20°. The reagent can be weighed in air, but it should always be handled in an efficient fume hood; great caution is advised because its vapors are toxic and extremely irritating to the eyes and nose. Although cyanogen bromide is generally commercially available (MC & B), a simple laboratory preparation has been described[3].

Cyanogen chloride could have been used in place of cyanogen bromide in the preparation of the 2-perimidylammonium salts, but it is less convenient to work with and tends to trimerize in the presence of small amounts of water[4].

Nucleophilic additions to cyanogen bromide are well known in the literature[5]. The carbon atom of cyanogen bromide is considerably more active towards nucleophilic attack than the carbon atom of the thiocyanate ion. This activity is manifested by the shorter reaction time required for the new preparation.

Another favorable factor in the cyanogen bromide reaction is that hydrogen bromide is formed as a product of the nucleophilic addition, and the desired compound precipitates out as the hydrobromide salt. The reaction of thiocyanate with 1,8-diaminonaphthalene produced 2-perimidylamine, rather than a salt; this is a serious disadvantage, because the free amine is susceptible to air oxidation and is generally less stable. The most important advantage of the cyanogen bromide reaction is that only one reaction product is formed. By contrast, thiocyanate reacts with 1,8-diaminonaphthalene to form either 2-perimidylamine or 2-thioperimidone. The result is that the yield of the desired product is reduced, and the by-product is an undesirable contaminant. The bromide salt of 2-perimidylammonium ion is slightly less soluble than the chloride salt. Nevertheless, it is easy to prepare an aqueous 0.5% solution of (PDA)Br by warming the solution (45–50°) and stirring. No attempt was made to investigate the nephelometric procedure for sulfate described by Stephen[1] but it seems probable that the bromide salt would be just as suitable as the chloride salt used earlier. It is quite important that the reagent solution (PDA)Br be stored in a dark glass bottle. Photodecomposition products form insoluble nuclei and promote crystallization of the reagent from the supersaturated solution.

The author wishes to express his appreciation for the help of Dr. Philip

W. West. The support of the National Science Foundation is also gratefully acknowledged in this project (125-20-8666).

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SHORT COMMUNICATION

Determination of antimony in galena by atomic-absorption spectrometry

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(Received 18th October 1972)

In the determination of antimony in galena, hydrochloric acid cannot be used to dissolve the sample because the chloride of antimony(III), as well as those of Ge(IV), As(III), Sn(IV) and Hg(II), are volatile at boiling temperatures[1]. If concentrated nitric acid is used, lead sulphate precipitates, and Sb, Bi, Ag, Cu, Ca and Sr are partially coprecipitated[1]. However, the lead sulphate can be easily dissolved in strong hydrochloric acid solution; it was found that no precipitate was formed even when the solution was diluted to 5% in hydrochloric acid; the solution remained clear on storage and when dilute sulphuric acid was added to an aliquot, no precipitate was formed. Accordingly, the galena could be dissolved with concentrated nitric acid, and then a hydrochloric acid solution of the sample at any desired acidity could be prepared.

Since some sulphide-forming elements (Sb, Bi, Ag) remain in solution only in strong hydrochloric acid, the extraction of antimony with methyl isobutyl ketone (MIBK) from such solutions was examined. An extraction procedure would not only concentrate the antimony, but would also minimize interferences, especially that of lead. Lead has been reported[2] to interfere in the determination of antimony by atomic-absorption spectrometry at 217.6 nm; Slavin and Sattur[3] suggested that antimony in the presence of lead should be determined at the resonance lines 206.83 or 231.15 nm.

Antimony in lead has been determined by a spectrophotometric method[4] in which the antimony is extracted into di-isopropyl ether from hydrochloric acid solution before its spectrophotometric determination with iodide. Meranger and Somers[5] determined antimony in titanium dioxide by atomic-absorption spectrometry after its extraction into MIBK from 10–14% (w/v) hydrochloric acid solution. Goto *et al.*[6] reported that antimony(III) and (V) are quantitatively extracted from hydrochloric acid solution less than 8 *M*, but that in the presence of 5 *M* sulphuric acid, they can be completely extracted from 0.5 *M* hydrochloric acid solution.

In the method proposed here, galena is dissolved in concentrated nitric acid; after removal of the excess, lead sulphate is dissolved by addition of hydrochloric acid, and the antimony is extracted into MIBK for measurement of the atomic absorbance at 217.6 nm.

Experimental

Instrumental settings. The controls on the Varian-Techtron AA4 used were

set as follows: lamp current 10 mA, slit-width 100 μm, air/acetylene 18/3.5 psig, wavelength 217.6 nm. A standard ASL hollow-cathode lamp was used as line source.

Stock antimony solutions, 500 and 50 p.p.m. Dissolve 0.2668 g of AR antimony potassium tartrate in 200 ml of distilled water. Prepare lower concentrations by dilution immediately before use.

Extraction of antimony(III) and (V) with MIBK. Antimony(III) and (V) were extracted from hydrochloric acid solutions of various concentrations into MIBK; antimony(V) was prepared by oxidation of antimony(III) with 1.0 ml of saturated bromine water. It was found that antimony(V) was more efficiently extracted into MIBK, as would be expected; the extraction of antimony(V) was quite constant in the range of 7.9–8.8 *M* hydrochloric acid solution (Fig. 1). All subsequent extractions were effected at 8.3 *M*.

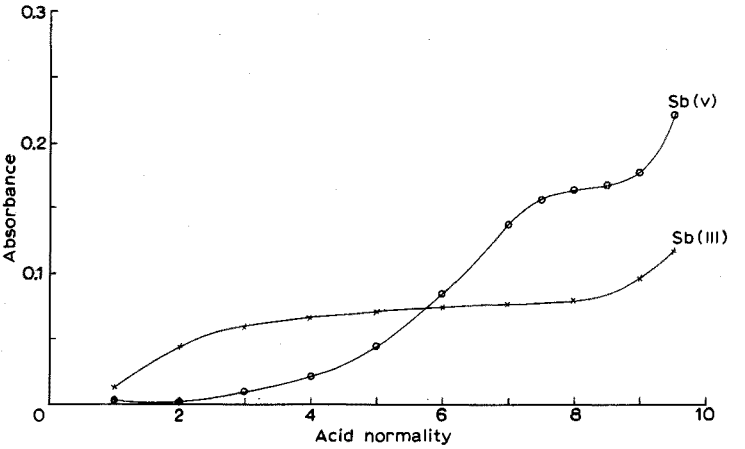

Fig. 1. Effect of acid concentration on the extraction of 250 μg of antimony with methyl isobutyl ketone (10 ml).

It was shown that 0.5 ml of saturated bromine water sufficed to oxidize 250 μg of antimony(III) with a 1-min reaction time. Variations in the extraction time from 0.5 to 4.0 min had no effect, nor had changes in the ratios of the volumes of MIBK to aqueous solution from 1:1 to 1:4.

Under the recommended conditions, 47.9% of antimony(III) and 86.0% of antimony(V) were extracted into the MIBK.

Effect of diverse ions. Metals which were likely to be associated with galena as sulphides were examined for possible interference. Known amounts of diverse ions were added to 250 μg of antimony(V). The presence of 300 mg of Pb and 30 mg of As(V), Bi, Cd, Cu(II), Hg, Mn, Sn and Zn did not interfere, but only up to 20 mg of Se(IV) and Te(IV), 9 mg of Au, Cr and Ni, 4 mg of Fe(III) and 2 mg of Ag could be tolerated. Errors of less than ±3% in absorbance were accepted as showing no interfering effect; this corresponds to about twice the standard deviation found for the determination of antimony in pure solution. Since a large concentration of lead could be tolerated, this indicated that lead was not extracted into MIBK[7] at this acid concentration; for lead interferes seriously in the determination of antimony in aqueous solution at 217.6 nm[2,3].

Calibration curve for antimony. Transfer by pipette 0.5–4 ml aliquots of standard antimony solution (50 μg ml^{-1}) into a series of 100-ml separating funnels. Add 30 ml of 8.3 *M* hydrochloric acid solution followed by 1 ml of saturated bromine water, and leave for 2 min. Extract the antimony with 10 ml of MIBK for 1 min. Separate the organic layer and measure the absorbance at 217.6 nm, using MIBK as blank.

Corrections were made for the reagent blank which gave a small absorbance value of 0.0055. The blank was prepared in the same manner without the antimony. A straight line calibration curve was obtained up to 12 p.p.m. of antimony. Above this concentration a gradual curve sloping towards the concentration axis was recorded.

Analysis of galena. Weigh the galena sample (0.1 g) accurately into a 100-ml beaker. Boil the samples with 10 ml of concentrated nitric acid until no more black particles remain. Add 1 ml of (1+1) sulphuric acid and boil the solution until white fumes are evolved. After cooling, add 30 ml of 8.3 *M* hydrochloric acid to dissolve the lead sulphate just by swirling. Then follow the same procedure as for the calibration curve.

Results and discussion

The galena samples used had not been analysed before, hence they were analysed together with a set of duplicates containing known amounts of antimony. The addition method provided a similar background matrix for the control sample. Good recoveries of added antimony were obtained (Table I).

TABLE I

RECOVERY AND DETERMINATION OF ANTIMONY IN GALENA SAMPLE

(All results are the average of duplicates)

Sample no.	*Antimony found in sample (μg)*	*Antimony added to sample (μg)*	*Total antimony found (μg)*	*Antimony recovered (μg)*	*% Error*
RG[a]	100	—	—	—	–
	100	25	125	25	0
	100	100	203	103	+3
RC 40	116	—	—	—	–
	116	50	165	49	−2
RC 50	108	—	—	—	–
	108	75	183	75	0
RC 55[a]	100	—	—	—	–
	100	100	206	106	+6
S 60	155	—	—	—	–
	155	150	300	145	−4

[a] 0.05 g of galena sample was used.

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SHORT COMMUNICATION

An indirect determination of uranium-235 by α-spectrometry

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(Received 16th October 1972)

Various methods have been reported for the determination of uranium-235 concentrations[1–3]. These include mass spectrometry, neutron activation, fission counting, delayed neutron counting, etc. Measurement of the uranium-235 concentration from a knowledge of the uranium-234 α-activity, a method which has not been reported earlier, is described in this communication.

Basic considerations

Theoretical calculations on the behaviour of uranium-234 in any diffusion separation plant based on a knowledge of separation factors have shown[4] that at low enrichment values there is a linear relationship between the ^{234}U and ^{235}U contents, when their concentrations expressed in atom per cent are plotted on a log–log scale, as shown in Fig. 1. Evidence for this relationship is provided by the

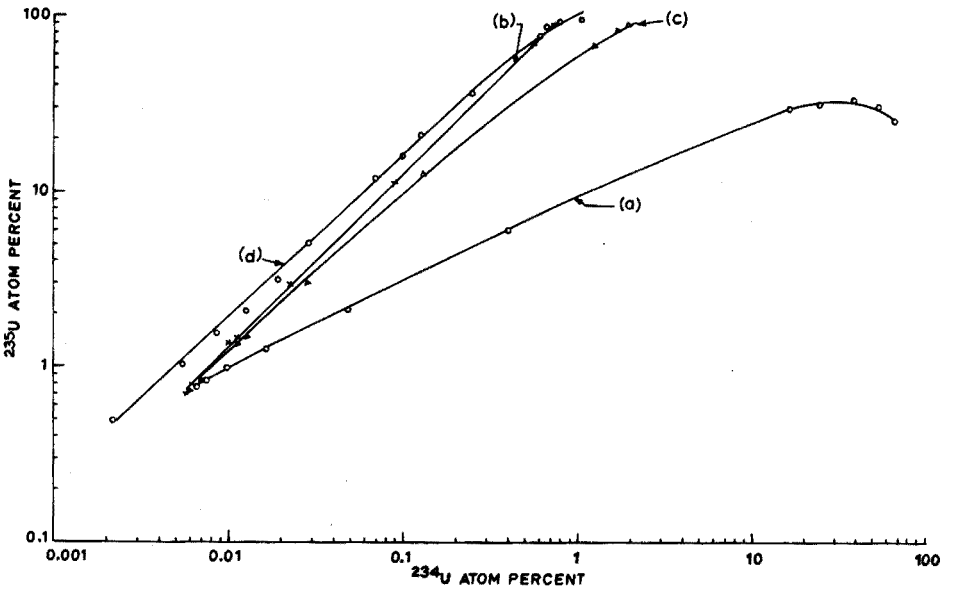

Fig. 1. Relation between ^{234}U and ^{235}U concentrations in atom per cent for different separation factors. (a) $^{235}U/^{238}U = 1.00424$; $^{234}U/^{235}U = 1.00414$; (b) $^{235}U/^{238}U = 1.0014$; $^{234}U/^{235}U = 1.000$; (c) $^{235}U/^{238}U = 1.0014$; $^{234}U/^{235}U = 1.0002$; (d) N.B.S.

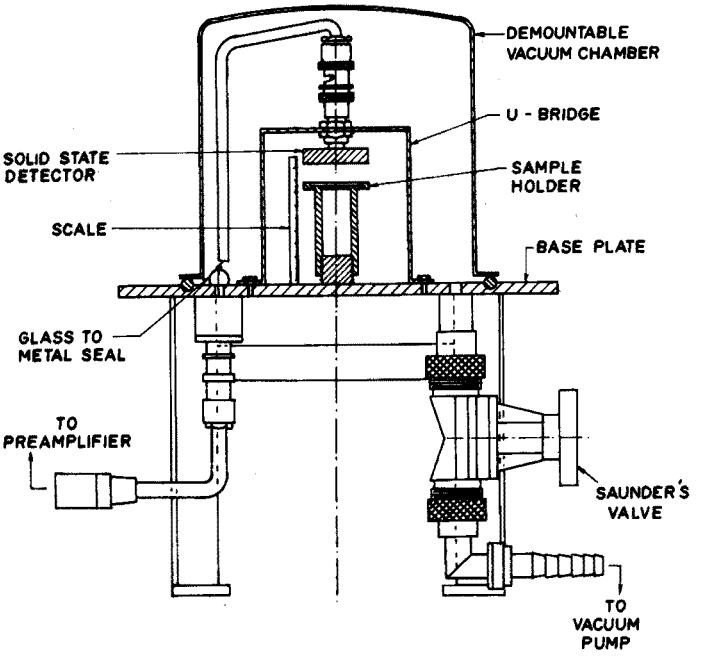

Fig. 2. Alpha chamber with solid state detector.

mass spectrometric data of enriched uranium oxides supplied by N.B.S., which are also included in Fig. 1.

It is therefore to be expected that the ^{234}U abundance expressed in terms of α-activity should also show a similar behaviour with respect to the concentration of ^{235}U in atom percent. The method therefore consists of electrodeposition of uranium, measurement of the α-activity of ^{234}U and computation of the ^{235}U content by means of a calibration curve.

Experimental

Reagents. All reagents were of recognised analytical purity.

Apparatus. α-Spectrometer. A gold-plated n-type surface barrier detector, made by the Technical Physics Division of this Research Centre, was used. It has an overall diameter of 2.5 cm, with an active area of 3.4 cm^2 and a depletion layer of 60 μm. It is mounted inside a demountable stainless steel vacuum chamber having an adjustable source mount (Fig. 2). The detector is connected to a 400-channel analyser through a charge-sensitive preamplifier and a low-noise mains amplifier, fabricated by the NISS section of the Electronics Division of this Research Centre. The system has an overall resolution of 1.4% for the 5.48-MeV α-particles from americium-241.

Electroanalyser. Controlled potential electroanalyser Model 40 (Fisher Scientific Co., U.S.A.).

Procedure. A convenient aliquot containing a known amount of uranium (*ca.* 200 μg) was transferred to a clean beaker, and evaporated to dryness on a water bath. The absorption and scattering factors influencing the α-intensity measure-

ments were controlled by keeping the sample weights within 50 μg of one another. The residue was taken up in a mixture of 1 ml of 0.01 *M* nitric acid and 2 ml of saturated ammonium oxalate solution as recommended by Cohen and Hull[5]. The solution was adjusted to pH *ca.* 3 and transferred to the electrolytic cell consisting of a copper disc cathode, and a platinum rod anode as described by Komura and Sakanoue[6]. The solution was diluted to 10 ml and electrolysed for 1 h at 6–8 V and 0.4 A. The solution was kept stirred by the platinum anode and its temperature was maintained at 80–90° during electrolysis. After electrolysis, the cell was disassembled, and the plated disc was washed with alcohol and dried. The disc was placed in the sample holder, below the detector, maintaining a 1-mm distance between the sample and detector. The chamber was then evacuated by a rotary pump for 1 h, and spectra were taken.

Results and discussion

Figure 3 shows a plot of the measured ^{234}U α-activity *vs.* ^{235}U concentration in two unknown samples E_1 and E_2; N.B.S. enriched uranium oxides and natural uranium were used as calibration points on this graph, which proves the basic assumption in the work. Analytical results for the two uranium samples of unknown enrichments are presented in Table I, and compared with values obtained by other

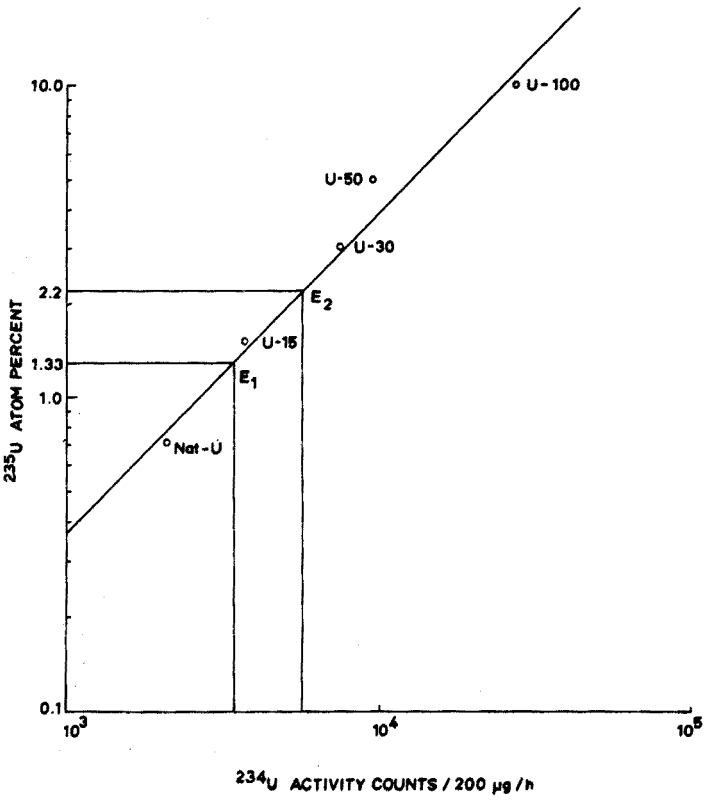

Fig. 3. Plot of measured α-activity *vs.* ^{235}U concentration.

TABLE I

COMPARISON OF THE RESULTS FOR ^{235}U BY α-SPECTROMETRY WITH THOSE BY OTHER METHODS

Sample no.	*^{235}U concentration (%)*		
	α-Spectrometry	*Mass spectrometry*	*Delayed neutron*
E_1	1.33	1.37±0.01	1.37
	1.30		
	1.32		
	1.29		
	Mean 1.31±0.02		
E_2	2.20	2.16±0.01	2.02
	2.30		
	2.34		
	2.28		
	Mean 2.28±0.08		

methods. The agreement between the values obtained by the present method and those by other methods is fairly satisfactory. The procedure is particularly suitable for the determination of uranium-235 concentration in process wastes.

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SHORT COMMUNICATION

Potentiometric studies with thallium(I)–heteropoly acid salt–epoxy resin membranes

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(Received 9th October 1972)

The larger monovalent cations form insoluble salts with the heteropoly acids. In previous communications[1,2] a cesium-sensitive electrode in which a cesium 12-molybdophosphate precipitate supported in an inert silicone rubber was used as active membrane, was described.

In the present communication the thallium(I) salts of molybdophosphoric and tungstophosphoric acids in epoxy resin supports were used to construct electrodes sensitive to thallium(I) ions.

Experimental

Chemicals. All chemicals used were of A.R. grade. The thallium heteropoly acid salts were prepared by precipitation of the salts with 12-molybdophosphoric and 12-tungstophosphoric acids from solutions containing excess of thallium nitrate. The precipitates were separated by centrifugation and dried at 260° for 24 h.

Apparatus and preparation of the membranes. The membranes were prepared by mixing equal masses (about 0.5 g) of the thallium heteropoly acid salt with Araldite. The mixture was spread out thinly (about 0.5 mm) on a piece of filter paper and left in the air to cure. After the membrane had dried and hardened a circular disc of 2.5 cm diameter was cut. The prepared membrane was then left in a 0.1 *M* thallium nitrate solution for equilibration for 6 days.

The membranes were mounted as described previously[2]. The reference solution in both cases was a 0.1 *M* thallium(I) nitrate solution. For use in potentiometric titrations, the membranes were fixed to one end of glass tubes.

Two Beckman fibre-junction saturated calomel electrodes were inserted into the solutions and all potential measurements were made with a Beckman Research model pH-meter. Although the filling solutions of the calomel electrodes were not changed, no problems arose from possible leakage of potassium chloride into thallium nitrate solutions. When not in use the calomel electrodes were stored in water. In the cell, when not in use, the test solution was replaced by a 0.1 *M* thallium nitrate solutions.

Study of direct potentiometry

Response of electrodes to thallium ions. The response of the membrane electrodes in pure thallium nitrate solutions is shown in Fig. 1. Activity coefficients were

calculated from the extended Debye-Hückel equation, $\log f = -0.51\, z^2 \mu^{\frac{1}{2}}(1+\mu^{\frac{1}{2}})^{-1}$.
The slopes of the calibration curves at 25° were as follows: for the Tl–molybdo-
calculated from the extended Debye–Hückel equation, $\log f = -0.51\, z^2 \mu^{\frac{1}{2}}(1+\mu^{\frac{1}{2}})^{-1}$.
brane TlWP 41 mV/pTl.

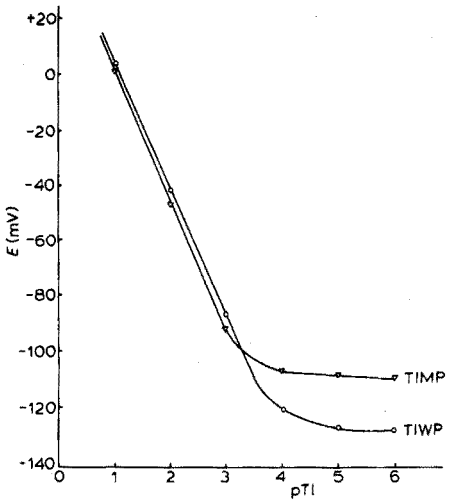


Fig. 1. Calibration curves for thallium electrodes at 25°.

The values are much smaller than the expected Nernstian value but both electrodes were suitable to determine thallium concentrations as low as 10^{-4} *M*. In general with ion-exchange membranes, deviations at high solution activities are caused by co-ion transference and at lower activities by competing hydrogen or hydroxyl ions[3]. Deviation from Nernstian behaviour may be attributed to incomplete permselectivity of the membrane[4], a factor which Helfferich[5] covered by an integral term in the function for membrane potential to account for the co-ion flux.

The prepared membranes lasted for about 5 months.

Effect of pH on the electrode potential. The pH dependence of the electrodes was studied for the pH range 1–6. The pH of the test solutions was adjusted by addition of nitric acid; higher pH values were not studied as heteropoly compounds begin to depolymerize and dissolve above pH 6. With both 10^{-2} and 10^{-4} *M* thallium(I) solutions, and for both electrodes, the potential increased with pH in the range 1–4, and then remained constant. All subsequent measurements were done at pH values between 4.5 and 5.5.

Effect of temperature on electrode response. The temperature coefficients of both the membrane electrodes were determined by measuring the cell potential in 10^{-2} and 10^{-4} *M* thallium(I) nitrate solutions between 0 and 40°. A rectilinear relationship was obtained; the observed temperature coefficient for both the measuring systems was 0.27 mV/degree.

Dynamic response characteristics. The response characteristics were evaluated by exposing the membranes to a rapid change in thallium nitrate concentration and recording the resulting e.m.f. *vs.* time function. Although response times were longer than with the commercially available electrodes a steady constant potential was

reached with both electrodes after 2 min. It has recently been mentioned[4] that perhaps too much emphasis has been placed on response times of electrodes. For direct potentiometric determination of activity response, times of some minutes are quite tolerable.

Effect of cations on electrode response. A systematic examination of the extent of interference of lithium, sodium, potassium, rubidium, cesium, ammonium, magnesium, calcium, barium and strontium ions on the response of the electrodes to thallium was undertaken. The cell potentials were measured in solutions that were 10^{-4} *M* in thallium ion and in solutions that were 10^{-4} *M* in thallium ion as well as 10^{-2} *M* in the foreign ion. Selectivity ratios K_{TlM} were calculated. K_{TlM} is defined by

$$E = E_0 + \frac{RT}{F} \ln (A_{Tl^+} + K_{TlM}(A_{Mm^+})^{1/m})$$

The values for the two electrodes are shown in Table I. The values of K_{TlM} for the TlMP-membrane electrode are much smaller than those for the TlWP-membrane electrode under comparable conditions. The values also show that the electrodes are not highly selective for thallium in presence of monovalent cations, but the electrodes could be used for the determination of thallium ion in solutions in which the monovalent cations are absent.

TABLE I

K_{TlM} VALUES FOR TlMP AND TlWP MEMBRANE ELECTRODES

Interfering cation	*TlMP*	*TlWP*
Li^+	10^{-2}	0.28
Na^+	10^{-2}	0.53
K^+	10^{-2}	0.66
Rb^+	0.065	0.80
Cs^+	10^{-2}	0.29
NH_4^+	10^{-2}	0.57
Ag^+	—	0.65
Mg^{2+}	10^{-3}	$2 \cdot 10^{-2}$
Ca^{2+}	10^{-3}	$2 \cdot 10^{-2}$
Sr^{2+}	10^{-3}	$1.3 \cdot 10^{-2}$
Ba^{2+}	10^{-3}	$1.2 \cdot 10^{-2}$

The response of the electrodes in non-aqueous media. The response of the membrane electrodes in thallium solutions containing various concentrations of methanol, ethanol, *n*-propanol and acetone was studied. In Table II, the effects of the various solvents on the slopes of the calibration curves are summarized. The results show no exceptional change in behaviour of the electrodes in these solvents. Response times of the electrodes were of the same order as those in water. The electrodes should be usable in solutions having a non-aqueous content of up to 25%.

Potentiometric titrations

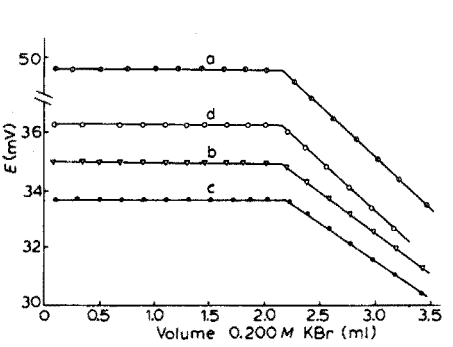
A series of precipitation titrations with the thallium tungstophosphate membrane electrode as indicator electrode were done. Thallium was precipitated with

TABLE II

EFFECT OF SOLVENT COMPOSITION ON CALIBRATION CURVES

Solvent composition (v/v %)	*Slope (mV/pTl)*	
	TlWP	*TlMP*
Water	40.0	36.0
10% Methanol	39.0	38.0
25% Methanol	40.0	39.0
10% Ethanol	40.7	37.0
25% Ethanol	42.0	39.0
10% *n*-Propanol	42.0	36.3
25% *n*-Propanol	44.7	40.0
10% Acetone	40.3	37.0
25% Acetone	46.3	38.0

potassium bromide, potassium chromate and sodium tetraphenylborate. The results are shown in Figs. 2–4. It can be seen that the curves do not have the classical form. This is probably due to the fact that the membranes are also sensitive to changes in activities of other monovalent cations; during titration the primary



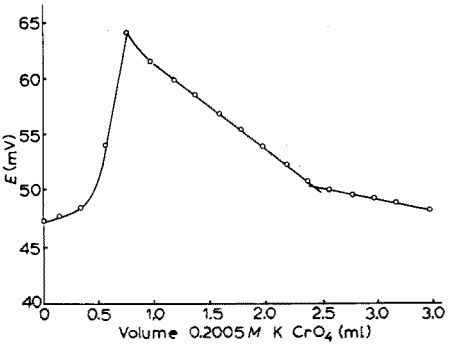


Fig. 2. The titration of 100.9 mg of thallium(I) with a 0.2008 *M* KBr solution. In 55 ml of water (a); in 25% (v/v) ethanolic solution (b); in presence of 19.6 mg of potassium (c); in presence of 11.5 mg of sodium (d).

Fig. 3. The titration of 100.9 mg of thallium(I) in 55 ml of water with a 0.2005 *M* K_2CrO_4 solution. End-point corresponds to formation of $TlKCrO_4$.

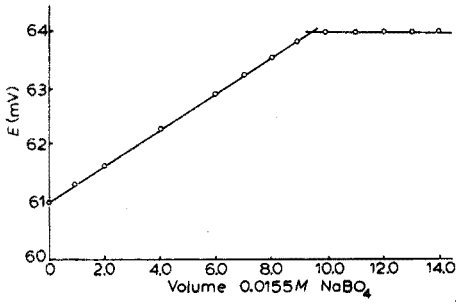


Fig. 4. The titration of 30.27 mg of thallium(I) in 50 ml of water with a 0.0155 *M* sodium tetraphenylborate solution.

cation is effectively removed from solution, but is replaced by another monovalent cation to which the electrode responds to a certain extent. However, the stoichiometric end-points of all the titrations were readily detectable, and the titrations are viable provided that an expanded-scale meter is available. When thallium(I) was titrated in the presence of silver(I) with bromide solution, the end-point corresponded to the total thallium–silver content, but the potential break was very small.

This work forms part of a doctoral thesis to be submitted by A.J.B. to the University of Stellenbosch and is published with the permission of his Promotor.

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SHORT COMMUNICATION

The spectrophotometric determination of palladium with 4-[(5-chloro-2-pyridyl)azo]-1,3-diaminobenzene

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(Received 26th October 1972)

Although many reagents have been employed for the spectrophotometric determination of palladium[1], the selectivity is rarely satisfactory. Recent reagents are arsenazo III[2], palladiazo[3] and 3-nitroso-2,6-pyridinediol[4]. In the present work, 4-[(5-chloro-2-pyridyl)azo]-1,3-diaminobenzene (5-Cl-PADAB) is used to determine microgram amounts of palladium. The synthesis of the reagent and its application to the analysis of trace amounts of cobalt has been previously reported[5]. The reagent also reacts with palladium over a wide pH range yielding a highly coloured complex.

In strongly acidic solution, only the cobalt–5-Cl-PADAB complex is stable but in moderately acidic media (pH 2–2.4 *M* hydrochloric acid), the cobalt and palladium complex are both stable. Moreover, the cobalt does not react with the reagent under controlled conditions. In the case of cobalt, the complex formed at pH 3.5–11.5 could be changed into a highly stable species which possessed remarkably increased absorptivity by the addition of mineral acid[5]. Therefore, with 5-Cl-PADAB a highly sensitive and selective determination of microgram amounts of palladium is possible under the optimal conditions established in this study.

Reagents and apparatus

5-Cl-PADAB solution. An ethanolic 0.08% (w/v) solution was prepared from the pure material. The solution was stable for several months if stored in amber bottle.

Standard palladium(II) solution. A stock solution was prepared by dissolving pure palladium chloride in water, adding 5 ml of concentrated hydrochloric acid and diluting to 250 ml with distilled water. This solution was standardized by the oxine gravimetric method and then diluted to give a standard solution containing 10 μg Pd ml^{-1}.

Buffer solutions. 0.2 *M* hydrochloric acid–0.2 *M* potassium chloride, 0.2 *M* acetic acid–0.2 *M* sodium acetate, and 0.2 *M* ammonium chloride–0.1 *M* ammonia mixtures were used for pH adjustment.

All other reagents used, including the platinum metal standard solutions and (1 + 1) hydrochloric acid solution, were made from high-purity materials or purified reagents, and all solutions were prepared with redistilled water.

Apparatus. Absorbance curves were measured with a Model EPS-3T Hitachi recording spectrophotometer with 1-cm cells; absorbances were measured with a

Model 139 Hitachi spectrophotometer with 1-cm cells. A Hitachi M5 type pH meter was used.

Absorbance curves

The absorbance curves of the reagent and its palladium complex in 1.2 *M* hydrochloric acid solution are shown in Fig. 1. The curves show two absorption maxima at 537 and 572 nm, respectively. Subsequent studies were made at 572 nm. The molar absorptivity of the palladium complex can be increased by addition of water-miscible organic solvents, *e.g.* ethanol; the maximal absorbance peak is then shifted to longer wavelength. The absorbance curves of the palladium complex in ethanolic aqueous solution are shown in Fig. 2.

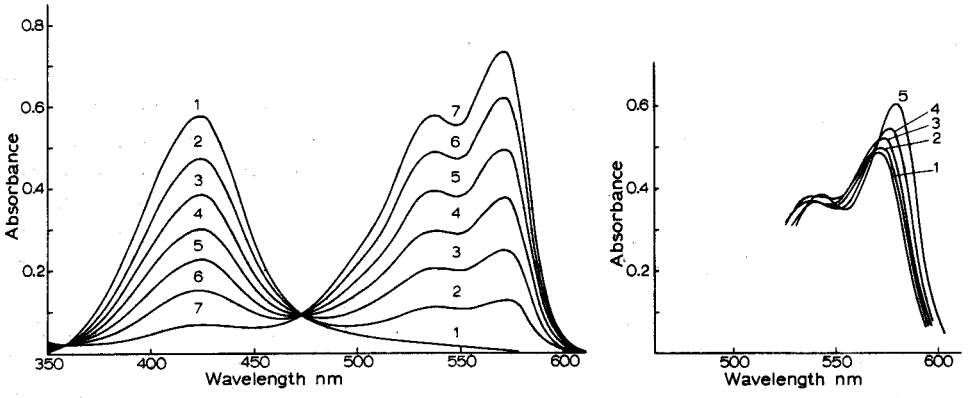

Fig. 1. Absorbance curves of 5-Cl-PADAB and its palladium complex in aqueous solution (1.2 *M* HCl). (1) 5-Cl-PADAB $1.2 \cdot 10^{-5}$ *M*; (2) 5.0 μg Pd, (3) 10.0 μg Pd, (4) 15.0 μg Pd, (5) 20.0 μg Pd, (6) 25.0 μg Pd, (7) 30.0 μg Pd, in 25 ml of solution.

Fig. 2. Absorbance curves of the palladium complex in ethanolic aqueous solution. 20 μg Pd/25 ml. Ethanol (v/v)%: (1) 4; (2) 10; (3) 20; (4) 40; (5) 80.

Optimal conditions

The effect of pH and acid concentration was studied with the results shown in Fig. 3. No change in absorbance was observed over the range pH 2 to 2.4 *M* hydrochloric acid solution. Subsequent studies were carried out with 5 ml of (1+1) hydrochloric acid per 25 ml (1.2 *M*).

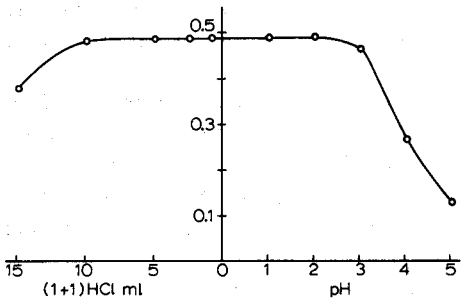

Fig. 3. Effect of pH. 20 μg Pd/25 ml.

The absorbances of a series of solutions containing 25 μg of palladium and 0.1–1.0 ml of 0.08% dye solution were measured. It was found that 0.5 ml of this dye solution sufficed to complex 25 μg of palladium; with higher reagent concentrations, the absorbance was essentially constant.

The minimum time for complete colour development of the complex was found to be 1–2 min at room temperature. The absorbance was then stable for at least 24 h.

Recommended procedure for the determination of palladium

To a 25-ml volumetric flask, transfer a suitable aliquot of acidic sample solution containing up to 25 μg of palladium, and add 0.5 ml of ethanolic 0.08% reagent solution. Then add 5 ml of (1+1) hydrochloric acid solution, dilute to volume and mix well. Measure the absorbance of the palladium complex produced at 572 nm against a reagent blank. Obtain the concentration of palladium from a standard calibration curve obtained under identical condition.

Results and discussion

Beer's law and sensitivity. The calibration graph proved to be linear over the range up to 1 p.p.m. of palladium. The effective molar absorptivities for the palladium complex were $6.5 \cdot 10^4$ l mol^{-1} cm^{-1} at 572 nm in aqueous solution and $8.06 \cdot 10^4$ l mol^{-1} cm^{-1} at 578 nm in 80% ethanolic aqueous solution. The sensitivity of the reaction as calculated from Beer' law was 0.00012 μg Pd cm^{-2} at 572 nm for $\log I_0/I = 0.001$.

Effect of foreign ions

Aluminum, beryllium, bismuth, cadmium, rare earths, magnesium, calcium, manganese, lead, thorium, titanium, uranium, zinc or zirconium in 5-mg amounts, vanadium(V), tungsten, nickel or chromium(III) in 1–2 mg amounts, and 0.5 mg of cobalt did not interfere. Five-fold amounts of iron(III) and two-fold amounts of copper did not interfere. Interference was caused only by chromium(VI). Common anions such as chloride, nitrate, acetate, sulfate and perchlorate did not interfere. Strong oxidizing agents interfered. The platinum metals did not react with reagent at room temperature; the tolerance limits for platinum metals are shown in Table I.

TABLE I

TOLERANCE FOR PLATINUM METALS

(Pd concentration, 20 μg/25 ml)

Metal	*Tolerance (mg/25 ml)*	*Metal*	*Tolerance (mg/25 ml)*
Pt(VI)	5.0	Os(VIII)	10.0
Rh(III)	5.0	Ru(III)	0.1
Ir(IV)	1.0	Au(III)	0.3

Synthetic samples of platinum metals which contained known amounts of palladium were analyzed, to assess the validity of the recommended procedure. Typical results are shown in Table II. Accordingly, 5-Cl-PADAB should be applicable for

TABLE II

DETERMINATION OF PALLADIUM IN SYNTHETIC MIXTURES

(Pd concentration, 20 μg/25 ml)

Other platinum metals (mg)	*Palladium found (μg)*
Pt(1), Rh(1), Ir(0.1), Au(0.1)	20.0
Pt(2), Rh(2), Ir(0.5), Au(0.1)	20.2
Pt(5), Rh(2.5), Ir(0.5)	20.4
Os(10), Pt(0.2), Rh(0.2)	21.0

the spectrophotometric determination of palladium in other platinum metals without separation. When larger amounts of these metals are present, the appropriate metals could be added to the reference solution.

Nature of complex

The empirical formula of the complex was studied by the continuous variations and mole ratio methods. The Job curves obtained indicated the formation of a complex of palladium in which the metal:ligand ratio is 1:1 (Fig. 4). The mole ratio method confirmed this conclusion.

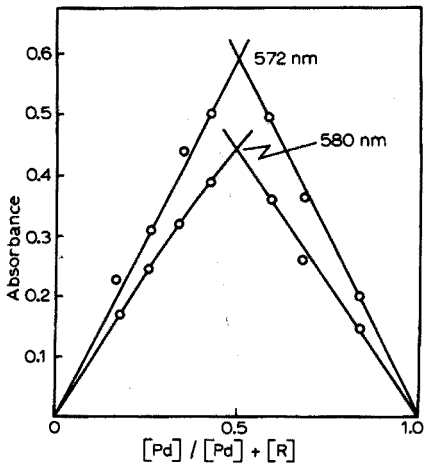

Fig. 4. Composition of palladium complex. $[Pd]+[5\text{-Cl-PADAB}]=1.9\cdot10^{-5}$ *M*, 1.2 *M* HCl solution.

The equilibrium constant of the reaction between palladium and 5-Cl-PADAB was calculated by Harvey and Manning's methods, on the assumption that complex formation proceeds according to the following scheme: $Pd^{2+}+R\rightleftarrows[PdR]^{2+}$, where R is the reagent. The K_{equil} was found to be $5.3\cdot10^{7}$ at 25° (1.2 *M* HCl).

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SHORT COMMUNICATION

A sensitive fluorimetric detection of carbohydrates with ethylenediamine sulfate

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(Received 10th October 1972)

During analytical studies of carbohydrates by means of fluorescence reactions with various amines, ethylenediamine sulfate was found to produce a stable fluorescence with excitation and emission maxima at 394 and 470 nm, respectively (Fig. 1), when heated with an aqueous solution of D-glucose. Since this reaction was specific for aldehydes and aliphatic polyhydroxyl compounds, it is considered to be suitable for carbohydrate analysis.

The use of this reaction for the paper-chromatographic detection of carbohydrates provided a sensitive method capable of detecting microgram quantities of carbohydrates. The fluorescent materials were formed simply by spraying with an aqueous 10% solution of ethylenediamine sulfate, followed by heating in an oven. The fluorescence was conveniently visualized as violet spots by irradiation with a mercury lamp which emitted the 365-nm light most abundantly.

Table I shows that the minimal detectable quantities of aldoses and ketoses were 0.1–0.5 μg, with a few exceptions. D-Glyceraldehyde was less sensitive (5 μg) and D-mannose, which was the most sensitive to this reagent, was detected to the minimum amount of 0.05 μg. A comparative study with other common reagents indicated that the minimal detection range of D-glucose with this reagent (0.1 μg) was

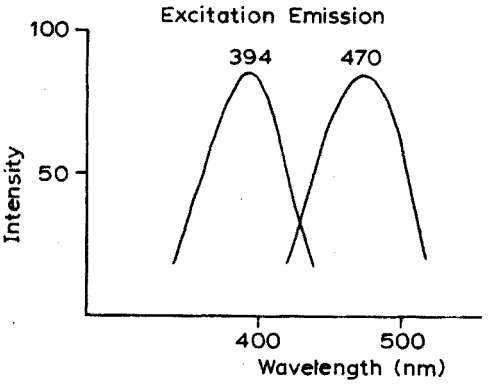

Fig. 1. Excitation and emission spectra of the fluorescent material formed in the reaction of D-glucose (10^{-4} *M*) with ethylenediamine sulfate (10^{-3} *M*) for 2 h at 90°.

TABLE I

MINIMAL DETECTABLE QUANTITIES OF CARBOHYDRATES[a]

Carbohydrate	*Limit of detection (μg)*	*Carbohydrate*	*Limit of detection (μg)*
D-Glyceraldehyde	5	D-Glucuronic acid	1
D-Erythrose	0.5	D-Glucuronolactone	5
D-Arabinose	0.1	D-Gluconic acid	50
D-Ribose	0.1	Glycerol	100
D-Xylose	0.1	D-Erythritol	100
D-Galactose	0.1–0.5	D-Dulcitol	>100
D-Glucose	0.1	D-Mannitol	>100
D-Mannose	0.05	D-Sorbitol	100
D-Fructose	0.1	Maltose	1
L-Fucose	0.5	Cellobiose	0.5
L-Rhamnose	0.1	Lactose	0.1
2-Deoxy-D-ribose	1	Gentiobiose	0.5
2-Deoxy-D-glucose	0.5	Gentiotriose	1
D-Galactosamine HCl	1–5	Sucrose	1–5
D-Glucosamine HCl	5	Raffinose	10

[a] Carbohydrates were chromatographed on Whatman No. 1 filter paper with 6:4:3 *n*-butanol–pyridine–water for 10 h. An aqueous 10% solution of ethylenediamine sulfate was sprayed, followed by heating at 120–130° in an oven for 10 min.

somewhat lower than that with aniline hydrogen phthalate[1] (1 μg), and was of the same order as that with alkaline silver nitrate[2]. Deoxy sugars were as sensitive as aldoses and ketoses to this reagent, whereas uronic acids and amino sugars seemed to be less sensitive (1–5 μg). The minimal detectable moles of reducing oligosaccharides were approximately equivalent to those of the component monosaccharides. The intensity of fluorescence obtained from non-reducing oligosaccharides was rather weak. Aldonic acids, as well as alcoholic sugars which do not contain carbonyl groups, were far less sensitive.

When the free base of the amine was used instead of the sulfate salt, the excitation and emission maxima shifted slightly, to 415 and 480 nm, respectively, and carbohydrates were detected rather weakly as blue-violet spots. The use of the hydrochloride salt was disadvantageous, because of the quenching effect exerted by the chloride ion. *n*-Pentyl- and *n*-hexylamines also gave fluorescence, but the intensity was so weak that the minimal detectable amounts of D-glucose were 10 and 1 μg, respectively.

There have been recent papers[3,4] on the interaction of ethylenediamine with some carbohydrates to form 1:1 ethylenediamine–carbohydrate complexes. The paper-chromatographic examination, however, indicated that the fluorescent material formed in the reaction of ethylenediamine sulfate with D-glucose stayed at the starting line, and was negative to alkaline silver nitrate[2]. In contrast, the complex, which had an absorption maximum at 330 nm, was detected as a mobile spot between that of D-glucose and the starting line. Consequently, the fluorescence was considered to be due to a compound different from the complex described in the literature[3,4].

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ANNOUNCEMENTS

Environmental Chemistry: Know-How and Chemicals in 1973–1978

October 1973, Brussels

A symposium on environmental chemistry will be organized by the International Business Contact Club in Brussels on October 24–25, 1973. Experts from 15 countries will present lectures on the 1973–1978 markets. Specialists in market research, research and development, sales and production from many different countries will attend. Further information can be obtained from: i.b./c.c. Administration, Nieuwelaan 65, B-1820 Strombeek, Belgium.

Short Summer Courses in X-Ray Spectrometry and X-Ray Powder Diffraction

A two-week short course in modern X-ray spectrometry will be offered at the State University of New York at Albany from June 4 to June 15, 1973. The course will be instructional and will develop the basic theory and techniques starting from elementary principles. No previous knowledge or experience is 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 $ 300.00 or for the entire two-week session at a registration fee of $ 550.00.

A one-week short course in modern X-ray powder diffractometry will be offered at the same University from June 18 to June 22, 1973. 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 bases 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 $ 300.00 payable in advance.

For further information and to register for these courses please communicate with: Professor Henry Chessin, State University of New York at Albany, Department of Physics, 1400 Washington Avenue, Albany, N.Y. 12222, U.S.A.

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BOOK REVIEWS

Treatise on Analytical Chemistry, Edité par I. M. Kolthoff et P. J. Elving, Part I, Vol. 10, Wiley–Interscience, New York, xvii + 594 pp., prix £9.85.

Ce volume comprend la fin de la section E intitulée: application des mesures, la section F: principes de l'instrumentation et la section G: préparation pour la recherche analytique et utilisation.

La section E est consacrée à l'analyse des gaz par conductibilité thermique. C'est l'occasion pour les auteurs d'en présenter les bases théoriques, les limites de sensibilité et la sélectivité tant instrumentale que chimique. Vient ensuite la partie pratique avec la description approfondie de l'appareillage. Le chapitre suivant est consacré à ce problème très important de l'analyse des gaz dans les métaux par les méthodes physiques et chimiques.

La section F débute par des généralités sur l'instrumentation en chimie analytique, puis un chapitre important a trait à l'automation.

Mais la partie la plus originale, à notre avis, est dans la section G: elle comprend deux chapitres qu'on rencontre très rarement dans les traités de chimie analytique. Le premier est une étude statistique qui permet, avec un minimum de résultats expérimentaux de tirer le maximum de renseignements. Le second chapitre est intitulé: Bibliographie (Literature) de chimie analytique. Il est le bienvenu. On sait en effet l'importance que revêt l'étude de la bibliographie qui précède toute recherche et toute mise au point. Il est donc très utile pour le chimiste d'avoir une liste des ouvrages à consulter. Lorsqu'on examine celle donnée à la page 6500 intitulée: Principaux périodiques de chimie analytique—il y en a 33—on se rend compte du nombre de disciplines qui font appel à la chimie analytique, qui s'étendent de la physique à la médicine.

On y lit entre autre que Analytica Chimica Acta est le journal officiel de la Section analytique de l'Union Internationale de chimie pure et appliquée.

Mais ce chapitre est mieux que cela car il donne des directives sur la façon de procéder. Il y a les sources primaires et les sources secondaires, les dictionnaires et les traités, les monographes et les brevets... Vraiment, c'est fort bien présenté et pour les chimistes comme pour les étudiants, c'est une aide précieuse et remarquable.

Denys Monnier (Genève)

P. Diehl, H. Kellerhals and E. Lustig, *Computer Assistance in the Analysis of High-Resolution NMR Spectra*, NMR—Basic Principles and Progress, Edited by P. Diehl, E. Fluck and R. Kosfeld, Vol. 6, Springer-Verlag, Berlin, 1972, 96 pp., price DM 48,— ($15.00).

Computer-assisted analysis of high-resolution n.m.r. spectra is now a routine technique which is available to the non-specialist; this volume provides the basic theory and outlines the various procedures currently available.

The opening chapter deals succinctly with the quantum mechanical theory of indirect spin coupling using the matrix representation of operators as this is particularly suited to computer methods. The following chapters outline the methods employed in analytical programmes from several laboratories, including auxiliary routines designed to assist in spectral assignment. The relative merits of the various approaches are discussed, and there are very useful sections devoted to the estimation of errors and the uniqueness of the solution. Readers with limited mathematical background should find the appendices on matrix diagonalisation, least-squares fitting, error statistics, and density matrix concepts, very useful. Other topics briefly discussed are the analysis of the spectra of partly orientated molecules, double resonance effects, and the analysis of spectra involving site-exchange.

The volume is very brief (only *ca.* 80 pages of text) therefore the coverage is necessarily sketchy in places. Thus the section on the modified Bloch equations is very scant and there is little discussion of the relevant computer methods; indeed the reader is given the erroneous impression that these programmes are limited to the simple two-spin case. However, for the most part this book provides an excellent summary of the theory and application of computer-assisted spectral analysis.

W. B. Jennings (Birmingham)

G. J. Moody and J. D. R. Thomas, *Selective Ion-Sensitive Electrodes*, Merrow Publishing Co. Ltd., Watford, England, 1971, vii + 140 pp., price £2.25 ($6.90).

This book gives an elementary introduction to selective ion-sensitive electrodes and is thus a valuable complement to existing review articles on the subject. It commences with a brief discussion of membrane potentials which gives the reader little idea, however, about the complexity of the topic. The next chapter deals with the concept of selectivity constants, which the authors are careful to point out are not true constants. Those methods used to determine selectivity constants are reviewed and a critical comparison of the results obtained by the different methods is given.

The following description of glass electrodes includes a short discussion of diffusion and phase boundary potentials, and of the effect of the structure of the glass on the selectivity. The chapter is devoted primarily to electrodes which follow ions other than hydrogen ions, despite the fact that the pH electrode is by far the most widely used of all ion-selective electrodes. In recent years, it has, however, become rather usual to omit this electrode from the category of selective ion-sensitive electrodes, presumably because it is no longer new.

The experimental procedures involved in the determination of activities and of free and total concentrations with ion-selective electrodes are dealt with in a later chapter. Known addition and known subtraction techniques (the latter formerly known as titration) for the determination of total concentrations are described in some detail. It would, however, have been helpful if the authors had identified these methods with the Gran method, which, apart from correcting for dilution, enables the attainment of increased precision through the inclusion of several titration points. No mention is, moreover, made, in the brief description of measur-

ing devices, of the fact that any voltmeter in conjunction with a high quality operational amplifier can provide an alternative to specific ion meters.

The three subsequent chapters deal in a clear and concise manner with different types of electrodes, *i.e.* homogeneous and heterogeneous solid-state electrodes and ion-exchange electrodes, the last category including the valinomycin electrode. Non-commercial electrodes are also described. The final one-page chapter on miscellaneous ion analysis could perhaps have been elaborated on or else omitted.

This book ought to be useful to analytical chemists with limited experience with selective ion-sensitive electrodes. The reader is provided with a brief introduction to the theory, an adequate coverage of current practical applications and an extensive compilation of data on electrode properties. This last feature means that the book can be used as a handbook even by more experienced workers in the field.

Daniel Jagner (Göteborg)

J. Tölgyessy, T. Braun and M. Kyrš, *Isotope Dilution Analysis*, International Series of Monographs in Analytical Chemistry, Vol. 49, Pergamon Press, Oxford, 1972, 194 pp., price £ 3.50.

The well known authors of this monograph devoted to isotope dilution analysis have packed it with a wealth of information. The principles of the whole range of isotope dilution methods are covered, including direct isotope dilution, reverse isotope dilution, derivative dilution, substoichiometric isotope dilution, and many of their variants. Critical appraisal of these methods with respect to their sensitivity, accuracy and range of application, helps to bring the real value of isotope dilution analysis into perspective. An interesting feature is the authors' scheme for systematically classifying isotope dilution methods. Because of the non-systematic nomenclature that surrounds this subject, it is often difficult to appreciate the principles a method uses or where it fits into the general framework simply from its name. The new scheme helps to clarify the position, although one may not agree that all the techniques encompassed by the scheme rely truly on isotope dilution.

Separation and quantification methods, and the importance of reagent and tracer purity, are discussed extensively in a chapter on experimental techniques.

The styles of the chapters on inorganic and organic applications are markedly different. The former plunges abruptly and without preamble into a large table of methods for the determination of 58 elements, followed by 5 specific methods, no reason being given for their particular selection. The latter is considerably more readable, being divided into sections on methods for different groups of organic compounds.

The book is completed with a brief chapter on stable isotope dilution analysis, and one on special uses of isotopes, such as studies of reaction rates, equilibrium phenomena, flow rates and the determination of radioactive contaminants.

The standard of this book is extremely variable. Starting excellently, it unfortunately fails to maintain this standard throughout. I would have welcomed more information on the determination of radioactivity, improved presentation of

the inorganic applications, and an expansion of the chapter on stable isotope dilution mass spectrometry.

J. W. McMillan (Harwell)

Solvent Extraction Reviews, Vol. 1, Edited by Y. Marcus, Marcel Dekker, Inc., New York, 1971, ix+256 pp., £ 7.80 or $ 19.50.

This is the first of a new series of critical reviews designed to meet the needs of those interested in liquid–liquid extraction, a field in which there have recently been extensive developments both in the academic aspects of the subject but even more so in diverse industrial and technological applications. The contents include accounts of the interactions of acidic organophosphorus extractants in the organic phase (63 pp.), the kinetics of metal extraction by organophosphorus acids (30 pp.), the industrial extraction of phosphoric acid (12 pp.), the solvent extraction of protactinium (46 pp.) and polonium (34 pp.), mixing equipment in liquid–liquid extraction (30 pp.), and mass transfer problems in liquid extraction (20 pp.). Each article has been written in depth with excellent bibliographies by specialists and for specialists.

The author and subject indexes have been carefully compiled and this series shows every sign of making a significant contribution to the literature of the subject. It will surely be a "must" for every researcher and chemist practising in this growing field.

H. Irving (Leeds)

PUBLICATIONS RECEIVED

Bibliography of Paper and Thin-Layer Chromatography 1966–1969 and Survey of Applications, Edited by K. Macek *et al.*, Elsevier Publishing Company, Amsterdam, 1972, xvi+991 pp., price Dfl. 200.00 (*ca.* £25.50).

This is a supplementary volume to the Journal of Chromatography, and follows the same lines as the earlier bibliography published in 1968. The first General Part covers books, reviews, principles and techniques. In the second Special Part, the references are listed according to compound classes. Both organic and inorganic applications are included.

Spectral Data and Physical Constants of Alkaloids, Vol. 7, Edited by J. Holubek *et al.*, Heyden and Son, London, 1972, 24 pp.+Cards 801–900, price £10.40 ($25.00, DM 94.00).

This volume covers 100 alkaloids, and forms part of a set of 8 volumes. Each card gives the structure, formula, m.p., refractive index and $pK_{80\%MCS}$ value, as well as information on source and occurrence. Infrared spectra are given in all cases; ultraviolet spectra are given for most alkaloids but are not always available.

Analytical Chemistry: Key to Progress on National Problems, Edited by W. Wayne Meinke and J. K. Taylor, N.B.S. Spec. Publ. 351, U.S. Government Printing Office, Washington, 1972, x+470 pp., price $3.50.

This volume contains the proceedings of the 24th Annual Summer Symposium held at Gaithersburg in June, 1971. The seven major papers presented cover the chemical analysis of things as they are (G. E. F. Lundell), solid-state research and electronics (R. A. Laudise), biomedical research and clinical chemistry (G. N. Bowers, Jr.), agricultural science (G. W. Irving and W. C. Schaefer), air pollution control (A. P. Altshuller), water pollution control (K. H. Mancy), and oceanography (J. H. Carpenter). Full reports of the discussion meetings after each paper are given. The book provides an excellent commentary on the importance of analytical methods in defining and solving current problems.

Kuster–Thiel–Fischbeck logarithmische Rechentafeln, Neubearbeitet von K. Fischbeck, W. de Gruyter, Berlin, 101 Aufl., 1972, xvi+313 S., Preis Werkstoff DM 26.00.

This new edition follows the same lines as earlier editions and provides very useful reference tables for chemists, pharmacists, and physicists. The material has been considerably revised and this edition should find the same wide following as its predecessors.

Hofmann–Jander qualitative Analyse, Herausgegeben von Helmut Hofmann, W. de Gruyter, Berlin, 4. Aufl., 1972, 362 S., Preis Kartoniert DM 14.80.

This is an extended and revised edition of the well-known text on qualitative analysis. The greatest part of the text deals with the properties and reactions of the different elements and ions, and then practical analysis is discussed. There are 32 tables to clarify the text.

J. H. van der Maas, *Basic Infrared Spectroscopy*, Heyden and Son, London, 2nd Ed., 1972, viii+109 pp., price £1.25 ($3.75, DM 11.25).

This paperback is a revised edition of the previous text published in 1969. The arrangement of chapters on fundamental theory, instrumentation, sampling and spectral interferences remains unchanged. The book provides a useful introduction to the subject for students.

B. W. Cook and K. Jones, *A Programmed Introduction to Infrared Spectroscopy*, Heyden and Son, London, 1972, xv+192 pp., price £1.50 ($3.90, DM 13.50).

This paperback is designed to teach the basic principles of infrared spectroscopy as well as its practice and routine applications. The student is taken in easy stages from the definition of infrared to the matching of spectra with given formulae, via instrumentation and basic principles. The programmed approach to the subject is perhaps more useful than a direct approach, particularly for young students and technical workers.

P. Ribereau-Gayon, *Plant Phenolics*, University Reviews in Botany, Vol. 3, Edited by V. H. Heywood, Oliver and Boyd, Edinburgh, 1972, xvi+254 pp., price £4.00.

This paperback is an efficient translation from the French edition of 1968, and has been brought up-to-date by the original author. The chapters are as follows: Conspectus of phenolic constituents; Chemistry of phenols and application to natural products; General methods of investigation; Phenolic acids and their derivatives; Flavones, flavonols and related compounds; Anthocyanins; Tannins; Metabolism and biological properties of phenolic constituents. Long sections on the separation and identification of the materials should be of interest to analysts.

H. Bennett and R. A. Reed, *Chemical Methods of Silicate Analysis*, Academic Press, London, 1971, xiv+272 pp., price £4.00 ($12.00).

This excellent book is essentially a third edition of Bennett and Hawley's *Methods of Silicate Analysis*, which last appeared in 1965. Very considerable revision has been done, and many new and faster chemical methods have been introduced. Sponsored by the British Ceramic Research Association, this handbook will remain for many years the standard text on the subject.

NATIONAL BUREAU OF STANDARDS PUBLICATIONS

J. Paul Cali, J. Mandel, L. Moore and D. S. Young, *Standard Reference Materials: A Referee Method for the Determination of Calcium in Serum*, NBS Spec. Publ. 260-36, May 1972, 136 pp., price $ 1.25.

C. H. Page and P. Vigoureux, *The International System of Units (SI)*, NBS Spec. Publ. 330, 1972 Edn., April 1972, 45 pp., price 30 cent.

H. L. Wagner, *Standard Reference Materials: Comparison of Original and Supplemental SRM 705, Narrow Molecular Weight Distribution Polystyrene*, NBS Spec. Publ. 260-33, May 1972, 30 pp., price 35 cent.

A. F. Clark, V. A. Deason, J. G. Gust and R. L. Powell, *Standard Reference Materials: The Eddy Current Decay Method for Resistivity Characterization of High-Purity Metals*, NBS Spec. Publ. 260-39, May 1972, 53 pp., price 55 cent.

American National Standard: Radiation Safety for X-ray Diffraction and Fluorescence Analysis Equipment, American National Standards Institute Subcommittee N43-1, NBS Handbook 111, June 1972, 20 pp., price 30 cent.

J. C. Richmond and J. J. Hsia, *Standard Reference Materials: Preparation and Calibration of Standards of Spectral Specular Reflectance*, NBS Spec. Publ. 260-38, May 1972, 57 pp., price 60 cent.

K. D. Mielenz and K. L. Eckerle, *Design, Construction and Testing of a New High-Accuracy Spectrophotometer*, NBS Tech. Note 729, June 1972, 60 pp., price 60 cent.

The above publications can be ordered from the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402. Orders from outside the U.S.A. should include an added 25% of price to cover mailing costs.

ERRATUM

A. Colombo, R. Vivian and E. Rodari, The vacuum-fusion determination of gases in silicon carbide, *Anal. Chim. Acta*, 62 (1972) 472–475.

The first three sentences on p. 472 of this paper should read as follows:

In the nuclear field, silicon carbide is used as coating material in the fabrication of coated particle fuels, and determination of its impurities, of which gases represent the largest part, is often required. To our knowledge, the only work on the subject is that of Paesold *et al.*[1], who carried out a gas analysis on one sample only, by means of the Balzers Exhalograph EA1. As this was part of a general work on the vacuum-fusion analysis of various oxides, precise details such as working temperature, weight and relative amount of samples with respect to the bath or flux employed, etc. were not given; nevertheless, their paper clearly indicated that, in comparison with the classical bath (80% Ni–20% Fe) or flux (Pt) techniques, the recovery of oxygen was almost doubled when the samples were thrown into the hot graphite crucible of the instrument in sealed graphite capsules.

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Short Communications

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