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SUMMARIES OF PAPERS PUBLISHED IN ANALYTICA CHIMICA ACTA

Vol. 30, No. 6, June 1964

THE EXTRACTION AND DETERMINATION OF TUNGSTEN WITH TETRAPHENYLARSONIUM CHLORIDE

A rapid, accurate and selective spectrophotometric method for tungsten is described. Tetraphenylarsonium chloride is used to form an insoluble ion-pair, tetraphenylarsonium thiocyanatotungstate, with tungsten previously reduced with tin(II) chloride in 6–9 *M* HCl. The ion-pair is formed by the addition of thiocyanate, extracted into chloroform, and the absorbance is measured at 406 mμ. The method is selective for tungsten under the conditions employed when niobium is masked with fluoride. The sensitivity is 12.58 μg W/ml of chloroform extract. The method has been applied successfully to 8 steels and 2 heat-resisting alloys which contain widely varying amounts of tungsten and other metals.

H. E. Affsprung and J. W. Murphy,

Anal. Chim. Acta, 30 (1964) 501–508.

SOLVENT EXTRACTION STUDIES OF ADDITION COMPOUNDS OF METAL HALIDES AND TRIPHENYLPHOSPHINE, -ARSINE AND -STIBINE

PART II. SPECTROPHOTOMETRIC DETERMINATION OF PALLADIUM

Palladium extracted with a cyclohexane solution of triphenylarsine from weakly acidic medium containing excess iodide and sulfite, can be determined spectrophotometrically by measuring the absorbance of the organic extract at 325 mμ. The optimum range of concentration was found to be 1 to 4 p.p.m. of palladium in the final dilution. Except for cyanide and large amounts of titanium(IV) and zirconium(IV), which inhibited the reaction, no other ion among the 41 tested interfered under the given conditions.

P. Senise and F. Levi,

Anal. Chim. Acta, 30 (1964) 509–514.

SYSTEMATIC ERRORS IN THE ABSOLUTE DETERMINATION OF DEUTERIUM IN ORGANIC COMPOUNDS

(in German)

The systematic errors which may affect the determination of the absolute deuterium content of organic compounds are studied. An empirical procedure for eliminating them is discussed.

P. Jordan,

Anal. Chim. Acta, 30 (1964) 515–518.

THE DETERMINATION OF MERCURY IN ORGANIC COMPOUNDS

The micro oxygen-flask combustion technique in combination with the sodium diethyldithiocarbamate titration is a rapid and useful method for determination of mercury in organic compounds. Chlorine and bromine do not interfere, iodine does. When the organic compound contains both mercury and chlorine, the chloride ions can be titrated argentometrically in the same absorption solution after the titration of mercury.

P. Gouverneur and W. Hoedeman,

Anal. Chim. Acta, 30 (1964) 519–523.

A COMPARISON OF THE PERFORMANCE OF AMMONIUM PHOSPHATE, TITAN YELLOW AND EDTA METHODS FOR THE DETERMINATION OF MAGNESIUM IN BLOOD SERUM

Detailed analyses were carried out on 3 specimens of serum to compare the precision and accuracy of 4 typical chemical methods for magnesium, and to determine whether wet-ashing or deproteinisation with trichloroacetic or acetic acid affected the results. Two ammonium phosphate precipitation methods involving spectrophotometry of phosphate in the precipitate, a spectrophotometric method with Titan Yellow and a visual EDTA titration method were studied. The analyses were designed to reveal systematic errors and the results were analysed statistically.

One ammonium phosphate method on untreated and wet-ashed samples and the EDTA method gave concordant and relatively precise results, which were used to estimate the true values. On this basis, another ammonium phosphate method involving overnight precipitation, gave consistently high results. The Titan Yellow method gave large random errors, particularly when proteins were not removed. The preliminary treatment of the samples did not appear to have any consistent effect.

E. J. Butler, D. H. S. Forbes, C. S. Munro and J. C. Russell,
Anal. Chim. Acta, 30 (1964) 524–536.

DETERMINATION OF IODINE IN COMMON SALT BY AN AUTOMATIC REACTION-RATE METHOD

An automatic reaction-rate method is described for the ultramicro determination of iodine in common salt. The method utilizes the acceleration of the reaction between ceric sulfate and arsenious acid by iodine. The time required for the reaction to consume a fixed amount of ceric ions is measured automatically and related directly to the iodine concentration. Measurements obtained in salt samples containing 3 to 7500 μg of iodine per 100 g were precise to within 2% or 0.3 μg of iodine, whichever is larger. Measurement times varied from a few sec to about 2 min.

T. P. Hadjiioannou,
Anal. Chim. Acta, 30 (1964) 537–542.

DETERMINATION OF SMALL QUANTITIES OF POTASSIUM AND SODIUM IN STONY METEORITIC MATERIAL, ROCKS AND MINERALS

A flame photometric method for the determination of potassium (0.005–0.1%) and sodium (0.01–1.0%) in stony meteorites and other silicate materials is described using a Beckman Model DU flame photometer. A simple scanning procedure eliminated background radiation and a small correction for the effect of magnesium on potassium radiation was calculated. Results obtained by this procedure agree well with an evaporative technique and isotope dilution determinations.

A. J. Easton and J. F. Lovering,
Anal. Chim. Acta, 30 (1964) 543–548.

DETERMINATION OF METHIONINE IN PROTEIN HYDROLYSATES BY REACTION WITH RANEY NICKEL AND INFRARED SPECTROSCOPY

An infrared spectrophotometric determination of methionine in protein hydrolysates is suggested. The method is based on the desulphurizing action of specially purified Raney nickel on methionine, during which methane is quantitatively liberated; methane is then measured at 3020.3 cm^{-1}. Cystine and cysteine do not interfere and can usually be determined from the difference between total sulphur and methionine sulphur. The method can be applied to protein hydrolysates, and to homogenates of internal organs. The time required for a single determination is about 40 min; the relative error is 1–2% down to a level of 200 μg.

C. P. IVANOV, B. V. ALEXIEV, M. KRSTEVA AND B. YORDANOV,

Anal. Chim. Acta, 30 (1964) 549–555.

THE SAMPLING OF HYDROGEN SULFIDE IN AIR WITH IMPREGNATED FILTER PAPER

A method is proposed for the quantitative collection of hydrogen sulfide in air on impregnated filter paper. An aqueous solution of potassium hydroxide, potassium zincate and glycerol is used as impregnating fluid. The stability of the collected sulfide and the efficiency of collection at different humidities, temperatures, hydrogen sulfide concentrations and air velocities were determined.

C. HUYGEN,

Anal. Chim. Acta, 30 (1964) 556–564.

THE ULTRAVIOLET SPECTROPHOTOMETRIC DETERMINATION OF CADMIUM BY THE DIETHYLDITHIOCARBAMATE METHOD

An ultraviolet spectrophotometric method for the determination of small amounts of cadmium is proposed. The method is based on measuring the absorbance at 262 mμ of the cadmium diethyldithiocarbamate chelate which has been extracted from basic solution with chloroform. The effect of solution variables and numerous diverse ions has been investigated.

D. F. BOLTZ AND E. J. HAVLENA, JR.,

Anal. Chim. Acta, 30 (1964) 565–568.

SPECTROPHOTOMETRIC DETERMINATION OF THE DISSOCIATION CONSTANTS OF N,N'-BIS(2-HYDROXYETHYL)-DITHIO-OXAMIDE

The dissociation constants of N,N'-bis(2-hydroxyethyl)dithio-oxamide were determined by a spectrophotometric method. The dissociation constants were calculated by means of a weighted least squares technique. N,N'-Bis(2-hydroxyethyl)dithio-oxamide was found to be a dibasic acid with a thermodynamic dissociation constant $pK_1 = 11.04$. At ionic strength $\mu = 0.5$, $pK_1 = 10.71$, and $pK_2 = 13.92$.

L. VAN POUCKE AND M. HERMAN,

Anal. Chim. Acta, 30 (1964) 569–575.

SPECTROPHOTOMETRIC DETERMINATION OF SUBMICRO QUANTITIES OF AJMALINE

A spectrophotometric method for the determination of 5–100 μg samples of ajmaline is described. Ajmaline gives a red colour with concentrated nitric acid, which is measured at 510 mμ after a definite time interval. The limits of interference from serpentine and ajmalicine are given. The method can be applied directly for the estimation of ajmaline in pharmaceutical preparations.

R. A. SHAH, N. FATIMA AND R. REHANA,

Anal. Chim. Acta, 30 (1964) 576–581.

THE SOLUBILITY OF AMMONIUM 12-MOLYBDOPHOSPHATE IN DILUTE ACIDS

An attempt has been made to clarify the position regarding the apparent solubility of ammonium molybdophosphate on prolonged standing with dilute acids. The solubility of ammonium molybdophosphate in nitric acid solutions of different concentrations was determined by radiochemical methods using ^{32}P and ^{99}Mo. On prolonged standing with aqueous solutions, ammonium molybdophosphate hydrolyses completely, mainly to phosphoric and molybdic acids. The precipitate dissolves in nitric acid at a much faster initial rate than in perchloric acid; the latter is suggested as an alternative wash solution.

D. W. ARCHER AND R. B. HESLOP,

Anal. Chim. Acta, 30 (1964) 582–589.

THE ION-EXCHANGE SEPARATION AND DETERMINATION OF SMALL AMOUNTS OF MAGNESIUM AND MANGANESE

(Short Communication)

A. A. ASHTON,

Anal. Chim. Acta, 30 (1964) 590–591.

THE SPARINGLY SOLUBLE SALTS OF LANTHANIDES WITH BENZENEPHOSPHONIC ACID AND BENZENEPHOSPHINIC ACID

(Short Communication)

A. K. MUKHERJI,

Anal. Chim. Acta, 30 (1964) 591–593.

POTENTIOMETRIC DETERMINATION OF MERCURY(II) WITH CYCLOHEXANEDIAMINOTETRAACETIC ACID

ANALYSIS OF BINARY MIXTURES

(Short Communication)

H. KHALIFA,

Anal. Chim. Acta, 30 (1964) 593–595.

THE EXTRACTION AND DETERMINATION OF TUNGSTEN WITH TETRAPHENYLARSONIUM CHLORIDE*

H. E. AFFSPRUNG AND J. W. MURPHY

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(Received November 7th, 1963)

Several procedures for the colorimetric determination of tungsten have been published. One of the most common and extensively used spectrophotometric procedures is the thiocyanate method. GOTTSCHALK[1] and MCDUFFIE *et al.*[2] have made rather complete studies of this determination, which is based upon the reduction of tungsten(VI) in concentrated hydrochloric acid to a lower oxidation state where a bright yellow complex with thiocyanate is formed. Some reducing agents which have been used will reduce the tungsten to the trivalent oxidation state but thiocyanate apparently oxidizes all of these forms to the pentavalent state, as is shown by GOTTSCHALK[1]. Spectrophotometric analysis may be carried out either on the aqueous solution or on an amyl alcohol or ether extract of the thiocyanate complex. Difficulty has been encountered with the method since the complex is unstable in these solvents and time measurements are often included in the recommended procedures. HOBART AND HURLEY[3] have discussed some of the advantages of the thiocyanate procedures. Since tetraphenylarsonium ion forms an insoluble ion-pair with the thiocyanate complex of tungsten which may be extracted into chloroform, it appeared that this type of extraction would allow a great improvement in the thiocyanate method for the determination of tungsten, for many of the difficulties in the aqueous method as well as extractions of the free acid into ether and amyl alcohol can be overcome by its use.

The use of tetraphenylarsonium ion as an extracting agent has been successful in other determinations by permitting the removal of many of the interferences, since the conditions for successful extraction of the onium ion-pair are usually much less critical than those for the free acid[4,5]. Also the stability of the onium ion extract in chloroform is usually much greater than is the case of the free acid in ethers and alcohols. Consequently, a method for the determination of tungsten was investigated which was based upon the extraction into chloroform of the tungsten thiocyanate complex with tetraphenylarsonium ion. Excellent results were obtained when the method was applied to a number of tungsten-containing alloys having widely varying amounts of tungsten and large amounts of other metals. When little difficulty is met in dissolving the sample, an analysis can be run from start to finish in less than an

* Work supported by the National Science Foundation.

hour. The ion-pair extraction is adequate to remove all interferences except niobium, and the interference of this ion can be completely masked by the use of a fluoride wash.

EXPERIMENTAL

Apparatus and materials

Beckman Model DU and DK-1 spectrophotometers were used for absorption spectra measurements.

Standard tungsten solutions were prepared by dissolving 5.00 mmoles of ignited tungstic oxide from K and K Laboratories, Jamaica, New York, in 20 ml of hot 5 *M* potassium hydroxide, filtering and diluting to 500 ml. Five ml of this solution were diluted to 500 ml to give a secondary stock solution of 0.0001 *M* tungsten.

Tetraphenylarsonium chloride, 0.025 *M*, solutions were prepared from the hydrochloride salt obtained from K and K Laboratories.

All other solutions were prepared from salts of analytical reagent quality.

Absorption spectra

In order to obtain the absorption spectrum of the colored complex the following procedure was used. A solution of tungsten(VI) in 12 *M* hydrochloric acid containing 0.5 μmole of tungsten was pipetted into a small beaker, 2 ml of 10% tin(II) chloride in concentrated hydrochloric acid were added and the mixture was heated nearly to boiling for 5 min. After the reduction the solution was cooled to 10–15° and transferred to a 60-ml separatory funnel equipped with a Teflon stopcock. Cold 6 *M*

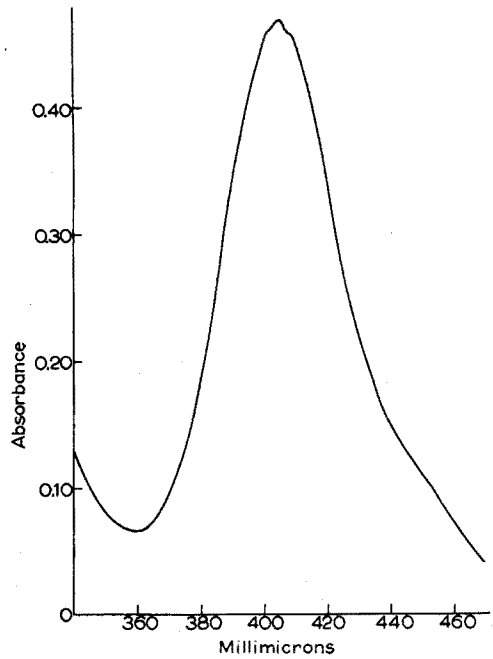

Fig. 1. Absorption spectrum of a chloroform extract of the complex.

hydrochloric acid (5–10 ml) was used to wash the beaker and complete the transfer. One ml of 0.025 *M* tetraphenylarsonium chloride was added and the mixture swirled. A white precipitate appears at this point, probably tetraphenylarsonium tetrachlorostannate(II), but it does not interfere with the determination. Next 3 ml of 1.5 *M* potassium thiocyanate were added, and upon swirling a yellow color could usually be seen in the precipitate. Finally 8 to 10 ml of chloroform were added and the mixture shaken vigorously. The two layers usually separated quickly and well, with the chloroform layer a bright yellow color. The chloroform was filtered, to remove suspended water droplets, into a 25-ml volumetric flask. The extraction was repeated with 10 drops of tetraphenylarsonium chloride and 5–10 ml of chloroform until the volumetric flask was full. The spectrum of the solution of the chloroform extract, given in Fig. 1, was obtained with the DK-1 spectrophotometer between 330 and 460 mμ. This spectrum corresponds very closely to that of the aqueous complex of thiocyanate with tungsten(V). The peak absorbance is shifted from 398 mμ, the value in water solution, to 406 mμ in chloroform, but the shapes of the curves are the same with shoulders in the same relative positions on both curves. Because of the close correspondence between the aqueous and chloroform spectra, it is probable that the absorbing species is the same in both cases, which indicates that the extracted material is the ion-pair $(C_6H_5)_4As^+W(OH)_2(SCN)_4^-$ with the same complex of tungsten as that reported by GOTTSCHALK[1] as the species present in aqueous solution.

Calibration

A series of solutions of increasing tungsten concentration were extracted in the manner described above. The absorbance of each was measured at 406 mμ, and a calibration curve of absorbance *vs.* concentration was prepared, assuming complete extraction of the tungsten into the chloroform. Beer's law was followed up to tungsten concentrations of $4.0 \cdot 10^{-5}$ *M* in the chloroform. At higher concentrations a negative deviation occurred. The absorptivity obtained from the straight line portion was 80.1. The most probable explanation for the deviation from Beer's law at higher concentrations is that polymerization of the tungsten complex occurs in the aqueous solution and prevents a satisfactory extraction. In any case this deviation did not introduce any difficulty in the subsequent application of the procedure to many complex materials, when the concentration of tungsten was kept below the limit of $4 \cdot 10^{-5}$ *M* in the chloroform extract.

Stability of extracted species

The chloroform solutions, extracted as described above, were stable for about 24 h. After this period of time most of them decreased in absorbance. When an oxidizing material is present in the chloroform the extract is very unstable and it starts turning red in a few minutes. Very small traces of oxidant will cause eventual formation of a red or reddish solution, which upon standing fades and leaves a yellow precipitate. For this reason it is mandatory to remove all nitrogen oxides from the solutions before an analysis is made. Nitric acid need not be removed, for it is not extracted into the chloroform to a significant extent. However, nitric acid solutions must not be left in contact with the chloroform extract since the yellow tungsten solution will be oxidized to a purple color upon standing in contact with an oxidizing material. It is essential that all glassware be kept as clean as possible, since small amounts of

oxidizing agents decrease the stability of the solution and can lead to spurious results.

If the extraction is performed in low hydrochloric acid or chloride ion concentration, it is incomplete; green crystals slowly form and precipitate, and the absorbance fades correspondingly. At very low hydrochloric acid concentrations the extraction is incomplete and precipitation of the green crystals is rapid. This precipitation also is apt to occur when the tungsten concentration is above $4 \cdot 10^{-5}$ M.

Interferences

Interference studies were made upon most of the elements which commonly occur in tungsten ores. No significant interferences were found with the procedures described above, except that of niobium. The interference studies were made by adding the appropriate element to an aliquot of the standard tungsten solution and carrying out the reduction and the extraction. As is shown in Table I, the niobium interference

TABLE I

INTERFERENCES IN THE EXTRACTION AND DETERMINATION OF W WITH $(C_6H_5)_4AsCl$

(Taken: 92.0 μg W; absorbance at 406 mμ = 0.298)

Interfering element	*Interfering element (μg)*	*A (±0.002)*	*W found (μg)*	*Error*	
				(μg)	*(%)*
V	250	0.297	91.6	−0.4	−0.3
Mo	1000	0.302	93.2	+1.2	+1.3
Co	1000	0.297	91.6	−0.4	−0.3
Cr	1000	0.296	91.4	−0.6	−0.6
Mn	1000	0.296	91.4	−0.6	−0.6
Ni	1000	0.297	91.6	−0.4	−0.3
Fe	7500	0.298	92.0	0.0	0.0
Ti	1800	0.300[a]	92.6	+1.4	+1.5
Ta	9100	0.296[a]	91.4	+0.2	+0.3
Nb[b]	37.2	0.298	92.0	0.0	0.0
Nb	37.2	0.462	106.8	+14.8	+16.1

[a] Theoretical A = 0.295, μg W taken = 91.2.

[b] $CHCl_3$ extract washed with 10 ml aqueous solution 3 M in $NH_4F \cdot HF$ and 6 M in NaCl.

can be removed by a fluoride wash of the extracted complex. Oxidizing agents such as the oxides of nitrogen interfere with the determination as discussed above; also vanadium-containing steels sometimes produce a precipitate of unknown composition which discolors the chloroform. All of these interferences may be removed by a preextraction of the acid solution of the steel or alloy sample with chloroform before the reduction is made. If a large amount of precipitate is present, it should be removed by filtration before the extraction since excessive amounts of solid material tend to emulsify the chloroform, making the extraction difficult to carry out. When titanium is present a precipitate of a thiocyanate complex is formed with the tetraphenylarsonium ion; however, prolonged shaking will eventually cause it to dissolve due to the removal of the tetraphenylarsonium ion from the aqueous layer. As is seen in Table I, the titanium did not interfere with the determination.

Analytical procedure

Weigh about 0.2 g of the sample into a beaker and treat with 25 ml of concentrated hydrochloric acid. Concentrated nitric and hydrochloric acids are added as necessary to dissolve the sample keeping the total nitric acid to a minimum to avoid excessive amounts of nitrogen oxides. When dissolution is complete, boil the solution slowly for about 10 min to remove nitrogen oxides. It is not necessary to remove the nitric acid. After boiling, transfer and dilute immediately to 100 ml with concentrated hydrochloric acid to prevent the possible precipitation of tungstic acid. If a small amount of insoluble material remains after the dilution, it should be removed by a pre-extraction of the solution with chloroform. Also the solution should be tested for complete removal of oxides of nitrogen by making an extraction on a portion of the sample with chloroform. If this extract has a yellow-brown color, the aliquot of sample should be repeatedly extracted until the chloroform layer is colorless.

Transfer 2 or 3 ml of the solution to be analyzed into a small beaker, add 2 ml of 10% tin(II) chloride, cover and heat just below boiling for about 5 min. Chill the solution and transfer to a small separatory funnel, preferably equipped with a Teflon stopcock. Use 6 *M* hydrochloric acid to make the transfer quantitative and to dilute the acid to 8 to 9 *M*. Add 1 ml of 0.025 *M* tetraphenylarsonium chloride solution and 3 ml of 1.5 *M* potassium thiocyanate solution and extract with chloroform in the manner described above. When niobium is known to be absent, filter the chloroform layer directly into a 25-ml volumetric flask and measure the absorbance. When niobium is present, transfer the initial chloroform extract to a second separatory funnel containing about 10 ml of fluoride wash solution (see below) and shake vigorously. Filter the washed portion of the extract into a 25-ml volumetric flask and continue the extraction in this manner until the flask is full. The fluoride wash seems to shift the position of maximum absorbance slightly to the short wavelength side, and it was found best to make the absorbance measurement at or near 400–403 mμ rather than 406 mμ where the maximum is found when the fluoride wash is not used. The absorbance of the solution is the same as that of the solutions of equal concentration which did not undergo the fluoride wash, and the same standard curve or absorptivity may be used to calculate the concentration of tungsten. The absorbance of solutions washed with fluoride solution may begin to decrease to a significant extent after about 30 min, so the measurement on these solutions should be made within this time period to assure accurate readings. The fluoride wash solution should be prepared immediately prior to use by dissolving 1.5–2 g of solid ammonium bifluoride in 10 ml of cold water in the funnel used for the washing operation.

DISCUSSION

The analysis consists of three parts: the solution and reduction of the sample, the formation of the complex and the extraction followed by the fluoride wash when necessary. In none of these steps are the conditions unusually severe. The reduction is best made in hot concentrated hydrochloric acid and the amount of tin(II) chloride is not critical, although a large excess is advisable to speed the reduction and to be sure that it is complete. Also, since some of the other elements such as iron and tantalum are reduced and their interference removed by the reduction, a large amount of tin(II) chloride is needed. The conditions for this reduction have been thoroughly discussed by GENTRY AND SHERRINGTON[6] and GELD AND CARROLL[5].

After reduction it is best to chill the solution in an ice bath to minimize decomposition of thiocyanate. The solution is then diluted with an equal volume of cold 6 *M* hydrochloric acid. For the extraction, better results are obtained when the tetraphenylarsonium chloride is added prior to the thiocyanate. A large excess of thiocyanate is necessary to obtain a quantitative extraction, and usually about 4.5 m-moles of potassium thiocyanate were added for every 0.04 mmole of tungsten expected. Although good results could be obtained with much less thiocyanate, the large excess insured a good extraction. The acid concentration controls very well the formation of many complexes which might interfere with the determination of tungsten. For example, the complex of cobalt is apparently not formed in acid concentrations above 5 *M*, while tungsten and niobium are effectively extracted only at these relatively high acid strengths.

The effect upon the efficiency of extraction as a function of the concentration of hydrochloric acid was investigated in the following manner.

(*1*) Five ml of 0.0001 *M* tungsten(VI) and 2 ml of 10% tin(II) chloride solution in 12 *M* hydrochloric acid were heated to reduce the tungsten.

(*2*) The reduced solutions were chilled and rinsed into the extraction funnel with 10 ml of cold hydrochloric acid solution; the concentration of hydrochloric acid in the wash solution was varied in order to control the final acid concentration.

(*3*) One ml of 0.025 *M* tetraphenylarsonium chloride and 3 ml of 1.5 *M* potassium thiocyanate were added to give a final volume in each case of 21 ml.

(*4*) 25 ml of chloroform were added, the mixture was well shaken, and a portion of the chloroform layer was filtered through a small paper into the spectrophotometer cell. The absorbance was measured and the percentage extraction calculated from the known amount of tungsten added.

The data are shown in Table II, where it is seen that the extraction is nearly complete for acid concentrations greater than 5 *M*.

TABLE II

VARIATION OF EXTRACTION EFFICIENCY WITH HCl CONCENTRATION

(Concentration W for complete extraction: 20 μM)

HCl concn. (M)	*% Extraction*
4	62.6
5	93.5
6	97.0
7	98.6
8	98.9
8.8	97.3

Although the removal of tungsten can be made quantitative in lower acid concentrations by repeated extraction, the extract is not stable. From 4 *M* hydrochloric acid green crystals are formed after about 20 min. When the extraction is made in 5 *M* acid the extract is stable for 6 h, and when the concentration is as great as 6 *M* no change in the absorbance is found for at least 24 h.

Accuracy

The accuracy and precision of the method were established by the analysis of a number of Bureau of Standards standard samples of steel and heat-resisting alloys. The data are given in Table III. As can be seen, the method is as accurate as any of the procedures reported upon by the Bureau of Standards and the precision as found from the standard deviation of the samples is very good.

TABLE III

ANALYSIS OF NBS STANDARD STEEL AND ALLOY SAMPLES

NBS	*% W*		*Difference*	*Standard deviation*	*Number of samples*	*Sample description and % composition*
	Reported	*Found*				
50a	18.25	18.22	−0.03	±0.25	5	Steel: Cr(3.5), V(1.0), Mn(0.4), C(0.6) <0.1 — Cu, Ni, Mo, As, Sn
50b	18.05	18.16	+0.11	±0.04	4	Steel: Cr(4.1), V(1.0), Mo(0.4), C(0.7), Mn(0.3) <0.1 — Cu, Sn, As
123b	0.18	0.176	−0.004	±0.002	4	Steel: Nb(0.75), Ta(0.2), Mo (0.2), Si(0.5), V(0.05), Ti(0.006)
132	6.29	6.26	−0.03	±0.05	5	Steel: Cr(4.1), V(1.6), Mo(7.1), Cu(0.15), Mn(0.25), C(0.8)
132a	6.20	6.19	−0.01	±0.07	7	Steel: Cr(4.2), V(1.9), Mo(4.5), Mn(0.3), C(0.8)
134	1.82	1.90	+0.08	±0.02	4	Steel: Cr(3.7), V(1.1), Mo(8.7), C(0.8), Cu(0.1)
153	1.58	1.61	+0.03	±0.02	6	Steel: Co(8.5), Mo(8.4), Cr(4.1), V(2.0), C(0.9)
155	0.517	0.501	−0.016	±0.01	3	Steel: Cr(0.5), Mn(1.24), C(0.9), Ni(0.1)
167	4.50	4.52	+0.02	±0.03	5	Heat-resisting alloy: Ni(20), Co(43), Cr(20), Mo(3.9), Nb(3.2), Ta(0.08), Fe(2.1)
168	3.95	3.99	+0.04	±0.01	4	Heat-resisting alloy: Ni(20), Co(41), Cr(20), Mo(4.0), Nb(3.0), Ta(1.0), Fe(3.4)

SUMMARY

A rapid, accurate and selective spectrophotometric method for tungsten is described. Tetraphenylarsonium chloride is used to form an insoluble ion-pair, tetraphenylarsonium thiocyanatotungstate, with tungsten previously reduced with tin(II) chloride in 6–9 *M* HCl. The ion-pair is formed by the addition of thiocyanate, extracted into chloroform, and the absorbance is measured at 406 mμ. The method is selective for tungsten under the conditions employed when niobium is masked with fluoride. The sensitivity is 12.58 μg W/ml of chloroform extract. The method has been applied successfully to 8 steels and 2 heat-resisting alloys which contain widely varying amounts of tungsten and other metals.

RÉSUMÉ

Une méthode spectrophotométrique, rapide, exacte et sélective est proposée pour le dosage du tungstène. Elle est basée sur la réaction du chlorure de tétraphénylarsonium avec le tungstène, préalablement réduit par le chlorure d'étain(II). Après addition de thiocyanate, on procède par extraction, dans le chloroforme, du thiocyanatotungstate de tétraphénylarsonium formé. Dans les conditions décrites, la méthode est sélective, à condition de masquer le niobium par un fluorure. Sensibilité: 12.58 μg/ml. Ce procédé a été appliqué à divers aciers et alliages, renfermant des quantités variées de tungstène.

ZUSAMMENFASSUNG

Eine schnelle, genaue und selektive, spektralphotometrische Methode zur Bestimmung von Wolfram wird beschrieben. Wolfram, das mit $SnCl_2$ in 6–9 *M* HCl reduziert wurde, bildet mit Tetraphenylarsoniumchlorid ein unlösliches Ionenpaar (Tetraphenylarsonium-thiocyanatowolframat). Das Ionenpaar wird durch Hinzufügen von Thiocyanat gebildet, mit Chloroform extrahiert und die Extinktion bei 406 mμ gemessen. Die Methode ist selektiv für Wolfram unter den angeführten Bedingungen, wenn Niob mit Fluorid maskiert wird. Die Empfindlichkeit beträgt 12.58 μg W/ml im Chloroformextrakt. Die Methode wurde erfolgreich bei Stählen und Legierungen angewandt, welche weit variierende Gehalte von Wolfram und anderen Metallen enthielten.

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SOLVENT EXTRACTION STUDIES OF ADDITION COMPOUNDS OF METAL HALIDES AND TRIPHENYLPHOSPHINE, -ARSINE AND -STIBINE

PART II. SPECTROPHOTOMETRIC DETERMINATION OF PALLADIUM

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In the first paper of this series, it was shown that palladium can be extracted very efficiently in the form of $PdI_2 \cdot 2(C_6H_5)_3M$, where M is P, As or Sb. A sensitive and specific test for palladium was developed, based on the extraction of the metal ion from a weakly acidic medium containing excess iodide and in the presence of sulfite, with a solution of triphenylstibine in cyclohexane[1].

Further studies showed that the same reaction can be applied very conveniently to the spectrophotometric determination of palladium. In this method, however, although the basic lines of the test procedure were followed, triphenylarsine was employed as reagent because more favorable experimental conditions were attained than with triphenylstibine.

EXPERIMENTAL

Apparatus and reagents

A Beckman spectrophotometer, model DU, and 10-mm silica cells were used.

Commercial cyclohexane was treated with 10% sulfuric acid and 1% oleum, dilute sodium hydroxide and permanganate solution, washed, dried over calcium chloride and distilled (b.p. 78°)[2].

Triphenylarsine, triphenylstibine and triphenylphosphine (E. Kodak and Fluka) were repeatedly crystallized from 95% ethanol.

All other chemicals were C.P. reagents.

Standard palladium solution

A stock solution containing approximately 1 mg Pd/ml was prepared by dissolving 0.50 g of $PdCl_2$ in 10 ml of 1 : 1 hydrochloric acid and diluting to 300 ml. Standardization was carried out gravimetrically with dimethylglyoxime. Working solutions were freshly prepared by proper dilution.

Spectral characteristics and choice of reagent

In Fig. 1 the absorption spectra of cyclohexane extracts are compared with those

of the solutions of the corresponding reagent in the same solvent. These spectra were found to correspond to those of the isolated $PdI_2 \cdot 2Ph_3M$ compounds when dissolved in cyclohexane, as previously mentioned[1].

The spectra of the antimony- and arsenic-containing compounds show a well-defined band in the near ultraviolet with a pronounced maximum at 333 and 325

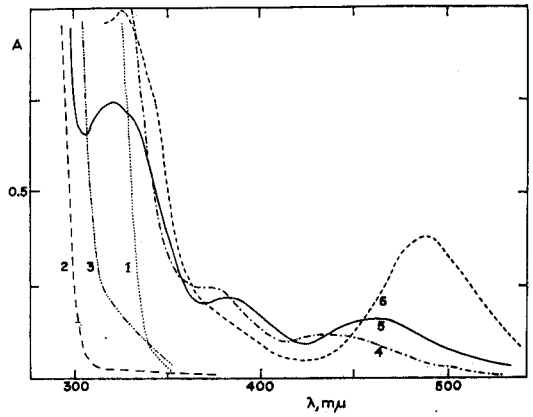

Fig. 1. Absorption spectra of palladium-containing extracts and corresponding extractant solutions. 1% Ph_3M in cyclohexane, M being 1,P;2,As;3,Sb. 4 p.p.m. Pd as $PdI_2 \cdot 2Ph_3M$ in 1% Ph_3M cyclohexane extracting solution, M being 4,P;5,As;6,Sb.

mμ respectively. Molar absorptivities were found to be 21,500 and 20,000 respectively. Curves 3 and 2, however, show that in this region and under the experimental conditions considered, the absorption of the free reagent is not insignificant in the first case while it is almost negligible in the second one.

In the visible range of the spectrum the absorption of the antimony compound is considerably more pronounced than in any other case studied as shown in curve 6. Molar absorptivity at the wavelength of maximum absorption, 490 mμ, was found to be 10,300.

These data led first to the selection of triphenylstibine as a reagent for the spectrophotometric determination, since it was intended to use either the ultraviolet or the visible band according to the amount of palladium present; this would allow the application of the method over a wide range of concentrations.

Experiments were then run in the presence of sulfite, as it was known[1] that this reagent was necessary in order to prevent interference of platinum. A white turbidity, however, was found to develop in the extract containing sulfur dioxide on exposure to light. Although many changes in the experimental conditions were tried, especially with the purpose of decreasing the amount of sulfur dioxide in the organic layer, this difficulty was not completely overcome.

For this reason, triphenylarsine was finally chosen as reagent and the method of determination was confined to the ultraviolet region of the spectrum.

Working conditions

The completeness of the reaction as well as the efficiency and rate of extraction

depend on several factors which were found to be to a certain extent interdependent. Thus the concentration of sulfite should be large enough to prevent interference of platinum but not so large as to affect the extraction of palladium; a decrease of pH speeds up extraction but if the hydrogen ion concentration is large the amount of sulfur dioxide evolved develops too high a pressure in the extraction tube and may thus produce mechanical losses. An increase of iodide as well as of reagent concentrations accelerates the extraction of palladium but may also help the analogous reaction of platinum.

For these reasons it was not possible to study the influence of each particular factor independently and the conditions of the procedure had to be established tentatively.

By careful adjustment of all experimental variables, the procedure given below was developed; this allowed complete recovery of palladium in numerous experiments.

Procedure

To 1.00 ml of the weakly acidic sample solution, in a ground-stoppered test tube, add 0.3 ml of 1% sulfuric acid, 1.0 ml of 15% sodium hydrogen sulfite solution and 0.2 ml of a 20% potassium iodide solution. Swirl to homogenize and let stand for about 20 min. Add 1.0 ml of a 1% solution of triphenylarsine in cyclohexane. Shake vigorously during 3 to 4 min. Centrifuge for a few sec to allow for phase separation and transfer the organic layer to a 5-ml volumetric flask with an extraction pipet[3]. Repeat the extraction with 1.0 ml of the reagent solution. Rinse the extraction tube and the pipet with pure solvent and make up to the mark.

Read the transmittance at 325 mμ against a blank run in parallel with all the reagents.

RESULTS AND DISCUSSION

Calibration curve and concentration range

By employing the procedure indicated above, a calibration curve was prepared which showed Beer's law to apply in all the ranges studied, *i.e.* up to 5 p.p.m. Pd in the measured solution. A Ringbom plot of the data showed the optimum concentration range to be 1 to 4 p.p.m. in the final dilution. The sensitivity, as required by SANDELL[4], was found to be 0.005 $\mu g/cm^2$.

Measured solutions showed high stability. Absorbance readings were reproducible over a period of a week after keeping both extract and blank in the dark.

Extraction efficiency

The extraction coefficient was estimated by extracting 2.5 ml of the aqueous phase, prepared exactly as indicated in the procedure given above, with 2.5 ml of a 1% solution of triphenylarsine in cyclohexane. The extract was transferred to a 5-ml volumetric flask and the volume completed with pure solvent.

Evaluation of the palladium content was made on the basis of the calibration curve previously prepared.

By averaging the results summarized in Table I one may conclude that at least 97% palladium should be extracted in a single pass.

TABLE I

EXTRACTION COEFFICIENT

(Experiments carried out with 2.5 ml of aqueous medium and 2.5 ml of organic phase)

Pd present (μg)	*Pd found (μg)*
1.0	0.98, 1.04, 0.67, 0.96[a]
5.0	4.8, 5.0, 4.8, 5.1
10.0	10.2, 10.5, 10.3, 10.0
20.0	20.1, 20.5, 19.7, 19.4
50.0	50.0, 48.0, 47.0, 48.5[b]

[a] 5.0 μg Pd were added to the extracts of this series in order to allow the final reading to fall within a more favorable range.
[b] Extracts were diluted to 25 ml.

TABLE II

INTERFERENCE STUDY

(Amount of each foreign ion present: 1000 μg)

Foreign ion	*Pd present (μg)*	*Pd found (μg)*	*Foreign ion*	*Pd present (μg)*	*Pd found (μg)*
Pt(IV)	5.0	5.1, 5.0	Sn(IV), Bi(III), Sb(III)	5.0	5.3, 5.2
	11.5	11.5, 11.9		20.0	20.6, 20.3
	20.0	19.8, 19.6			
	25.0	25.0			
			Pb(II)[a], U(VI)	5.0	4.7, 4.7
Ru(III), Rh(III)	5.0	5.2, 5.3		20.0	19.5, 20.0
	20.0	20.0, 20.5			
			As(III), Be(II), Mn(II), Th(IV)	5.0	5.2, 5.2
Os(IV), Ir(III)	5.0	4.8, 5.0		20.0	19.9, 19.7
	20.0	20.0, 20.3			
Fe(III), Co(II), Ni(II)	5.0	5.2, 5.0	Ti(IV)[b]	5.0	5.0, 4.9
	20.0	20.5, 20.6		20.0	20.3, 19.8
Au(III)	5.0	5.2, 5.0	Zr(IV)[b]	5.0	4.9, 5.2
	20.0	20.3, 19.8		20.0	20.3, 19.8
Cu(II), Ag(I)	5.0	4.7, 4.7	V(III)	5.0	5.1, 5.3
	20.0	20.0, 19.7		20.0	19.4, 20.5
Cd(II), Zn(II), Hg(II)	5.0	5.1, 4.7	Cr(III)	5.0	4.8, 4.7
	20.0	19.6, 19.6		20.0	19.4, 19.5
Al(III), Ga(III), In(III), Tl(I)	5.0	5.4, 5.3	Mo(VI), W(VI)	5.0	5.0, 4.9
	20.0	19.3, 20.1		20.0	20.2, 20.0
			Cl^-, Br^-, N_3^-, SCN^-	5.0	4.9, 5.1
Ge(IV)	5.0	5.2, 5.3		20.0	20.6, 20.5
	20.0	19.3, 19.2			

[a] Let stand a few min after acidifying with sulfuric acid to allow complete precipitation of lead sulfate before adding iodide.
[b] No sulfite was added.

Interference study

Results of experiments carried out with 1000 μg of foreign ion are shown in Table II. In addition to these data, more information was collected in determinations carried out with different amounts of interferences. Thus the platinum content was lowered to 500 μg in some experiments; the influence of niobium and tantalum was studied with only 250 and 300 μg respectively. No interference was found to occur in all these experiments. Titanium and zirconium were found to inhibit partially the extraction of palladium, the maximum tolerable amounts being 50 and 30 μg, respectively. Nevertheless as shown in Table II, experiments carried out without sulfite gave results as expected. Cyanide was found to inhibit the reaction completely.

Precipitations occurring while carrying out the procedure were ignored.

Precision study

Two series of experiments of 20 independent determinations, with 5.0 and 20.0 μg Pd in the aqueous phase, were performed in order to estimate the precision of the method. With 5.0 μg Pd in 5.0 ml of aqueous phase, the average absorbance was 0.183, with an average deviation of 0.0065. For 20.0 μg Pd in 5.0 ml of aqueous phase, the average absorbance was 0.757 with an average deviation of 0.012. The standard deviation for the first set was 0.0086 and for the second one 0.0013 in terms of absorbance values. For a single measurement the probable errors were 0.006 and 0.009, which in terms of palladium concentration indicates that a single determination should be correct to $\pm$ 0.16 and 0.24 μg Pd respectively.

Remarks

The results reported confirm that unless platinum is present, it should be possible to carry out the determination of palladium without the addition of sulfite. This would allow, for instance, the determination of small concentrations of palladium in the presence of very large amounts of zirconium or titanium.

In this connection, one might also expect that sulfite could be likewise eliminated by employing triphenylstibine as reagent and measuring the absorption of the organic phase at the 490 mμ maximum of the visible band. Platinum as well as a number of metal ions would not interfere and the color of the palladium-containing extract is stable in the absence of sulfur dioxide. Accordingly, studies were undertaken, which led to a method of simultaneous determination of platinum and palladium; this will be reported in a further paper.

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SUMMARY

Palladium extracted with a cyclohexane solution of triphenylarsine from weakly acidic medium containing excess iodide and sulfite, can be determined spectrophotometrically by measuring the absorbance of the organic extract at 325 mμ. The optimum range of concentration was found to be 1 to 4 p.p.m. of palladium in the final dilution. Except for cyanide and large amounts of titanium(IV) and zirconium(IV), which inhibited the reaction, no other ion among the 41 tested interfered under the given conditions.

RÉSUMÉ

Une méthode est proposée pour le dosage spectrophotométrique du palladium. On procède par extraction au moyen d'une solution de triphénylarsine dans le cyclohexane, en milieu légèrement acide, renfermant un excès d'iodure et de sulfite. Le domaine de concentration optimum est situé entre 1 et 4 p.p.m. de palladium dans la solution finale. Des 41 ions examinés, seuls les cyanures et de fortes quantités de titane(IV) et de zirconium(IV) peuvent gêner, dans les conditions décrites.

ZUSAMMENFASSUNG

Palladium wurde mit einer Lösung von Triphenylarsin in Cyclohexan aus schwach saurem Medium, das Jodid und Sulfit im Überschuss enthält, extrahiert und spektralphotometrisch durch Messen der Extinktion der organischen Phase bei 325 mμ bestimmt. Der optimale Konzentrationsbereich lag bei 1–4 p.p.m. Palladium in der Endlösung. Von 41 geprüften Ionen stören nur Cyanid und grosse Mengen Titan(IV) und Zirkonium(IV), die die Reaktion unter den gegebenen Bedingungen hemmen.

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SYSTEMATISCHE FEHLER BEI DER ABSOLUTBESTIMMUNG DES DEUTERIUMS IN ORGANISCHEN VERBINDUNGEN

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Im Zusammenhang mit der Entwicklung der massenspektrometrischen und der kernmagnetischen Molekülstrukturaufklärungsmethoden, kommt es immer häufiger vor, dass Absolutbestimmungen des Deuteriumgehaltes organischer Verbindungen zu Vergleichs- bzw. Kontrollzwecken verlangt werden.

Bei den hier betrachteten Analysenmethoden geht in der Regel der eigentlichen Messung des Isotopengehaltes eine Verbrennung der Substanz voran, nach welcher der Wasserstoff zunächst als Wasser gebunden vorliegt. Die Adsorptions- und Austauscheigenschaften des letzteren, in Zusammenhang mit dem stets mehr oder weniger komplizierten Röhren- und Gefäss-System einer Verbrennungsapparatur, verursachen bekanntlich, bereits bei den Relativbestimmungen des Deuteriums, Gedächtniseffekte und eine erhöhte Streuung der Resultate, sowohl für den Wasserstoffwert bei der Elementaranalyse als für den Deuteriumwert bei der Isotopen-Messung.

Demgegenüber war kein wesentlicher systematischer Fehler bei der Isotopenbestimmung, nach einer passenden Konditionnierung der Verbrennungsapparatur, zu erwarten. Insbesondere war nicht ohnehin ersichtlich, auf welche Art und Weise systematische Deuteriumverluste hätten auftreten sollen, da nach der erwähnten Konditionnierung der Apparatur nur noch reinster trockener Sauerstoff in die letztere eingeleitet wird.

Nachdem wiederholte Vergleiche der mittels der drei eingangs erwähnten Methoden erhaltenen Resultate vermuten liessen, dass die Isotopen-Elementaranalysenmethode um 10 bis 15% zu tiefe Deuteriumwerte lieferte, wurde letztere einer eingehenden Kontrolle unterworfen, die den Gegenstand des vorliegenden Berichtes bildet.

DIE URSACHEN DER DEUTERIUM-VERLUSTE UND DEREN BEHEBUNG

Die zur Diskussion stehende Apparatur gehört der von ANDERSON *et al.* beschriebenen Type[1], mit einer zusätzlich angebrachten Thermostatierung des Manometers zur Stabilisierung der Druckwerte. Für das Verbrennungsrohr wurde schon früher Quarz durch Supremaxglas ersetzt, was zu einer Verbesserung der Wasserwerte führte. Die Erfahrungen, welche über mehrere Jahre mit drei für ^{14}C und ^{2}H eingesetzten Verbrennungsanlagen betriebsmässig gesammelt wurden, führten zu folgenden Schlüssen:

(*1*) Die stöchiometrischen C-Werte entsprechen der diesbezüglich von ANDERSON

et al. angegebenen Genauigkeit. Standardabweichung der Einzelmessungen: $(C_{th} - C_{gef.})/C_{th} = 0.4\text{–}0.5\%$.

(*2*) Die ^{14}C-Werte (spezifische Radioaktivitäten) weisen eine Standardabweichung der Einzelmessung von 1 bis 1.5% für die Gesamtanalyse (Verbrennung + Messung als CO_2 im Proportionalzählrohr) auf.

(*3*) Die H-Werte weisen eine etwas hohe Streuung auf. Standardabweichung der Einzelmessungen $(H_{th} - H_{gef.})/H_{th} = 2\text{–}3\%$. Zudem muss der empirische Faktor, welcher bei der Berechnung der Wassermengen aus den Manometerablesungen auftritt, von Zeit zu Zeit um 1 bis 2% modifiziert werden.

(*4*) Nachdem die Messung des Deuteriums nach einer neuen Methode[2] vorgenommen wurde betrug die Standardabweichung der Einzelmessung von 1 bis 1.5%.

Für die Kontrolle der mit diesen Anlagen erhaltenen Absolutwerte für das $^2H/^1H$-Isotopenverhältnis organischer Verbindungen, wurde als Testsubstanz ein deuteriertes Diphenyl-Präparat genau bekannter Isotopenzusammensetzung verwendet.

Als Ausgangssubstanz für die Herstellung des betreffenden Präparates wurde Diphenyl-d_{10} der Firma Merck (Canada) verwendet, dessen garantierter Deuterium-Mindestgehalt mit 98 At.-% angegeben wurde, und das vor Gebrauch sublimiert wurde, wobei ein geringer, leicht gelber Rückstand zurückblieb; Smp. vor/nach Subl.: 70.4/70.9°. Der Deuteriumgehalt dieser Substanz wurde massenspektrographisch und durch kernmagnetische Resonanz ermittelt. Das Massenspektrum lieferte einen Wert von 99.1 ± 0.1 At.-% D, das NMR-Spektrum einen solchen von 99.1 ± 0.08 At.-% D. Demnach wurde für das Diphenyl-d_{10} ein Wert von 99.1 ± 0.05 At.-% D angenommen. Dieses Produkt wurde dann mit aus Alkohol umkristallisiertem und sublimiertem, nicht indiziertem Diphenyl (Smp. 69.0°) durch gemeinsames Lösen und Rekristallisieren bis auf 11.62 At.-% D isotopisch verdünnt.

Die Untersuchung ging so vor sich, dass etwa 50 Verbrennungen vorgenommen wurden, wobei mehrere für Deuteriumverluste in Frage kommende Faktoren geprüft wurden. Zur Abklärung der Frage, ob die Deuteriumverluste wirklich im Laufe der Verbrennungen, und nicht etwa erst bei den darauffolgenden Isotopenverhältnisbestimmungen auftraten, wurden ferner eine Reihe von Analysen durchgeführt, bei welchen deuteriertes Wasser als Testsubstanz diente, *d.h.*, nach der Standardprozedur "verbrannt" wurde. So zeigte es sich eindeutig, dass die Deuteriumverluste tatsächlich mit den Operationen der eigentlichen Elementaranalyse in Zusammenhang standen.

Die ersten Analysen, welche nach der bisher verwendeten Prozedur ausgeführt wurden, lieferten tatsächlich Deuteriumwerte, die selbst nach 3 Konditionnierungs-Verbrennungen noch um 14% zu tief lagen. Es zeigte sich gleich, dass die *manometrische Bestimmung des Wassers* einen empfindlichen Einfluss auf die Senkung und die Streuung der Deuteriumwerte ausübte. In der Tat stellt das Manometer einerseits, samt Ballastvolumen und Zuleitungen etwa ein Drittel der Gesamtglasfläche dar, mit welcher das Wasser im Laufe einer Analyse in Kontakt kommt. Andererseits ziehen die mit der Bestimmung des Wasserwertes verbundenen Operationen eine Verdoppelung der Verweilzeit des Wassers in der Apparatur nach sich. Da die Chemiker in der Regel mehr Gewicht den C-Werten als den H-Werten beimessen, wurde auf die stöchiometrische Bestimmung des Wassers verzichtet. Doch könnte diese ohne weiteres nach wie vor ausgeführt werden, allerdings mit einer Erhöhung der Streuung der D-Werte um 0.3 bis 0.5%.

Die Messung der *mit dem Sauerstoffstrom in die Apparatur eingeführten Wassermenge* ergab Leerwerte von 0.03 mg Wasser für eine Standardverbrennung, wobei für die Reinigung des Sauerstoffs die Vorschriften von ANDERSON *et al.* genau befolgt wurden. Diese Wassermenge wurde durch Füllen des U-Rohres unmittelbar vor dem Verbrennungsrohr mit Molekularsieben, unter Kühlung auf −80°, um einen Faktor 15 reduziert. Die Molekularsiebe werden regelmässig durch Heizen an Ort und Stelle auf 300° unter Vakuum regeneriert.

Es zeigte sich ferner, dass die an sich recht kurzzeitige *Entfernung des Verbrennungsrohr-Verschlusses zur Einführung des Schiffchens* bereits genügt, um eine Wasseraufnahme von 0.025 mg zu verursachen. Dies liesse sich nur durch einen unverhältnismässig höheren apparativen bzw. Arbeitsaufwand vermeiden. So haben wir es vorgezogen, diesem Effekt durch eine passende rechnerische Korrektur zu begegnen.

Es ist anderseits wohlbekannt, dass *Eis bei Trockeneis/Aceton-Temperatur immer noch einen Dampfdruck besitzt, der u.U. empfindliche Wasserverluste verursachen kann.* Wir haben unsererseits experimentell festgestellt, dass während einer Analyse etwa 0.04 mg Wasser aus der Falle durch den Sauerstoffstrom mitgerissen werden und zwar lediglich durch Verdunsten, unabhängig von den Verlusten, die auf eine unvollständige Kondensation als Folge einer immer zu befürchtenden Nebelbildung auftreten.

Es entsteht so ein Deuterium-Defizit, welches, je nach der anschliessend verwendeten Isotopenbestimmungsmethode, bis zum Endresultat übertragen wird. Dieser Faktor wurde zusammen mit dem vorangehenden durch eine passende rechnerische Korrektur bei der Analysenauswertung berücksichtigt.

Die beiden soeben erwähnten Effekte der Wasser-Zufuhr und -Abfuhr durch den Sauerstoffstrom heben sich in Bezug auf die Massenbilanz des Wassers zu einem guten Teil gegenseitig auf, wogegen sie sich hinsichtlich der Isotopenzusammensetzung summieren. Deshalb bietet ein befriedigender Nullwert für das Wasser bei einer Blankanalyse noch keine Gewähr für die Genauigkeit einer Deuterium-Absolutbestimmung.

Die unvermeidbaren Deuteriumverluste sind einer exakten Berechnung nich zugänglich. Doch kann eine hinreichend genaue Korrektur sehr einfach auf Grund der Annahme ermittelt werden, dass alles so vor sich geht, als ob das bei einer Analyse entstehende Wasser jeweils mit einer konstanten Menge unindizierten Fremdwassers im Laufe der Analyse vermischt würde. Es ergibt sich somit für den Korrekturfaktor: $f = (H + h)/H$, wobei H die in der Analysenprobe vorhandene Wasserstoffmenge darstellt, und h, eine Konstante, welche für die Apparatur und den Analysengang charakteristisch ist, und durch Verbrennung einer Eichsubstanz ermittelt werden muss. Die Konstanz von h wurde durch die Analyse von Proben, deren Gewichter zwischen 1 mg und 10 mg lagen, experimentell geprüft. Es wurde der Wert $h = 0.028$ mg H_2 gefunden, was einer austauschenden Menge Wasser von 0.25 mg entspricht.

Die mittlere Abweichung der Deuteriumeinzelbestimmung beträgt nun $\pm$ 1%, wobei durch Anpassung der Konstanten h für die Beseitigung des früher auftretenden systematischen Fehlers gesorgt wurde.

ZUSAMMENFASSUNG

Es werden die systematischen Fehler untersucht, welche bei der Absolutbestimmung des Deuteriums in organischen Verbindungen u.U. auftreten können. Eine Prozedur zur Beseitigung derselben wird angegeben.

SUMMARY

The systematic errors which may affect the determination of the absolute deuterium content of organic compounds are studied. An empirical procedure for eliminating them is discussed.

RÉSUMÉ

Les erreurs systématiques qui peuvent éventuellement intervenir lors de la détermination de la teneur absolue en deutérium de composés organiques sont étudiées. On indique une méthode pour y remédier.

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THE DETERMINATION OF MERCURY IN ORGANIC COMPOUNDS

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For the determination of mercury in organic material, the sample is usually decomposed by tube combustion or wet oxidation methods[1–4]. These procedures, however, are rather complicated and time-consuming. More attractive for reasons of simplicity and speed seemed to us the micro oxygen-flask combustion technique as described by SCHÖNIGER[5]. During the past few years this technique has received the attention of many micro analysts; a recent review shows its many applications[6].

Few reports have appeared on its use for the determination of mercury. SOUTHWORTH *et al.*[7] determine the mercury(II) ions formed by combustion and subsequent oxidation, by amperometric titration with EDTA. Visual EDTA titration was used by VICKERS AND WILKINSON[8]. The present report confirms the suitability of the oxygen-flask technique for mercury determination in organic material. For the analysis of the mercury(II) ions eventually formed, we used the simple visual titration method with sodium diethyldithiocarbamate, based on work by WICKBOLD[9] and elaborated by ROTH AND BECK[10]. It was found that chlorine, if simultaneously present in the sample, can be titrated immediately after the mercury determination in the same combustion run.

Analysis of mercury(II) solutions

The titration method of ROTH AND BECK[10] is based on the fact that on addition of sodium diethyldithiocarbamate to an ammoniacal solution (pH *ca.* 9) containing both mercury(II) and copper(II) ions, first the white mercury(II) carbamate complex is precipitated quantitatively and next the yellow-brown copper(II) carbamate complex. In actual titrations a copper(II) solution is added as the indicator. The colour change from white to yellow-brown is particularly sharp, even with a 0.005 *N* carbamate solution, if a few ml of chloroform are present to concentrate the colour.

The carbamate solution is not stable and should be standardized every day against standard mercuric sulfate solution. The normality of the solution was found to decrease by about 0.4% per day; this value was also observed by WICKBOLD[9].

Analysis of mercury-containing compounds

For the decomposition of the organic material the oxygen-flask technique was adopted, the products of combustion being absorbed in concentrated nitric acid. The first results for phenylmercuric acetate were low (70–90% recovery); it was observed, however, that on adjusting the pH of the nitric acid absorption solution to the re-

quired value of 9 by addition of ammonia before titration, a white precipitate appeared, probably an oxyamidomercury compound, which escaped titration with the carbamate. A mere increase of the amount of nitric acid in the absorption liquid gave no improvement. When, however, ammonia was added after 15 min of refluxing of the nitric acid absorption liquid, the precipitate did not appear and complete recovery was obtained.

A number of mercury-containing compounds were analysed by the above technique; the sample size was of the order of 8 to 10 mg. The results are recorded in Table I; the research samples were mainly alkylmercuric acetates.

TABLE I

ANALYSIS OF ORGANIC MERCURY COMPOUNDS

Compound	*Grade of sample*	*Mercury (%w)*		
		Calculated	*Found*	*Mean*
Phenylmercuric acetate	Analytical standard	59.57	59.28–59.86	59.57[a]
Mercurous acetate	Unknown purity	77.26	77.18; 76.89	77.03
A	Research sample	—	74.51; 74.23	74.37
B	do.	—	84.17; 84.56	84.37
C	do.	—	80.33; 79.87	80.10
D	do.	—	77.84; 77.73	77.79
E	do.	—	70.71; 71.26	70.99
F	do.	—	71.47; 71.13	71.30
G	do.	—	63.43; 63.97	63.70
H	do.	—	59.64; 59.75	59.70

[a] Mean of 7 results.

Analysis of compounds containing both mercury and halogen

ROTH AND BECK[10] state that chlorine, bromine and iodine in such quantities as are present in organic compounds do not interfere with the mercury determination via wet oxidation and subsequent carbamate titration. In the present method, this was confirmed for chlorine and bromine (Table II). This suggests that the solubility product of mercury(II) carbamate is small enough to be exceeded in spite of the low mercury(II) concentration created by the sparingly dissociated mercury(II) chloride or bromide. When iodine was simultaneously present, difficulties were encountered in that the mercury titration was very indistinct and recoveries were low (80–90%).

TABLE II

ANALYSIS OF ORGANIC COMPOUNDS CONTAINING MERCURY AND CHLORINE OR BROMINE

Compounds	*Mercury (%w)*	
	Calculated	*Found*
Phenylmercuric acetate (with added *p*-chlorobenzoic acid)	59.57	59.80 59.43
p-Chloromercuribenzoic acid	56.16	56.33 56.01 56.38
Phenylmercuric acetate (with added bromobenzoic acid)	59.57	59.86 59.74

It was interesting to find that chlorine could be determined along with mercury in compounds containing both these elements. Such simultaneous determination, often presenting a problem in other methods[11], can be effected by first precipitating the mercury as the carbamate as usual, then filtering the chloroform layer and next titrating the liberated chloride ion with silver nitrate solution (Table III).

TABLE III

DETERMINATION OF MERCURY AND CHLORINE IN *p*-CHLOROMERCURIBENZOIC ACID

Mercury (%w)		*Chlorine (%w)*	
Calculated	*Found*	*Calculated*	*Found*
56.16	56.01	9.93	9.82
	56.33		9.66

EXPERIMENTAL

Apparatus

A 250-ml flask of Pyrex or Jena glass with a ground joint BS B24, was fitted with a stopper (BS B24) carrying a fused-in platinum wire (30 mm long, 0.8 mm diam.) with a 15 × 20 mm piece of 40-mesh platinum gauze attached in the usual way.

A detachable metal wire gauze jacket, fitting round the conical flask[12], was used as a safety measure during the combustion.

Reagents

Copper(II) acetate solution. To 100 mg of copper(II) acetate add 2.5 ml of 20% (v/v) sulfuric acid, and dilute to 100 ml with distilled water.

Mercury(II) sulfate solution, standard. To prepare a *ca.* 0.01 *N* solution, transfer 1.4834 g of dry salt to a 1-l volumetric flask, add 20 ml of 20% (v/v) sulfuric acid and, after complete dissolution, dilute to 1 l with distilled water.

Sodium diethyldithiocarbamate solution, standard (ca. 0.01 N). Dissolve 2.5 g of the trihydrate in 1 l of distilled water. Standardize daily against the standard mercury(II) sulfate solution, as described below for determination of mercury.

All reagents were of analytical reagent grade.

RECOMMENDED PROCEDURES

Sampling and combustion

The amount of sample should not exceed 30 mg and should not contain more than 10 mg of mercury or 3.5 mg of chlorine. Weigh the sample to the nearest 0.01 mg on to the filter-paper wrapper (Schleicher and Schüll 589-2 paper cut in the usual way), fold up and clamp in the platinum gauze so that the fuse remains free and in line with the suspension wire.

Introduce 3 ml of 65% nitric acid (s.g. 1.42) into the previously *dried* flask and install in the safety jacket. Pass a rapid stream of oxygen through the flask for about 30 sec. Ignite the end of the fuse using any small flame fed with halogen-free fuel, and immediately insert the stopper. Keep the flask firmly closed and invert it to prevent the flame from touching the wall or the bottom of the flask. As the combustion slows down, tilt the flask to promote combustion of the final particles. Then shake the flask for 1 min.

Allow the flask to stand for 15 min if both mercury and chlorine are to be determined; for mercury alone, 5 min is sufficient. Remove the stopper and wash both the stopper and the gauze with 2 ml of 65% nitric acid. Install a water-cooled condenser on the flask and boil the contents for 15 min on a hot plate. Cool to room temperature and rinse the condenser, the platinum gauze and the stopper with distilled water, collecting the washings in the flask.

Determination of mercury

Add 10 ml of 10% (w/v) tartaric acid solution and 1 ml of the copper acetate solution. Adjust the pH of the solution to 9–10 with 25% (v/v) ammonia (using universal indicator paper) and add 5 ml of chloroform. Titrate with the standard diethyldithiocarbamate solution using magnetic stirring. The end-point of the titration is indicated by the permanent yellow-brown colour of the chloroform layer.

Determination of chlorine

Filter the solution through ash-free filter paper to remove the chloroform. Wash the filter with distilled water. Neutralize the solution with 30% (v/v) nitric acid using methyl red indicator, and add 12 drops in excess. Titrate the chloride potentiometrically with standard 0.01 *N* silver nitrate solution.

Blank determinations

A blank determination is required for the chlorine determination only. It is obtained by carrying out the entire procedure, omitting the sample.

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SUMMARY

The micro oxygen-flask combustion technique in combination with the sodium diethyldithiocarbamate titration is a rapid and useful method for determination of mercury in organic compounds. Chlorine and bromine do not interfere; iodine does. When the organic compound contains both mercury and chlorine, the chloride ions can be titrated argentometrically in the same absorption solution after the titration of mercury.

RÉSUMÉ

La microméthode de combustion, avec flacon d'oxygène, combinée avec un titrage au diéthyldithiocarbamate de sodium constitue une méthode rapide et pratique pour le dosage du mercure dans les composés organiques. Le chlore et le brome ne gênent pas, contrairement à l'iode dont la présence est nuisible. Lorsque la substance organique renferme simultanément mercure et chlore, on peut doser dans une même solution le chlore par titrage argentométrique, après titrage du mercure.

ZUSAMMENFASSUNG

Als eine schnelle und bequeme Methode zur Bestimmung von Quecksilber in organischen Verbindungen wird die Verbrennung im Sauerstoffkolben mit darauffolgender Titrierung mit Natriumdiäthyldithiocarbamat beschrieben. Chlor und Brom stören im Gegensatz zu Jod die Bestimmung nicht. Liegen gleichzeitig Quecksilber und Chlor vor, so können nach der Titration des Quecksilbers die Chlorionen in derselben Lösung argentometrisch titriert werden.

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A COMPARISON OF THE PERFORMANCE OF AMMONIUM PHOSPHATE, TITAN YELLOW AND EDTA METHODS FOR THE DETERMINATION OF MAGNESIUM IN BLOOD SERUM

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The importance of magnesium deficiency in human and veterinary medicine has grown considerably in recent years and consequently there has been an increased interest in methods for the determination of this element in blood serum and plasma. This has resulted in the publication of a large number of procedures, most of which are variations of chemical methods. The majority of these papers contain sufficient results from replicate analyses and recovery experiments to give an estimate of the precision, but tests for systematic errors have seldom been reported and few attempts have been made to compare the performance of different methods on an adequate scale or to determine what influence a particular modification to a method has on the results. There is, therefore, little information in the literature to guide the analyst in the choice of a method.

This paper describes a series of comparative tests which were carried out on 4 chemical methods that are representative of those in common use. Two of these were variations of the ammonium phosphate precipitation method involving the spectrophotometric estimation of phosphate in the precipitate, one was a spectrophotometric method using Titan Yellow and the other an EDTA titration method with a visual end-point. The tests were designed to give a measurement of the precision of each method, to detect systematic errors, and to determine whether preliminary treatment of the sample, *i.e.* wet-ashing or deproteinisation by precipitation with trichloroacetic or acetic acids, had any influence on the results.

EXPERIMENTAL

Methods

Ammonium phosphate. One of the ammonium phosphate methods (A) had been in routine use at Moredun Institute for several years and was derived from that described by GREEN AND ALLCROFT[1] by making the following modifications.

(*1*) The entire procedure was standardised with aliquots of a solution of 'Specpure' magnesium sulphate instead of calibrating the final stage with potassium dihydrogen phosphate.

(*2*) The measurement of colour intensity was carried out with a Unicam SP350 spectrophotometer, at 680 mμ in 1-cm cells, instead of with a visual colorimeter.

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(3) The precipitation of magnesium ammonium phosphate was allowed to take place for 3 h in a refrigerator instead of overnight at room temperature.

The second ammonium phosphate method (B) differed in that precipitation of magnesium ammonium phosphate was allowed to take place overnight in the refrigerator and the precipitate was washed 3 times with 33% ammonia instead of twice with this solution and once with an alcoholic ammonia solution.

The standard curves were calculated by the method of least squares and over their linear regions were described by regression equations of the following general form: μg Mg = (slope $\times$ absorbancy) + intercept. The values obtained for the slopes and intercepts and their standard deviations are shown in Table I. In both procedures the intercept was a characteristic of the system, presumably reflecting the solubility of the precipitate, and was not due to a failure to correct for the blank value or to differences in the optical characteristics of the spectrophotometer cells. A small but significant difference was found in the slopes of the two curves (P = 0.05–0.01). Larger differences were shown by the intercepts and scatter of the points, both being greater for method B ($P = < 0.001$).

TABLE I

CHARACTERISTICS OF STANDARD CURVES OF METHODS USED FOR THE ESTIMATION OF MAGNESIUM IN SERA

(The values shown are expressed as μg Mg)

Method	*Slope* $\pm$ *S.D.*	*Intercept* $\pm$ *S.D.*	*Standard error of fit*
Ammonium phosphate A (standardised with Mg)	71.50 $\pm$ 0.56	1.99 $\pm$ 0.54	$\pm$0.63
Ammonium phosphate B (standardised with Mg)	70.09 $\pm$ 0.14	3.59 $\pm$ 0.96	$\pm$1.01
Ammonium phosphate (standardised with phosphate)	74.12 $\pm$ 1.64	$-$0.70 $\pm$ 1.47 (Not significant)	$\pm$2.28
Titan Yellow (standardised on one occasion)	155.9 $\pm$ 2.82	$-$1.02 $\pm$ 0.41	$\pm$0.76
Titan Yellow (standardised over 4 months)	159.3 $\pm$ 3.54	$-$1.87 $\pm$ 0.52	$\pm$1.03

When the method was calibrated by taking equivalent amounts of phosphate through the final stage, as is the usual practice, the curve obtained (Table I) differed from those described above in that it passed through the origin and had a different slope (P = 0.05–0.01). The use of this curve would introduce negative errors which would vary according to the magnesium content of the sample and the analytical procedure used. For example, with method A the mean error would vary from about 3 to 15% when analysing the range of samples usually encountered.

Titan Yellow. The Titan Yellow method studied was similar to that described by HEAGY[2]. Minor modifications were made as follows.

(*1*) 1 ml of a 0.02% solution of the reagent was used instead of 1.5 ml of a 0.05% solution in order to reduce the absorbancy of the blank to approximately 0.55.

(*2*) The final volume was fixed at 10 ml instead of 12.5 ml.

(*3*) Absorbancies were measured against water at 510 mμ with a Unicam SP 1400 spectrophotometer (1-cm cell) instead of with an Evelyn instrument.

Since the reagent is unstable in solution, three standards, *viz.* 10, 20 and 30 μg Mg, were included in each series of determinations and a new standard curve was plotted each time. The mathematical characteristics of the linear portions of these curves are shown in Table I. When the results obtained over a period of 4 months were used for the calculation of the curve, the deviation of the points from it was significantly greater than that of points determined on the same day ($P = 0.05$–0.01). This reflects the instability of the Titan Yellow solution and changes in other factors which affect its reaction with magnesium. The intercepts were found to be due to the imperfect matching of the optical cells. No interference from amounts of calcium likely to be present in serum could be detected, which is in agreement with the findings of several other workers.

EDTA. The EDTA method used was identical with that described by TODD[3]. Calcium was removed by precipitation with oxalate and the magnesium in the supernatant liquid was titrated with EDTA using Eriochrome Black T as an indicator. A separate calibration factor was determined for each series of analyses. The mean of the values obtained for this factor throughout the investigation was 17.6 μg Mg/ml EDTA solution. Its standard error was ± 1.22 μg Mg which reflects changes in the concentration of the solution and differences between the serum samples.

Treatment of samples. The ammonium phosphate and Titan Yellow methods were applied to untreated serum and also to samples which had been wet-ashed by the method described by MIDDLETON AND STUCKEY[4], or had been deproteinised by precipitation with acetic acid and heat as described by HUNTER[5], or with trichloroacetic acid. The latter method was similar to that used by HEAGY[2] except that the precipitate was separated by centrifugation instead of by filtration. 25-ml samples of serum were wet-ashed and the solution made up to 50 ml. Appropriate aliquots were then taken for analysis by the various procedures.

Precautions were taken to prevent contamination of the sample by adventitious traces of magnesium during storage and analysis. These included the cleaning of glassware with nitric acid and the removal from the laboratory of important sources of magnesium such as face powder and asbestos[6].

Serum samples

Large specimens of blood were collected in Pyrex 2-l beakers from 3 sheep when they were slaughtered. These were allowed to stand for 24 h at room temperature and the serum was then separated by centrifugation to give specimens A, C and D. After mixing thoroughly each specimen was transferred to 2-oz. screw-capped glass bottles and stored at about —20° until required.

Design of tests

Besides providing a comparison of precision and accuracy, the experiments were designed to detect systematic errors which were not proportional to the amount of serum taken for analysis. This was achieved by carrying out replicate analyses on

different amounts of serum in such a way as to keep the influences of these variables orthogonal to each other, thereby facilitating the statistical examination of the results for evidence of variations with sample size. Tests of this kind have been advocated by YOUDEN[7,8] who has drawn attention to the information that they can provide and for this reason has deplored the common practice of using the same amount of sample when replicate estimations are carried out to determine the precision of the method. Furthermore, the estimate of the precision obtained in this way is often unrealistic even when no systematic errors are present.

In our experiments analyses were carried out in triplicate on 3 different amounts of each serum sample, making a total of 9 determinations by each procedure on each sample. Serum D was a smaller specimen than the others and was insufficient for the complete series of 117 analyses. The amounts used were 0.5, 1.0 and 2.0 ml for the ammonium phosphate and EDTA methods, and 0.2, 0.4 and 0.8 ml for the Titan Yellow method.

The results were assessed statistically using conventional techniques such as the analysis of variance procedure which was used to detect systematic errors, and Student's 't' test[9-13]. The latter was used for comparing groups of results whose variances were not significantly different as shown by the variance ratio or F test. When the variances differed the Brehrens–Fisher test was applied[14].

RESULTS

Internal variation of results

The mean values and standard deviations for each set of 9 results are shown in Table II, and their variances are summarised in Table III. It can be seen that those obtained with the EDTA method had by far the smallest scatter, the variance being much lower than that of any other set of results ($P = < 0.001$). Of these, the results for untreated serum obtained with ammonium phosphate method A lie next in order of increasing variance, followed by those for wet-ashed samples which were analysed

TABLE II

SUMMARY OF RESULTS OBTAINED FOR THE MAGNESIUM CONTENT OF SERA

(The figures shown are the mean values and standard deviations of the 9 results obtained for each serum by each procedure and are expressed as mg Mg/100 ml serum)

Serum	*Treatment*	*Method*			
		Ammonium phosphate		*Titan Yellow*	*EDTA*
		A	*B*		
A	None	1.70 ± 0.11	1.90 ± 0.16	2.02 ± 0.26	1.68 ± 0.03
	Deproteinised with TCA	1.92 ± 0.32	1.69 ± 0.47	1.52 ± 0.12	—
	Deproteinised with HAc	1.74 ± 0.14	1.91 ± 0.36	1.46 ± 0.35	—
	Wet-ashed	1.68 ± 0.14	2.11 ± 0.37	1.79 ± 0.14	—
C	None	2.52 ± 0.08	2.95 ± 0.33	3.34 ± 0.30	2.54 ± 0.05
	Deproteinised with TCA	2.71 ± 0.20	2.56 ± 0.56	2.89 ± 0.28	—
	Deproteinised with HAc	2.44 ± 0.35	3.17 ± 0.41	2.81 ± 0.29	—
	Wet-ashed	2.58 ± 0.21	3.13 ± 0.50	2.87 ± 0.13	—
D	None	1.95 ± 0.09	2.31 ± 0.46	—	1.96 ± 0.06
	Deproteinised with TCA	—	2.00 ± 0.38	—	—
	Deproteinised with HAc	—	2.12 ± 0.33	—	—

TABLE III

VARIANCES OF RESULTS OBTAINED FOR THE MAGNESIUM CONTENT OF SERA

(The figures shown were calculated from the combined results for the different sera)

Treatment	*Method*			
	Ammonium phosphate		*Titan Yellow*	*EDTA*
	A	*B*		
None	0.010	0.117	0.077	0.003
Deproteinised with TCA	0.072	0.225	0.046	—
Deproteinised with HAc	0.071	0.135	0.102	—
Wet-ashed	0.032	0.195	0.018	—

with the Titan Yellow method and the former method respectively. The remainder had such a wide scatter that they would be unacceptable for most purposes. There was a marked difference between the two ammonium phosphate methods in this respect, with method B generally giving a much greater variance. The preliminary treatment of the sample did not appear to have a consistent effect on the precision of different methods. For example, wet-ashing increased the scatter in the case of ammonium phosphate method A, but decreased it when the Titan Yellow method was used ($P = 0.01$–0.001).

Systematic errors

The standard deviations and variances shown in Tables II and III reflect any systematic errors present as well as those of a random nature and in order to detect the presence of the former, the results were examined by the analysis of variance procedure. In the first instance, the results obtained by one analytical procedure for all the sera were pooled for this purpose; to illustrate the way in which the calculations were carried out the results obtained with ammonium phosphate method B on TCA-

TABLE IV

RESULTS OBTAINED WITH AMMONIUM PHOSPHATE METHOD B FOR SERA TREATED WITH TRICHLOROACETIC ACID (mg Mg/100 ml)

Serum	*Replicates*	*Volume serum taken (ml)*		
		0.5	*1.0*	*2.0*
A	1	2.45	1.63	1.46
	2	2.10	1.50	1.35
	3	2.25	1.40	1.06
	Mean	2.27	1.51	1.29
C	1	3.30	2.53	2.11
	2	3.30	2.30	2.08
	3	3.30	2.20	2.08
	Mean	3.30	2.34	2.09
D	1	2.75	1.95	1.66
	2	2.10	1.80	1.75
	3	2.45	1.88	1.63
	Mean	2.43	1.88	1.68

TABLE V

ANALYSIS OF VARIANCE OF RESULTS OBTAINED WITH AMMONIUM PHOSPHATE METHOD B FOR SERA TREATED WITH TRICHLOROACETIC ACID

Source of variance	*Sum of squares*	*Degrees of freedom*	*Variance*	*F*
Sera	3.6676	2	1.8338	71.4[a]
Amounts	4.7485	2	2.3743	80.6[a]
Interaction	0.1859	4	0.0465	1.8[b]
Residual (replicates)	0.4623	18	0.0257	—
Total	9.0643	26	—	—

[a] Highly significant ($P = <0.001$).
[b] Not significant ($P = >0.05$).

treated sera are given in Table IV and their statistical analysis is shown in Table V. A significant interaction term was obtained for 3 sets of results, *viz.* the ammonium phosphate method B for untreated sera, the Titan Yellow method for wet-ashed sera and the EDTA method for untreated sera, indicating that the influence of the amount of serum taken for analysis on the result varied from serum to serum, and in these cases the analysis of variance was repeated using the results for each serum separately as shown in Table VI.

The conclusions drawn regarding the influence of sample size on the result are summarised in Table VII from which it can be seen that a systematic error was found consistently in the results obtained with ammonium phosphate method B. In each case the apparent magnesium content of the serum decreased with increasing sample size as shown by the results given in Table IV. On the other hand there was no evidence of this error in the results obtained with the other ammonium phosphate procedure.

A variation of this kind is easier to detect when the random errors are small and

TABLE VI

ANALYSIS OF VARIANCE OF RESULTS OBTAINED WITH AMMONIUM PHOSPHATE METHOD B ON UNTREATED SERA

Serum	*Source of variance*	*Sum of squares*	*Degrees of freedom*	*Variance*	*F*
A	Amounts	0.1378	2	0.0689	5.7[a]
	Replicates	0.0723	6	0.0120	—
	Total	0.2101	8	—	—
C	Amounts	0.8570	2	0.4285	70[b]
	Replicates	0.0350	6	0.0059	—
	Total	0.8920	8	—	—
D	Amounts	1.5803	2	0.7902	37[b]
	Replicates	0.1278	6	0.0213	—
	Total	1.7081	8	—	—

[a] Significant ($P = 0.05$–0.01).
[b] Highly significant ($P = <0.001$).

TABLE VII

VARIATION OF RESULTS OBTAINED FOR THE MAGNESIUM CONTENT OF SERA WITH SAMPLE SIZE

Treatment	*Statistical significance of variation*			
	Method			
	Ammonium phosphate		*Titan Yellow*	*EDTA*
	A	*B*		
None	[a]	A[b], C and D[c]	[a]	A and D[b], C[d]
Deproteinised with TCA	[a]	[c]	[a]	—
Deproteinised with HAc	[a]	[c]	[a]	—
Wet-ashed	[a]	[c]	A[b],C[a]	—

[a] Not significant ($P = >0.05$).
[b] Significant ($P = 0.05$–0.01).
[c] Highly significant ($P = <0.001$).
[d] Very significant ($P = 0.01$–0.001).

this is illustrated by the results obtained with the EDTA method which had a small scatter, the coefficient of variation being 1.8–3.1%, and showed significant changes with sample size. However, these variations did not show a consistent pattern and were probably due to random rather than systematic errors. Only one set of results obtained with the Titan Yellow method (for wet-ashed serum A) showed a significant variation of this kind but again it was not consistent with the change in sample size and may therefore be attributed to random errors.

Accuracy of methods

For each serum there was good agreement between the values for the magnesium content obtained with 3 procedures, *viz.* ammonium phosphate method A with untreated and wet-ashed samples and the EDTA method, and this agreement was much closer than that between any other results. Since the internal scatter of the former results was relatively small, and the results were obtained with basically different methods, it was felt that they would give the best estimate of the true magnesium content of the sera. The means of these results for each serum were therefore taken as the most accurate values and are compared with those obtained by the other procedures in Table VIII. This comparison is also shown for sera A and C in Fig. 1 which is a graph of the type suggested by YOUDEN[8], the estimated true values being used to draw the quadrants.

It can be seen that on this basis none of the procedures gave consistently low results and some gave large positive errors for both sera. Those involving ammonium phosphate method A generally gave more accurate results than the Titan Yellow or other ammonium phosphate procedures. Three of the Titan Yellow procedures gave mean results within 15% of the true values but the fourth, carried out on untreated samples, was much less accurate ($P = <0.001$). Except for this procedure, 3 of those involving the use of ammonium phosphate method B gave the least accurate results of all. On the other hand, the fourth procedure involving this method, in which the samples were deproteinised with trichloroacetic acid, gave extremely close agreement with the true values but in view of the large internal variation in these results which was noted above, this was obviously due to the fortuitous choice of sample size.

TABLE VIII

DEVIATION OF MEAN VALUES FROM THE ESTIMATED TRUE MAGNESIUM CONTENT OF SERA

(The differences shown are expressed as mg Mg/100 ml serum)

Treatment	*Serum*	*Estimate of true Mg content*[a]	*Method*			
			Ammonium phosphate		*Titan Yellow*	*EDTA*
			A	*B*		
None	A	1.69	+0.01[b]	+0.21[e]	+0.33[e]	−0.01[b]
	C	2.55	−0.03[b]	+0.40[e]	+0.79[e]	−0.01[b]
	D	1.96	−0.01[b]	+0.35[e]	—	0.00[b]
Deproteinised with TCA	A	1.69	+0.23[e]	0.00[b]	−0.17[e]	—
	C	2.55	+0.16[d]	+0.01[b]	+0.34[e]	—
	D	1.96	—	+0.04[b]	—	—
Deproteinised with HAc	A	1.69	+0.05[b]	+0.22[d]	−0.23[e]	—
	C	2.55	−0.11[b]	+0.62[e]	+0.26[e]	—
	D	1.96	—	+0.16[b]	—	—
Wet-ashed	A	1.69	−0.01[b]	+0.42[e]	+0.10[d]	—
	C	2.55	+0.03[b]	+0.58[e]	+0.32[e]	—

[a] The estimated true values for sera A and C were obtained by averaging the pooled results obtained with ammonium phosphate method A for untreated and wet-ashed sera and with the EDTA method. Wet-ashed samples of serum D were not analysed with the ammonium phosphate method and the true value was derived from the other two sets of results.
[b] Not significant ($P = >0.05$).
[c] Very significant ($P = 0.01$–0.001).
[d] Significant ($P = 0.05$–0.01).
[e] Highly significant ($P = <0.001$).

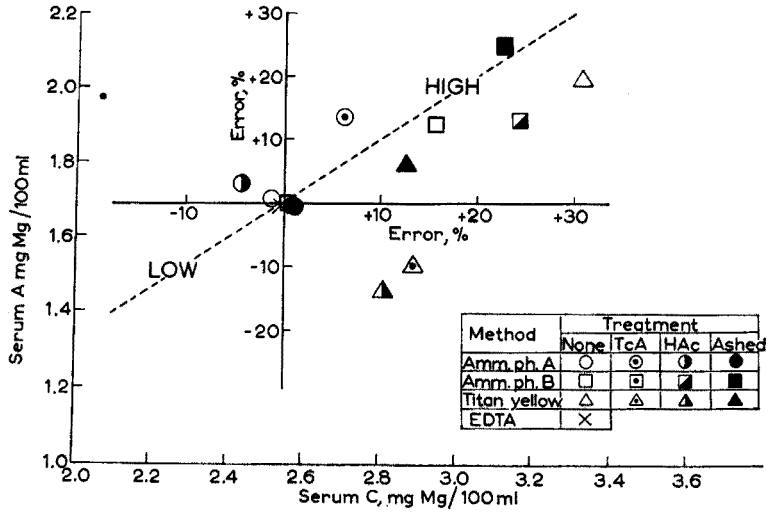

Fig. 1. Mean values obtained for the magnesium content of sera A and C in comparison with the estimated true values. The broken line indicates equal errors.

The general character of the results given by each method can be seen in Fig. 2, which is a histogram of the deviations of the individual results for sera A and C from the estimated true values. For the construction of this diagram the treatment of the sample was ignored. It shows clearly the wide scatter of the results given by ammonium phosphate method B and the Titan Yellow method, and the preponderance of positive errors in comparison with the results which were taken for estimating the true values. Most of the latter results lie within $\pm 5\%$ of the true values; all those outside these limits were obtained with ammonium phosphate method A.

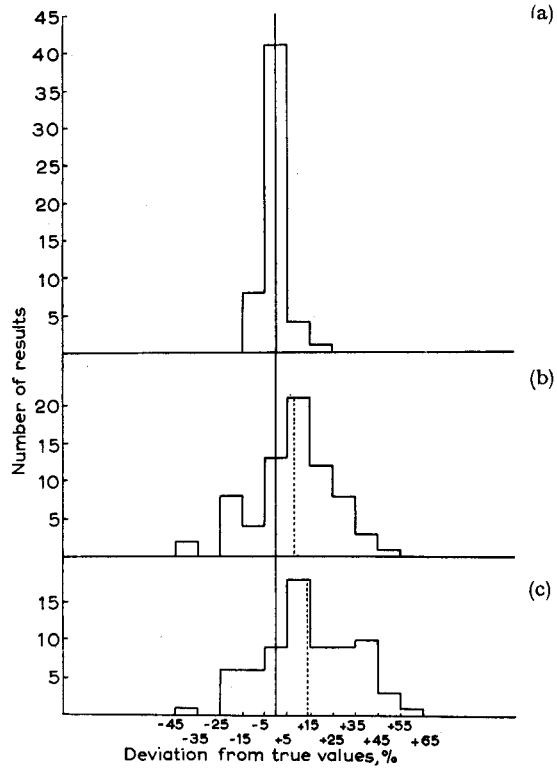

Fig. 2. Histograms of percentage deviations from the estimated true values for the magnesium content of sera A and C. Mean values are indicated by dotted lines. (a) Results taken for estimating true values (ammonium phosphate method A and EDTA); (b) Titan Yellow method; (c) ammonium phosphate method B.

The preliminary treatment of the sample did not appear to have any consistent effect on the results obtained by different methods. When the ammonium phosphate method B was used, the results for wet-ashed samples were on the whole significantly higher than those for the untreated samples or those which had been deproteinised with trichloroacetic acid or acetic acid (Table IX), but this effect was not shown by the results obtained with the other methods. With the other ammonium phosphate method, the results for samples which had been deproteinised with trichloroacetic acid were significantly higher than each of the other three sets of results ($P = 0.05$–0.001).

TABLE IX

DEVIATION OF MEAN VALUES FOR THE MAGNESIUM CONTENT OF SERA FROM THOSE OBTAINED FOR WET-ASHED SAMPLES WITH THE SAME METHOD

(The differences shown are expressed as mg Mg/100 ml serum)

Treatment	*Serum*	*Method*		
		Ammonium phosphate		*Titan Yellow*
		A	*B*	
None	A	+0.02[a]	−0.21[a]	+0.23[b]
	C	−0.06[a]	−0.18[a]	+0.47[c]
Deproteinised with TCA	A	+0.24[a]	−0.42[b]	−0.27[c]
	C	+0.13[a]	−0.57[b]	+0.02[a]
Deproteinised with HAc	A	+0.06[a]	−0.20[a]	−0.33[b]
	C	−0.14[a]	+0.04[a]	−0.06[a]
Mean difference		+0.06[a]	−0.24[b]	+0.01[a]

[a] Not significant ($P = >0.05$).
[b] Significant ($P = 0.05$–0.01).
[c] Highly significant ($P = <0.001$).

DISCUSSION

Although many procedures have been devised for the determination of magnesium in serum or plasma few attempts have been made to compare their performance on an adequate scale. The investigations which have been carried out have consisted of the analysis of a series of serum samples from different subjects by the selected procedures, often without replication. Several authors appear to have regarded a comparison of results for groups of different normal subjects as a comparison of methods but it is clear that biological variations must be eliminated in investigations of this kind. In the experiments described in this paper a different approach was explored, which involved detailed studies on a few samples rather than single analyses on a larger number of samples. By varying the amount of sample taken for analysis it was possible to detect systematic errors which otherwise might have remained hidden. YOUDEN[7,8] has also drawn attention to the fact that such experiments are seldom carried out and has discussed the information that they can provide.

The results obtained with ammonium phosphate method B provide a good illustration of the value of this approach to the problem. Variation of the amount of serum taken for analysis had a marked effect on the results, which generally increased as the sample size was decreased, and this error was not removed by ashing or deproteinisation of the serum. The results approached the estimated true values most closely when 1 or 2 ml of serum was used and it is relevant to note that these are the amounts of untreated serum or plasma which are generally taken when the method is used for routine work on humans or large animals. This variation was studied in more detail with another specimen of untreated serum and the results are plotted in Fig. 3, which shows clearly the enormous increase in the error when the sample size falls below 1 ml. It is frequently necessary to analyse samples of this size when studying children or small animals, and it is clear that very inaccurate results would be obtained with this

method. A plot of the apparent amount of magnesium in the sample against the sample size gave a rectilinear regression line with a highly significant intercept, which was described by the expression: $\mu g\ Mg = 18.26 \times$ ml serum $+ 8.68$.

This error accounts for about 90% of the variation in the results shown in Tables II and III and Fig. 2, and when it is removed during the analysis of variance, the residual variance, due to replication, is similar to that of the results obtained during standardisation and also with the other ammonium phosphate method on untreated samples. Thus the precision of method B is satisfactory and comparable with that of method A when a fixed volume of serum or plasma is taken for analysis, which of course is the usual practice.

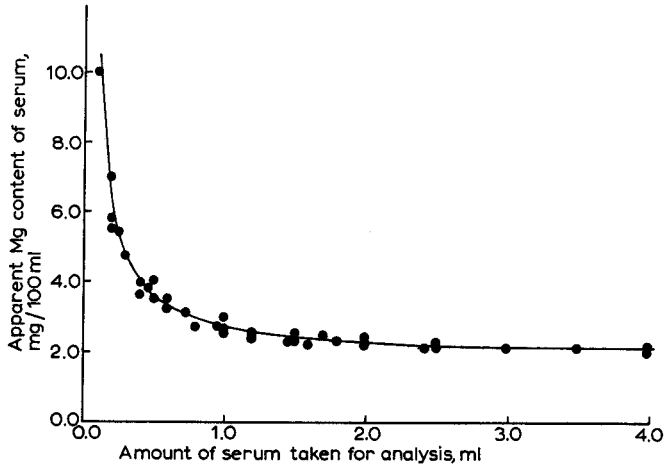

Fig. 3. Effect of sample size on the results obtained with ammonium phosphate method B.

No systematic error of this kind was evident in the results obtained with ammonium phosphate method A which differed in that the precipitation of magnesium ammonium phosphate was allowed to take place for 3 h at about 4° instead of overnight at this temperature, as is frequently the custom, and an alcoholic ammonia solution was used instead of 33% aqueous ammonia for the final washing of the precipitate. It is possible that both stages contribute to the error associated with method B but it is likely that the precipitation stage is more important, since it was noticed that with this method the precipitates were much more sticky and difficult to suspend in the washing fluid than those obtained with method A. This was not apparent during the determination of the standard curve which was similar to that for method A indicating that the interfering factors were derived from the serum. The effect of the time and temperature of precipitation on the results has also been investigated by SIMONSEN, WESTOVER AND WERTMAN[15] who apparently obtained similar values when precipitation was carried out for 2 and 16 h at 4°, but since they used the same amount of serum throughout (probably 2 ml) their results would probably not reveal the error described above.

Of the methods tested, the ammonium phosphate method A and the EDTA titration method gave the most precise and accurate results and these were in good agreement with each other, a finding which is consistent with the results obtained by other authors[16-18]. The values obtained with ammonium phosphate method A on untreated serum were practically identical with those for wet-ashed samples but deproteinisation increased the random errors and decreased the accuracy. The main disadvantages of this method, which is usually performed on untreated serum, are that it is tedious and requires a considerable degree of manipulative skill. Considering the nature of these operations and the small amounts of magnesium involved, it is perhaps surprising that it gives such good results.

The EDTA method was applied only to untreated serum samples, none of which was particularly pigmented, and the end-points were detected visually. Several workers have found that with pigmented samples the end-points are difficult to detect in this way and this has led to the development of spectrophotometric titration procedures. It is therefore likely that in routine use the errors associated with this method would be considerably larger than those found in our investigation. If such errors were not serious, the EDTA method would be preferable to the ammonium phosphate method because of its speed and simplicity.

A low degree of precision and very inaccurate results with a preponderance of positive errors were obtained with the Titan Yellow method, particularly when it was applied to untreated serum as was recently advocated by SPARE[19]. A considerable proportion of the scatter in the results appeared to be due to random errors, some of which undoubtedly arose from the high blank value. Other authors have compared this method with the ammonium phosphate[20] and EDTA methods[21,22] by analysing a series of serum samples and in two cases the Titan Yellow method again gave higher results[20,21].

SUMMARY

Detailed analyses were carried out on 3 specimens of serum to compare the precision and accuracy of 4 typical chemical methods for magnesium, and to determine whether wet-ashing or deproteinisation with trichloroacetic or acetic acid affected the results. Two ammonium phosphate precipitation methods involving spectrophotometry of phosphate in the precipitate, a spectrophotometric method with Titan Yellow and a visual EDTA titration method were studied. The analyses were designed to reveal systematic errors and the results were analysed statistically.

One ammonium phosphate method on untreated and wet-ashed samples and the EDTA method gave concordant and relatively precise results, which were used to estimate the true values. On this basis, another ammonium phosphate method involving overnight precipitation, gave consistently high results. The Titan Yellow method gave large random errors, particularly when proteins were not removed. The preliminary treatment of the samples did not appear to have any consistent effect.

RÉSUMÉ

Les auteurs ont effectué une étude du dosage du magnésium dans le sérum sanguin, en comparant 4 méthodes: 2 méthodes par précipitation au phosphate d'ammonium, avec spectrophotométrie du phosphate précipité; une méthode spectrophotométrique au jaune titane et une méthode par titrage à l'EDTA. Ils ont également examiné l'influence du traitement préliminaire des échantillons: minéralisation par voie humide ou déprotéination au moyen d'acide trichloracétique ou d'acide acétique.

ZUSAMMENFASSUNG

An 3 Serumproben wurden detaillierte Analysen ausgeführt, um die Genauigkeit und Reproduzierbarkeit von 4 chemischen Methoden zur Magnesiumbestimmung zu vergleichen. Ferner sollte

bestimmt werden, ob die Nassveraschung oder Deproteinisation mit Trichloressig- oder Essigsäure die Ergebnisse beeinflussen. Zwei Ammoniumphosphatfällungsmethoden einschliesslich der spektralphotometrischen Bestimmung des Phosphats im Niederschlag, eine spektralphotometrische Methode mit Titangelb und eine visuelle EDTA Titrationsmethode wurden untersucht.

Eine der Ammoniumphosphatmethoden, bei der mit unbehandelten und nassveraschten Proben gearbeitet wurde, und die EDTA-Methode gaben übereinstimmende und relativ genaue Werte. Sie wurden zur Bestimmung der wahren Werte benutzt. Auf dieser Basis ergab die andere Ammoniumphosphatmethode, bei der der Niederschlag über Nacht stehengelassen wurde, übereinstimmend hohe Ergebnisse. Die Titangelbmethode ergab grosse zufällige Fehler besonders dann, wenn die Proteine nicht beseitigt wurden.

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DETERMINATION OF IODINE IN COMMON SALT BY AN AUTOMATIC REACTION-RATE METHOD

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Very few methods have been described in the literature for the determination of iodine in common salt[1]. An automatic reaction-rate method for the ultramicro determination of protein-bound iodine and iodine in natural waters was described recently[2,3]. The same general automatic procedure is well suited for the determination of iodine in common salt.

The method utilizes the acceleration of the reaction between ceric sulfate and arsenious acid by iodine. The time required for the reaction to consume a fixed amount of ceric ions and therefore for the absorbance to decrease by a preselected amount (about 0.06 unit) is measured automatically and related directly to the iodine concentration.

Two methods for the determination of iodine in salt were used. The direct method permits the use of a calibration curve obtained with pure solutions of potassium iodide in distilled water and was applied to the analysis of solutions containing less than 0.5% sodium chloride. The standard addition method was applied to the analysis of solutions containing more than 0.5% sodium chloride.

Rapid operation, easily mastered technique, and high sensitivity are the main features of the automatic method. Measurements obtained on salt samples containing 3 to 7500 μg of iodine per 100 g were precise to within 2% or 0.3 μg of iodine per 100 g, whichever is the greater. The relative error for the recovery of 40 to 100 μg of iodine added per gram of iodized salt was within $\pm 3\%$. Measurement times varied from a few sec to about 2 min.

EXPERIMENTAL

Apparatus and reagents

These were the same as previously described[3] with the following additions.

Working iodide solution II, 2000 p.p.b. It is prepared from stock solution I by appropriate dilution.

Working iodide standards III, 50, 100, and 150 p.p.b. They are prepared from the 1000 p.p.b. of iodide solution by appropriate dilution and are used for the preparation of the calibration curve.

All reagents were prepared in triple distilled water.

PROCEDURE

Procedure for common salt

Dissolve 2.50 g of common salt in about 30 ml of water. Filter the solution through filter paper previously washed thoroughly with hot water (or centrifuge). Wash the filter with small increments of water and dilute the solution to 50 ml. From the 5% salt solution prepare 10 ml of 0.5% salt solution by dilution with water.

(1) Direct method. Use 3.00 ml of the 0.5% salt solution for analysis and carry out the measurement by the low-range procedure, as previously reported[3].

If the measurement time is larger than 120 sec, more accurate results are obtained if the 5% salt solution is analyzed by the standard addition method. Both the low-range and the high-range procedures can be used.

(2) Standard addition method. (a) Low-range procedure. In the method of standard addition, measurements are taken for 3 solutions, solution A being the unknown salt solution and solutions B and C containing in addition 20 and 10 p.p.b. of iodide respectively. To prepare solution B, transfer 0.250 ml of the 2000 p.p.b. of iodide solution into a 25-ml volumetric flask using a 0.25-ml syringe and dilute to the mark with solution A. To prepare solution C, mix 5 ml of solution A with 5 ml of solution B. Carry out the measurement according to the low-range procedure[3]. *(b) High-range procedure.* Solution A is the unknown salt solution and solutions B and C contain in addition 100 and 50 p.p.b. of iodide respectively. Solutions B and C are prepared as previously described, but the 10,000 p.p.b. of iodide solution is substituted for the 2000 p.p.b. of iodide solution. Carry out the measurement as previously reported[3].

Procedure for iodized salt

Prepare a 0.1% (w/v) solution of iodized salt. Use 3.00 ml of this solution for analysis by the high-range procedure[3].

Determination of blank

As previously reported[3].

Calibration curves

For the low-range procedure, use the standard solutions containing 3, 9, and 15 p.p.b. of iodide; for the high-range procedure, the standards containing 50, 100, and 150 p.p.b. of iodide.

Calculations

When the direct method is used, the concentration of the salt solution is read directly in p.p.b. of iodide from a calibration curve obtained by plotting linearly reciprocal times *vs.* iodide concentration of the standards.

In the standard addition method a working curve is obtained by plotting reciprocal times *vs.* iodide concentration of solutions A, B and C. Solution A, the salt solution without added iodide, is plotted as 0 p.p.b. of iodide and its actual concentration is obtained from the intercept of the working curve with the abscissa. The blank should be subtracted from this value.

The iodine content of the salt is calculated from the concentration of the salt solution by multiplying by the appropriate dilution factor.

RESULTS AND DISCUSSION

Table I shows the results as read directly from the readout dial of the automatic system for a typical series of determinations. In the same Table, the values obtained by the direct method for iodine in two salt samples — common and iodized salt — are compared with those obtained by the standard addition method. The 1 to 2% reproducibility for each sample is typical of the precision of the direct method. The agreement between the two methods is within 1 to 2% which is typical of many

TABLE I

AUTOMATIC RESULTS FOR IODINE IN SODIUM CHLORIDE

Sample	*Procedure*		*Direct time readout*[a] *(sec)*	*Iodine (p.p.b.)*	
	Low range	*High range*		*Standard addition method*	*Direct method*
3 p.p.b. iodide	+		153.0		
9 p.p.b. iodide	+		65.2		
15 p.p.b. iodide	+		41.6		
0.2% (w/v) NaCl A (common salt)	+		68.0	8.6	8.6, 8.4
0.2% (w/v) NaCl A + 10 p.p.b. iodide	+		33.2		
0.2% (w/v) NaCl A + 20 p.p.b. iodide	+		22.3		
50 p.p.b. iodide		+	96.5		
100 p.p.b. iodide		+	48.2		
150 p.p.b. iodide		+	31.7		
0.1% (w/v) NaCl B (iodized salt)		+	66.6	72.0	71.2, 72.0
0.1% (w/v) NaCl B + 50 p.p.b. iodide		+	40.0		
0.1% (w/v) NaCl B + 100 p.p.b. iodide		+	28.0		

[a] Average of two values.

analyses performed using commercially available salt samples. Both methods can be used for the analysis of salt containing more than 100 μg of iodine per 100 g; for the analysis of salt containing smaller amounts of iodine only the standard addition method should be used. The direct method should be preferred, whenever possible, because it is faster and requires smaller amounts of samples; thus in the analysis of iodized salt as little as 3 mg of sample might be sufficient for a complete analysis.

Iodized salt in the United States contains about 1 part of potassium iodide to 10,000 parts of salt. This amount corresponds to 76.5 p.p.b. of iodine when a 0.1% solution is prepared and such a solution falls within the 50 to 250 p.p.b. of iodine high-range procedure. However, if the iodine content of the iodized salt is much

smaller* a more concentrated salt solution (up to 0.5%) should be used for the analysis.

The rate of the catalytic reaction has a large temperature coefficient of about 5.5% in the neighborhood of 25° under the conditions described.

The reciprocal of the average readout values from Table I are plotted against concentration in Fig. 1. Calibration curve 1 (scale A) is for the low-range, and calibration curve 2 (scale B) is for the high-range method. Since the ceric solution II decomposes slowly (about 5% per day), the calibration curves should be rechecked every 2 to 3 h with at least 2 standard iodide solutions. Blank values should be established for each new batch of reagents, and should be redetermined occasionally as a check on contamination.

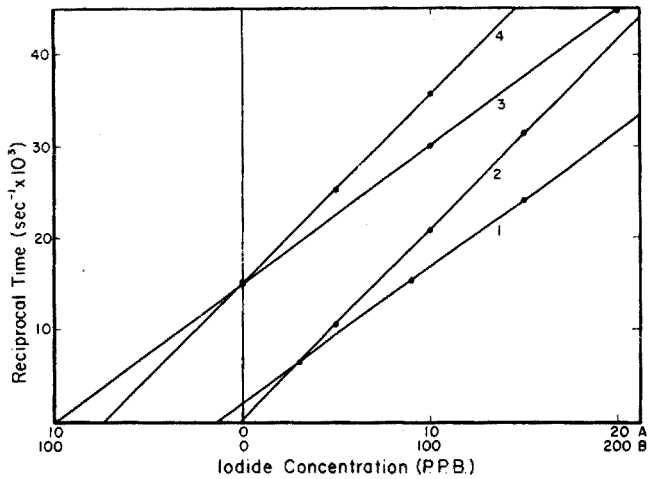

Fig. 1. Plot of reciprocal time *vs.* iodide concentration. (1) Calibration curve for the low range; (2) calibration curve for the high range; (3) common salt; (4) iodized salt.

TABLE II

DETERMINATION OF IODINE IN COMMON SALT

% *NaCl (w/v)*	*Procedure*		*Iodine (p.p.b.)*		*Iodine (μg per 100 g NaCl)*
	Low range	*High range*	*Standard addition method*	*Direct method*	
0.2	+		8.6		430
0.2	+			8.5	425
0.5	+		21.5		430
0.5	+			21.5	430
1.0		+	42.5		425
5.0		+	218		436
1.0		+		41.6	416
5.0		+		178	356

* In December, 1947, the Goitre Sub-Committee of the Medical Research Council in England recommended an average addition to table salt of 1 part of potassium iodide to 40,000 parts of salt[1].

To determine the maximum chloride concentration which can be present when the direct method is used, solutions of various concentrations of the same salt samples were analyzed by both methods. Results for one salt sample (U.S.A., Morton's plain salt) are shown in Table II. It can be seen from these data that the results obtained by the two methods agree within 1 to 2% as long as the solution contains less than 0.5% sodium chloride, and this is the maximum amount of sodium chloride that can be present when a calibration curve is used. With more concentrated salt solutions the reaction velocity is reduced because of a decrease in activity of the components of the system[4] and this results in longer measurement times for the same iodine concentration. Consequently, the results obtained by the direct method are smaller than those obtained by the standard addition method. The difference in results obtained by the two methods increases as the sodium chloride concentration increases; the difference was only about 2% for the 1% sodium chloride solution, but increased to 18% for the 5% solution.

TABLE III

RECOVERY OF IODIDE ADDED TO IODIZED SALT

Sample no.	*Iodide concentration (μg/l)*				*Recovery (%)*
	Originally present[a]	*Added*	*Total*	*Found*[a]	
1	71.5	40	111.5	113	104
1	71.5	80	151.5	152	101
2	65.5	50	115.5	114.5	98
2	65.5	100	165.5	164.5	99
3	61.5	50	111.5	112.5	102
3	61.5	100	161.5	160.2	99

[a] Average of two determinations.

The principal natural impurities in common salt are usually calcium and magnesium salts. The proportion of each of these salts may reach, but seldom exceeds, 2%. The effect of these salts on the catalytic reaction was negligible even at the 5% level[1]. The effect of bromide on the ceric-arsenite reaction was determined by the addition of potassium bromide to sodium chloride solutions which had already been analyzed. It was found that the reaction rate remained practically the same with amounts of bromide corresponding to 0.2% bromide in sodium chloride. The 0.2% bromide content is much higher than the average 0.02% bromide found in sodium chloride from sea water[5].

Iodized salt from three companies was found to contain 0.0080 to 0.0094% potassium iodide; the manufacturer's value was 0.01%.

The standard addition method was applied to the analysis of 2 samples of reagent-grade sodium chloride. They were found to contain 3.5 and 6.0 μg of iodine per 100 g respectively. At these extremely low iodine levels (1 part of iodine to 25,000,000 parts of sodium chloride) the results were precise to within 0.3 μg of iodine per 100 g.

The proposed method was checked for accuracy by adding known amounts of iodine to solutions of iodized salt which had already been analyzed. Iodate or iodide standards were added in amounts which increased the concentration by 40 to 100 μg of iodine per liter. The recovery of iodine varied from 98 to 104% with an average of 100.2% whether the addition was made as iodide or iodate (Table III).

Since iodate itself does not act as a catalyst but must first be reduced to a lower oxidation state by reaction with arsenious acid[6], conditions are arranged so as to ensure that the time measurement starts after the reaction rate approaches the value to be expected for the iodine present in the sample. This is accomplished by setting the comparator zero adjust at 7.00 so that the premeasurement time is at least 20 sec.

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SUMMARY

An automatic reaction-rate method is described for the ultramicro determination of iodine in common salt. The method utilizes the acceleration of the reaction between ceric sulfate and arsenious acid by iodine. The time required for the reaction to consume a fixed amount of ceric ions is measured automatically and related directly to the iodine concentration. Measurements obtained in salt samples containing 3 to 7500 μg of iodine per 100 g were precise to within 2% or 0.3 μg of iodine, whichever is larger. Measurement times varied from a few sec to about 2 min.

RÉSUMÉ

L'auteur décrit une méthode automatique pour le dosage ultramicro de l'iode dans le sel. Ce procédé est basé sur l'accélération par l'iode de la réaction entre le sulfate cérique et l'acide arsénieux. Le temps nécessaire à la réaction d'une quantité déterminée de cérium est mesuré automatiquement et rapporté directement à la concentration en iode. La précision obtenue est de 2% ou 0.3 μg d'iode pour des échantillons renfermant 3 à 7500 μg d'iode pour 100 g de sel.

ZUSAMMENFASSUNG

Eine Ultramikrobestimmung von Jod in Kochsalz wird beschrieben. Die Methode stützt sich auf die Beschleunigung der Reaktion zwischen Cersulfat und arseniger Säure durch Jod. Die Zeit, die für die Reaktion zum Verbrauch einer bestimmten Menge von Cerionen benötigt wird, wird automatisch gemessen und dient als Mass für die Jodidkonzentration. Messungen mit Salzproben, die 3 bis 7500 μg Jodid in 100 g enthielten, ergaben eine Genauigkeit von 2% oder 0.3 μg Jodid. Die Messzeiten variierten von wenigen Sek bis zu etwa 2 Min.

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DETERMINATION OF SMALL QUANTITIES OF POTASSIUM AND SODIUM IN STONY METEORITIC MATERIAL, ROCKS AND MINERALS

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Normal wet chemical methods are unsuitable for the determination of potassium and sodium in stony meteoritic material owing to the low level in which they are present. A major advance in the accurate determination of these elements was made by an evaporative technique[1]. With this technique, the potassium and sodium were evaporated away from the major part of the matrix thereby reducing the quantity of interfering elements in the flame photometric determination that followed. Before this technique was developed isotope dilution was the only suitable method for the determination of such low levels. During the investigation of the application of the flame photometric method to the determination of potassium and sodium in minerals and rocks ($K = <0.1\%$ and $Na = <2.0\%$), the present procedure was found to allow determination of these low levels without either chemical or evaporative separation after a simple dissolution of the sample with hydrofluoric and sulphuric acid.

A Beckman Model DU flame photometer was used mainly because the volume of solution available was required to be kept to a minimum; moreover, this instrument allowed scanning of the spectra about the 768 mμ potassium and 588 mμ sodium peaks. This procedure was found to be essential as opposed to the normal single reading of the radiation at the appropriate peaks.

EXPERIMENTAL

Potassium is the more difficult of the 2 alkalies to determine since it is usually present in smaller quantities than sodium. The phenomena from which errors would arise from a single reading of the potassium radiation at 768 mμ are: (*1*) Background radiation, and (*2*) matrix effect.

(*1*) At low levels of potassium the slit width is required to be increased as shown in Fig. 1 and with an increase in slit width it was found that the background radiation increased rapidly. When a concentration of 1 g of stony meteoritic material per 100 ml of solution was used the widest slit width used was 0.2 mm; at this setting water contributed only a small portion of the background radiation.

To eliminate the background radiation on samples and standards the transmission was read at 725, 768 and 825 mμ. Shown in Fig. 2 are the readings obtained on a sample of the achondrite Shalka (0.008% K). A large percentage of the total transmission at 768 mμ is contributed by background radiation and therefore this reading

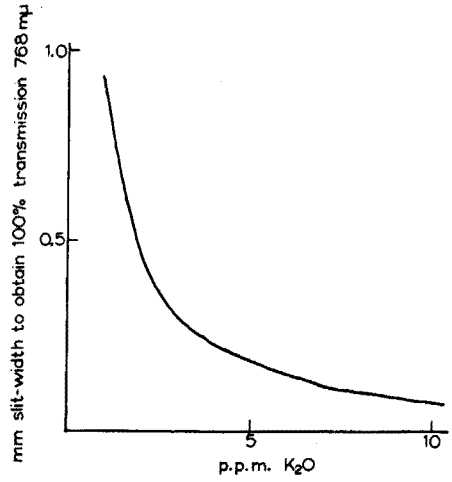

Fig. 1. Variation in slit width with concentration of K_2O.

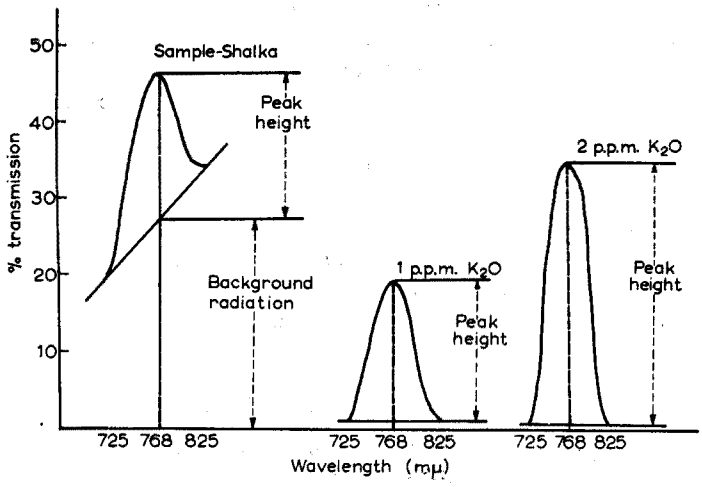

Fig. 2. Spectra of sample and standards (K_2O).

alone has no relationship to the potassium content of the sample. The peak heights of the sample and standards were obtained by plotting the readings at 725 and 825 mμ and subtracting the intersection at 768 mμ from the total transmission reading. In all cases the peak height of the sample was compared with those of standards above and below the concentration of the sample.

Since the sodium content of stony meteorites is substantially greater than that of potassium a smaller width was used and the background radiation was therefore less. In the cases where sodium was $<0.05\%$, background radiation was eliminated by using the same procedure as for potassium but taking readings at 580, 588 and 600 mμ. Since the sodium peak was found to be symmetrical either reading was deducted from the total transmission at 588 mμ to obtain the peak height.

(*2*) The major elements that normally interfere with the flame photometric determination of potassium are iron, calcium, aluminium and magnesium, iron and magnesium being dominant in chondrites. Using standard potassium solutions (10 p.p.m. K_2O) to which iron had been added, it was found that the increase in transmission was largely due to background radiation and that when this was deducted the enhancement was mainly eliminated. Both aluminium and magnesium depressed the radiation of potassium to a larger extent when present singly than in a mixture of all three elements (Fe, Al and Mg).

Calcium had no effect when present as a saturated calcium sulphate solution, the form in which it would be present in the calcium-rich achondrites, provided that the background correction was made.

The interference of each element in a multiple mixture was not found to be additive; magnesium caused the main depression although then only to the extent of about half of the value exhibited when alone (Table I). This behaviour only allowed an

TABLE I

EFFECT OF MAGNESIUM ON POTASSIUM AND SODIUM RADIATION

MgO (p.p.m.)	*% Depression of peak height*	
	K_2O (10 p.p.m.)	*Na_2O (50 p.p.m.)*
100	0	<1
500	5	1
1000	11	1
2000	11	1
3000	13	1
4000	16	1

empirical correction to be calculated for the depressive effect of magnesium in the presence of other elements and required a knowledge of the approximate magnesium content of the sample solution (in p.p.m.). A tentative correction was calculated by halving the percentage depression obtained from Table I for the magnesium content of the sample solution. The validity of this correction was tested against solutions containing 10 p.p.m. K_2O to which had been added various quantities of Mg, Fe, Al and Ca. The results obtained (Table II) show this correction to be sufficiently valid so that when applied the potassium found is within $\pm 5\%$ of the true value. This correction was calculated and applied to all chondritic samples, the maximum correction applied to any one sample being 6%. It must be emphasized that these findings only apply to measurements made on a Beckman DU instrument, using a hydrogen–oxygen flame and a sample concentration of 1 g per 100 ml.

TABLE II

EFFECT OF OTHER ELEMENTS

Elements added (p.p.m.)		*K_2O(p.p.m.)*	
		Present	*Found with correction (MgO depression)*
MgO	2000	10.0	9.9
Fe	1000		
MgO	1000	10.0	10.2
Fe	1000		
MgO	2000	10.0	10.4
Fe	250		
MgO	1300		
Fe	1000	10.0	10.3
Al	750		
MgO	1000		
Fe	1000	10.0	10.0
Al	1000		
$CaSO_4$	Saturated		

PROCEDURE

Weigh the sample (1 g) of finely ground stony meteorite into a platinum basin which has been freed of potassium and sodium by repeated boiling with distilled water. Add 25 ml of hydrofluoric acid and 1 ml of concentrated sulphuric acid, evaporate to dryness on a water bath and fume off most of the residual sulphuric acid. Allow to cool and dissolve the residue in distilled water. If calcium is high in the sample, calcium sulphate will precipitate out. Centrifuge off any residue using a clean glass tube. Transfer the solution to a well washed 100-ml volumetric flask, make up to volume and mix well. Preferably the determination is made on the same day to avoid leaching of potassium and sodium from the flask.

Potassium

The sample and suitable standards, prepared from fused K_2SO_4 (A.R. grade), are placed in the flame alternatively until stable transmission readings are obtained at a wavelength of 768 mμ. The transmissions of the sample and standards are then also read at 725 and 825 mμ. A number 3 resistor is used. The slit width is set so that the highest standard solution used to bracket the sample gives a transmission reading high on the scale, *e.g.* 80–90%. A blank is prepared from 25 ml of hydrofluoric acid (Baker Analyzed Reagent 48.8%) and 1 ml of concentrated sulphuric acid (A.R.) and in this work was found to be 3 μg K_2O.

Sodium

The sample and suitable standards prepared from Na_2SO_4 (A.R. grade) are placed in the flame and the transmissions are read at a wavelength of 588 mμ. The same solutions are then read at 580 and 600 mμ. A number 2 resistor is used. The slit width is set in the same way as for potassium. The blank for sodium was found to be 10 μg Na_2O.

Calculations were made as previously described in the text.

Rocks and minerals

A similar procedure may be used for the determination of potassium and sodium in rocks and minerals. Although the accuracy is not sufficiently high for the result to be used for potassium–argon dating, it is useful as an indication of the potassium content of the material where K is $< 0.1\%$.

Where K is $< 0.01\%$ and approximately 0.5 g of material has been used for the dissolution, the potassium present in the solution will be < 1 p.p.m. This is also the case where only a semi-micro (50–100 mg) quantity of material is available for analysis. In this case a satisfactory procedure is to set the slit width to 0.7 mm and the sensitivity knob to the extreme left. Then a series of standard potassium solutions (0.1–1.0 p.p.m.) are placed in the flame and their peak heights measured. A calibration curve may then be drawn and used for conversion of the peak heights of the samples to potassium content of the solutions. Although it is necessary to check one or two points on this curve each time the instrument is used, provided that the jet is kept free the curve appears to be constant within experimental limits ($\pm$ 2% transmission). The previously described correction for the depressive effect of magnesium is applied to the results as before.

RESULTS AND DISCUSSION

Certain inhomogeneities appear to exist within stony meteorites with regard to potassium and sodium which makes comparison with previous work difficult. However taking the extreme case of the Johnstown achondrite, although the actual amounts of potassium and sodium differ by a factor of 10 the Na : K ratio is essentially the same as previously reported. In spite of these difficulties the results obtained on

TABLE III

COMPARISON OF POTASSIUM AND SODIUM RESULTS IN CHONDRITIC AND ACHONDRITIC MATERIAL

Name	*% K*			*Ref.*	*% Na*			*Ref.*
	Our method	*Edwards and Urey*[1]	*Others*		*Our method*	*Edwards and Urey*[1]	*Others*	
Forest Vale	0.075	—	0.081	2	—			
Indarch	0.084	0.088	0.09	2	0.80	0.75	0.75	5
Orgueil	0.031	0.056	0.0347 0.0343	7	0.21	0.55		
Bishopville	0.086	0.083	0.02	2	0.85	1.0 1.16	0.8	4
Frankfort	0.026	0.021						
Goalpara	0.007	0.006			0.018	0.04		
Johnstown	0.009	0.0008	0.002 0.001	2	0.028	0.003		
Juvinas	0.031	0.038	0.024 0.039	2	0.308	0.33		
Moore County	0.021	—	0.019	6				
Nakhla	0.115	0.102			0.38	0.44		
Shergotty	0.122	—	0.15	3	0.91		1.0	4
Stannern	0.069	0.060 0.069			0.40	0.43	0.6	4

both chondrites and achondrites show close agreement with the evaporative technique[1] and isotope dilution results as shown in Table III.

Comparison of results obtained on minerals using this procedure with those given by isotope dilution, shown in Table IV, gives a fair agreement considering the low level of potassium measured. The proposed procedure, although not giving a high degree of accuracy, will give a clear indication of the potassium content which is sufficient for most geochemical purposes and preliminary examination of material for potassium–argon age work.

TABLE IV

COMPARISON OF FLAME PHOTOMETRIC AND ISOTOPE DILUTION DETERMINATIONS ON MINERALS

Mineral	% *K*		*Reference*
	This work	*Isotope dilution*	
Hypersthene	0.024	0.009	7
Pyroxene	0.025	0.017	7
Pyroxene	0.015	0.017	7
Pyroxene	0.18	0.15	7
Plagioclase	0.57	0.48	7
Clinopyroxene	0.11	0.09	7
Clinopyroxene	0.002	0.002	8
Clinopyroxene	0.007	0.003	8
Clinopyroxene	0.005	0.004	8
Enstatite	0.007	0.003	8

SUMMARY

A flame photometric method for the determination of potassium (0.005–0.1%) and sodium (0.01–1.0%) in stony meteorites and other silicate materials is described using a Beckman Model DU flame photometer. A simple scanning procedure eliminated background radiation and a small correction for the effect of magnesium on potassium radiation was calculated. Results obtained by this procedure agree well with an evaporative technique and isotope dilution determinations.

RÉSUMÉ

Une méthode par photométrie de flamme est décrite pour le dosage du potassium et du sodium, dans des météorites pierreux et autres silicates. On utilise un photomètre de flamme Beckman, modèle DU. Les résultats correspondent bien avec ceux obtenus par dilution isotopique.

ZUSAMMENFASSUNG

Eine flammenphotometrische Methode zur Bestimmung von 0.005–0.1% Kalium und 0.01–1.0% Natrium in Meteoritengestein und anderen silikatischen Materialien wird beschrieben. Der Einfluss von Mg, Fe, Al und Ca wird untersucht. Die Ergebnisse stimmen gut mit denen der Isotopenverdünnungsmethode überein.

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DETERMINATION OF METHIONINE IN PROTEIN HYDROLYSATES BY REACTION WITH RANEY NICKEL AND INFRARED SPECTROSCOPY

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Methionine is encountered in most proteins, though only in relatively small amounts. It participates in biochemical processes in living cells as a source of methyl groups, and therefore its determination in biological products is of great interest. Many different methods have been suggested: *e.g.* differential oxidations with fuming nitric acid and sodium peroxide[1]; a polarographic method, based on the action of iodine on methionine to form an active polarographic product[2], which is only applicable to pure methionine; a colorimetric method based on the reaction of methionine with sodium nitroprusside[3]. This last method has been widely applied to protein materials after various modifications[4–7]. Other possible procedures are based on ninhydrin reaction[8], alkoxy group determination[9,10], the catalytic effect on the azide–iodide reaction[11], and on microbiological methods[12]. Of course, there are many other methods by which methionine could be determined, like other amino acids, after a suitable separation based on ionophoresis, adsorption or partition.

Despite the variety of existing methods for methionine determination, a close study shows that none of them meets all the needs of accuracy, rapidity, sensitivity and specificity. Thus, the development of a method for methionine determination which would fulfil these requirements to a higher extent than previous methods should be of interest.

Some years ago, a method was developed for the determination of sulphur in organic compounds, based on the fact that Raney nickel reacts quantitatively with organic sulphur and binds it as a nickel sulphide[13]; the hydrogen sulphide formed on addition of sulphuric acid, was absorbed in alkaline solution and determined iodometrically or polarographically. This method is applicable to the amino acids, cystine and methionine. In the latter case, as would be expected, methane is also evolved. This suggested the possibility of a selective determination of methionine based not on determination of hydrogen sulphide as for other sulphur-containing compounds, but on the amount of methane separated during desulphurization. If the interaction of methionine with Raney nickel proceeds quantitatively as follows:

$$CH_3S{\cdot}CH_2{\cdot}CH_2{\cdot}\underset{\displaystyle NH_2}{\underset{|}{CH}}{\cdot}COOH + Ni(H) \rightarrow CH_3{\cdot}CH_2{\cdot}\underset{\displaystyle NH_2}{\underset{|}{CH}}{\cdot}COOH + CH_4 + NiS$$

then the amount of methionine may be determined from the amount of methane

separated. Since the methane can be separated from methionine only, the method should be applicable in the presence of other sulphur-containing amino acids.

Initially, we attempted to develop a method analogous to the ZEREWITINOFF determination of active hydrogen. However, when the flask was charged with a methionine solution and a Raney nickel suspension, and the volume of gas which separated on moderate heating of the reaction mixture, was measured, results were very high. Under the conditions used, the gas measured consisted not only of methane, but also of hydrogen, which was obviously desorbed from the Raney nickel. Attempts to eliminate hydrogen partially from the Raney nickel (without lowering its desulphurizing or hydrogenating capacity) so that it would not separate hydrogen on reaction with methionine, produced only unsatisfactory and unreliable results. Nor was the utilization of solutions which would absorb hydrogen, successful. Palladium was not tested because it would have led to a considerable complication of the apparatus. Accordingly, we decided to determine the methane in the gaseous mixture obtained after the desulphurization, by infrared spectroscopy. Some preliminary results of these investigations have already been communicated[14] and the present paper comprises a final report on the procedure.

EXPERIMENTAL

Reagents

An alloy containing 50% nickel and 50% aluminium (E. Merck, Germany) was used to obtain the Raney nickel suspension.

Methionine, chromatographically pure for scientific purposes, was obtained from Th. Schuchardt (Germany). The other amino acids used were of analytical grade.

Casein was obtained by HAMMARSTEN's method[15]. Trypsin was obtained by the activation of crystalline trypsinogen in the presence of calcium salts[16]; the product was twice recrystallized and then dialyzed and lyophilized. The pepsin was thrice recrystallized, then dialyzed and lyophilized. The bovine serum albumin was produced by Roth (Germany). The egg albumin was obtained as described by FORSYTHE AND FOSTER[17]. Zein was prepared as described by MASON AND PALMER[18], and hordein was obtained by the method of OSBORNE[19].

All other reagents used were of analytical grade.

Apparatus

The reaction between the Raney nickel and the methionine solutions or the protein hydrolysates was done in the apparatus shown in Fig. 1.

The methane determination was carried out with an infrared spectrophotometer (UR-10 Zeiss, 1958), which registered absorption curves in coordinate wave number/percentage transmittance. The 100-mm long gas cell employed had potassium bromide windows.

Preparation of the catalyst

In initial tests of the method for methionine with Raney nickel, prepared by the method of PAVLIK AND ADKINS[20], exceedingly high results were obtained for the methane and so the methionine. At first it was assumed that this was due to the use of ethanol in the preparation of the Raney nickel, for certain alcohols split out a methoxy group, probably in the form of methane[21], at a temperature of 250°. How-

ever, when the Raney nickel was prepared without alcohol, high results were still obtained. It was therefore assumed that the methane came from a reaction of aluminium carbide, contained in the initial alloy, with water on heating; in fact, when the gas obtained by heating a sample of the initial alloy with water was measured in

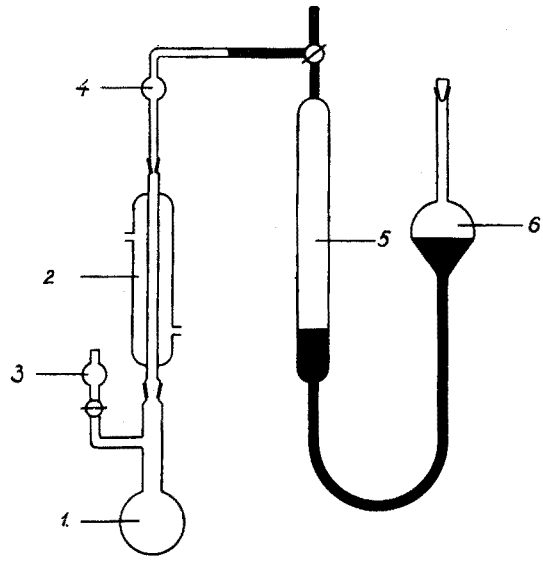

Fig. 1. Apparatus for desulphurization of methionine.

the spectrophotometer, a considerable amount of methane was registered. In order to eliminate aluminium carbide, the initial alloy was boiled with distilled water for 20 min; it was then left to cool for several hours before being washed 5 times with distilled water. A control sample of the alloy thus treated showed a complete absence of methane and aluminium carbide. The use of alcohol in the preparation of the catalyst and in the course of the determination should nevertheless be avoided, since it has been shown that under the conditions of the estimation adopted, ethanol separates a small amount of methane.

A sample (50 g) of the alloy was boiled in distilled water, and had already begun to be partially corroded; this was added in portions over a period of 20–30 min with mechanical stirring, to 250 ml of 5% sodium hydroxide solution. During the addition of the alloy, the temperature of the mixture was maintained at about 50°. After the addition of the last alloy portion, the stirring was continued for another 30 min at the same temperature. The catalyst thus prepared was washed with distilled water until the pH was 8–8.5 and kept under distilled water in a stoppered bottle. It should be borne in mind that under these conditions of preparation, the entire alloy is not corroded; additional corrosion occurs during the determination, and ensures a high activity of the catalyst. The removal of the aluminium carbide from Raney nickel must be checked for each new batch of catalyst.

The Raney nickel was measured in the following way. The bottle containing the

catalyst was vigorously shaken and part of the suspension was poured out into a buret. After waiting for 3 to 5 min, for the sludge to sink to the lower part of the buret, the tap of the buret was turned on and the volume desired measured out.

Methionine determination

A sample containing 0.2–10 mg of methionine was dissolved in 5 ml of distilled water in the reaction flask 1 (Fig. 1), and several drops of 0.1 *N* sodium hydroxide added. The flask was connected to the reflux condenser 2 and the capillary 3-way tap was placed in position to join the flask to the gas pipet 5. Then the side-arm 3 was charged with 2–3 ml of Raney nickel sludge. By means of reservoir 6, a reduced pressure was created in the system, and the catalyst was drawn into the flask by opening the tap on 3. The mixture was then heated to boiling; if strong foaming occurs, the heating should be done with greater care. During the heating, the pressure in the system should be a little lower than atmospheric. After boiling for 15–20 min, the flask was slightly cooled. The side-arm was then connected by means of rubber tubing with a funnel containing a saturated solution of sodium chloride; a screw-clip was placed at the bottom end of the tubing. By raising the funnel and releasing the screw-clip and the tap, the entire apparatus (the flask, the reflux condenser and the tube above it to the capillary tap) was filled with liquid. Some cotton was placed beforehand in chamber 4 to prevent any particles of the catalyst from reaching the capillary tap.

The gas, collected in the gas pipet, was then transferred to the gas cell of the spectrophotometer. The cell was previously evacuated at a water pump to about 15 mm Hg, so that, when the gas was transferred, the cell remained under reduced pressure. Then the cell was placed in the spectrophotometer and the pressure in the cell was equalized to atmospheric by brief opening of the cock. Finally the absorption curve of the methane was recorded in the range 2990–3040 cm^{-1}.

The same technique was employed for the analysis of hydrolysates; the acid hydrolysate was previously neutralized with a concentrated sodium hydroxide solution, a small crystal of phenolphthalein being dropped into the acid solution.

The hydrolysis of proteins was carried out with 6 *N* hydrochloric acid in a sealed ampoule at 105° for 16 h. The hydrolysis of homogenates from heart, kidneys and liver of a Syrian golden hamster was carried out with the same hydrochloric acid in an open flask at 135° for 20 h. The moisture content of the products studied was determined in all cases in parallel tests.

The determination of total sulphur in mixtures of methionine and cystine or in protein hydrolysates was done as described previously[13].

RESULTS

Numerous determinations on pure methionine by the above procedure were carried out for the construction of a calibration graph. The experimental plot of extinction *vs.* mg of methionine proved to be linear up to 10 mg of methionine (the highest amount tested) and passed through the origin.

Experiments for the methionine determination in the presence of cystine were further conducted. It was proved that the presence of large amounts of cystine had no effect on the methionine determination.

Methionine was also determined in an artificial mixture with other amino acids,

taken in much larger amounts. The composition of one such mixture was as follows: 0.64 mg of methionine, 31.1 mg of arginine, 44.0 mg of asparagine, 4.4 mg of D,L-isoleucine, 28.2 mg of D,L-leucine and 20.3 mg of L-tyrosine. In all cases, the methionine determination gave good results and no unwanted gases were released.

After these preliminary investigations, the method was applied to hydrolysates of proteins of vegetable and animal origin. The results obtained are shown in Table I.

TABLE I

METHIONINE CONTENT IN HYDROLYSATES OF PROTEINS

Protein	*Methionine found (g) per 100 g of protein*	*Literature data*
Egg albumin	5.20	5.2[22]
Bovine serum albumin	1.00	0.9[a]
Total casein	2.86	2.8[23]
Pepsin	1.66	1.7[24]
Trypsin	1.28	1.27[a]
Zein	1.68	1.7[4]
Hordein	1.35	1.4[25]

[a] Owing to the lack of reliable literature data, the methionine content of the bovine serum albumin was estimated at a content of 4 methionine residues for a molecular weight of 66,000; the methionine content in the trypsin was estimated at two residues for a molecular weight of 23,500.

TABLE II

METHIONINE CONTENT IN HOMOGENATES OF INTERNAL ORGANS OF GOLDEN HAMSTER

Organ	*Methionine found (g) per 100 g of homogenate*
Heart	1.73
Kidney	1.45
Liver	2.18

Finally, the methionine content in homogenates of internal organs of the Syrian golden hamster (*Mesocricetus auratus*) was also determined. The results obtained are shown in Table II. The methionine contents shown in these Tables are the mean of at least 2 determinations; differences between separate determinations were never larger than 0.04%.

DISCUSSION

The infrared spectroscopic determination of the methane liberated by the interaction of methionine with Raney nickel, was chosen for the following reasons.

(*1*) Among all the gases which may occur in the gaseous mixture after the desulphurization of amino acids or of protein hydrolysates (H_2, O_2, N_2, CH_4 and CO_2) only methane and carbon dioxide are determined by infrared methods; and carbon dioxide absorbs in a spectral zone different from that of methane.

(*2*) Methane gives quite intensive absorption lines, which makes it possible to determine very small amounts. The most intensive line was chosen for the determination — the Q branch of the asymmetrical stretching vibration at 3020.3 cm^{-1}.

Since the intensity of the lines depends on instrumental conditions (dispersion of the prism, spectral slit width and certain parameters of the amplifier), an empirical calibration graph was constructed.

The method suggested is essentially specific for methionine among other amino acids, including those containing sulphur. The determinations made show that cystine and cysteine do not influence the determination. In addition, when, as normally happens, the protein contains only cystine and cysteine along with methionine in the sulphur-containing amino acid group, then the cystine-cysteine can be determined from the difference between the total sulphur (*e.g.* as described previously[13]) and the methionine sulphur. Among the few interferences must be considered djenkolic acid (3,3'-(methylene-dithio)dialanine) which would probably liberate methane on desulphurization with Raney nickel. This acid is rarely encountered but would explain the higher methionine content found for bovine serum albumin (Table I), which is known to be rich in djenkolic acid[26].

The relative error of the determination of methionine in protein hydrolysates is 1–2%; taking into account the difficulties in the purification of proteins, this accuracy must be considered as very satisfactory. The time required for a single determination is about 40 min.

An important advantage of the method is that it allows the determination not only of free methionine, but also of its oxidation forms, sulphoxide and sulphone; the sulphoxides and sulphones are readily hydrogenated by Raney nickel to the corresponding sulphides, which are then desulphurized[27]. Because of this, the results of the determination are not affected by any oxidation processes which might occur during the treatment of the solutions. Moreover, it becomes possible to determine methionine in hydrolysates of proteins and peptides, which have previously been subjected to oxidation with performic acid. Naturally, it is better to carry out the determination before oxidation, since in the course of oxidation, part of the methionine could be destroyed.

The least amount of methionine which could still be determined with the accuracy mentioned above, was 200 μg. Further investigations are in progress, to scale the method down to the 20 μg level or less.

The preliminary results for determinations of methionine in non-hydrolysed proteins showed, as would have been expected, that not all methionine residues react with Raney nickel. These results, together with results for the desulphurization of non-hydrolysed proteins under various conditions[28] and the results for micro-structural investigations, should provide indications of the reactivity towards Raney nickel of various methionine and cystine residues in the protein molecule. This should, in turn, provide information on the macro-structure of the corresponding protein.

The method suggested could probably be extended for other compounds containing the groups CH_3S–, C_2H_5S– and C_3H_7S–, as well as to the corresponding disulphides. Moreover, since Raney nickel reduces as well as desulphurizes, the degree of oxidation of the sulphur would have no significance, and the corresponding sulphoxides, sulphones, vinyl- and propenyl thioethers, etc. could also be determined. At present, a method for the determination of the antibiotic allicin and its precursor, alliin, by infrared measurement of the propane liberated by treatment with Raney nickel is being studied.

SUMMARY

An infrared spectrophotometric determination of methionine in protein hydrolysates is suggested. The method is based on the desulphurizing action of specially purified Raney nickel on methionine, during which methane is quantitatively liberated; methane is then measured at 3020.3 cm^{-1}. Cystine and cysteine do not interfere and can usually be determined from the difference between total sulphur and methionine sulphur. The method can be applied to protein hydrolysates, and to homogenates of internal organs. The time required for a single determination is about 40 min; the relative error is 1–2% down to a level of 200 μg.

RÉSUMÉ

Les auteurs proposent une méthode par spectrophotométrie infra-rouge, pour le dosage de la méthionine dans des hydrolysats de protéïnes. La méthode est basée sur l'action désulfurisante du nickel-Raney (spécialement purifié) sur la méthionine. Le méthane libéré est mesuré à 3020.3 cm^{-1}. La cystine et la cystéine ne gênent pas. Leur teneur peut être calculée par différence entre le soufre total et le soufre de la méthionine.

ZUSAMMENFASSUNG

Eine infrarotspektralphotometrische Bestimmung von Methionin in Proteinhydrolysaten wird vorgeschlagen. Die Methode beruht auf der Entschwefelung des Methionins mit speziell gereinigtem Raney-Nickel, währenddessen Methan quantitativ gebildet und bei 3020.3 cm^{-1} gemessen wird. Cystin und Cystein stören nicht und können auf übliche Weise aus der Differenz zwischen dem Gesamt- und dem Methioninschwefel bestimmt werden. Die Zeit für eine Bestimmung beträgt etwa 40 Min, der relative Fehler 1–2% bis zu einer unteren Grenze von 200 μg.

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THE SAMPLING OF HYDROGEN SULFIDE IN AIR WITH IMPREGNATED FILTER PAPER

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In previous papers methods were described for the sampling of sulfur dioxide[1] and hydrogen fluoride[2] in air by drawing the air through impregnated filter paper. In these methods an aqueous solution of potassium hydroxide with added glycerol or triethanolamine was the impregnating fluid used. In the present paper it is shown that the same impregnated filters can be used for the collection of hydrogen sulfide in air if potassium zincate is added to the impregnating fluid to retain the hydrogen sulfide as zinc sulfide.

In the above-mentioned papers it was explained that the impregnated filter paper method will be most useful in sequential samplers fitted with impregnated tape and to replace impingers in field work. This is also true for the proposed method for hydrogen sulfide. It is comparable to the lead acetate paper method (see, *e.g.* JACOBS[3]), but the visual or photometric inspection of the formed fleck must be replaced by, for instance, the methylene blue method. This is more complicated but more precise and more sensitive.

EXPERIMENTAL

Filters

Filters (Whatman no. 1, diam. 5.5 cm) were prepared by impregnating them with 0.5 ml of fluid and heating at 100° until they were just dry. The impregnating fluid was prepared by dissolving 11 g of zinc oxide in 60 g of molten potassium hydroxide and dissolving this mixture—after cooling—in 360 ml of distilled water and 30 g of glycerol.

Some filters were exposed for a month to air with a relative humidity of about 50%.

Preparation of known concentrations of hydrogen sulfide in air of known temperature and humidity

The apparatus used is shown schematically in Fig. 1.

The necessary amount of air was supplied by a pump and valve. It was completely dried by silica gel and filtered. Known quantities of water were evaporated on a heated mica plate in the air-stream. The air-flow was measured, the excess air vented and the air-flow again measured. Then a known flow of hydrogen sulfide was added

and finally the temperature adjusted by passing the air through a few meters of teflon tube immersed in a waterbath. The humidity and hydrogen sulfide concentration were calculated from the air, water and hydrogen sulfide flows. The procedure was checked for temperature and humidity with a wet and dry bulb hygrometer. The hydrogen sulfide was generated in a Kipp apparatus. Its purity was determined iodometrically, or occasionally by potentiometric titration with silver nitrate.

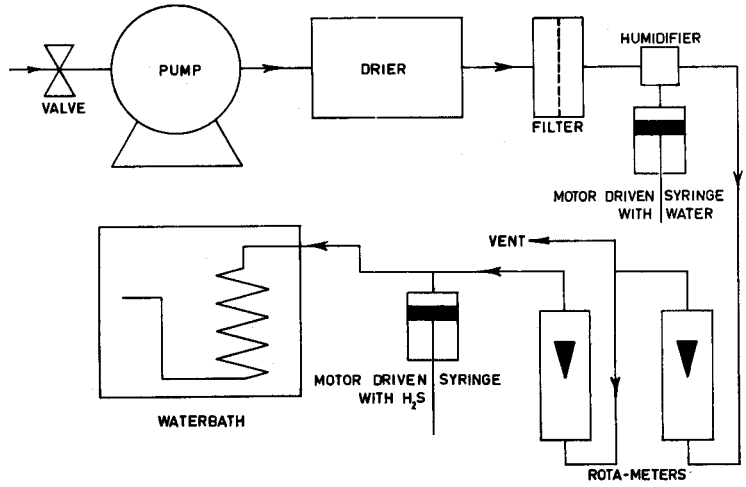

Fig. 1. Schematic of apparatus for preparation of known concentrations of hydrogen sulfide in air of known humidity.

Determination of the collection efficiency

As sketched in Fig. 2 the collection efficiency of a filter was determined by potentiometric titration[4] of the amount of hydrogen sulfide leaking through the filter.

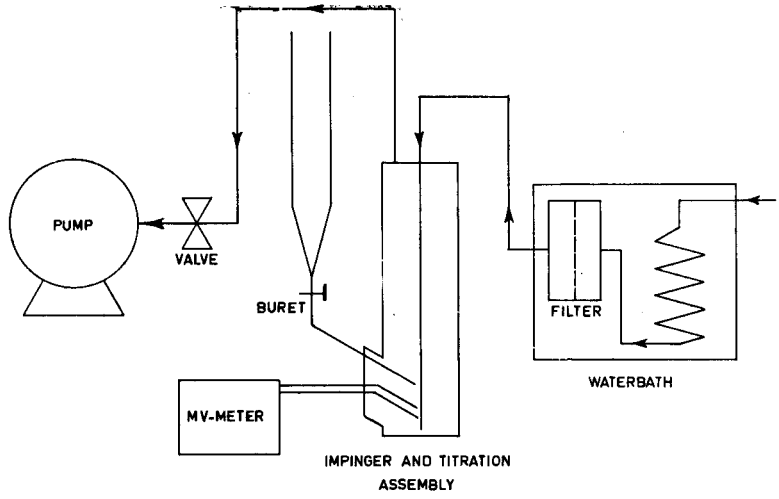

Fig. 2. Schematic of sampling apparatus.

The impregnated filter was clamped between plastic rings in a holder. The rings restricted the effective diameter to 4 cm. The filter holder was immersed in the water-bath used for the teflon tubing mentioned above.

After passing through the filter the air was bubbled through an impinger containing 0.1 *N* sodium hydroxide. Immersed in the solution were a silver/silver sulfide electrode and a sodium acetate salt bridge to a calomel electrode. The amount of 0.01 *N* silver nitrate necessary to keep the potential at the equivalence point was added from a buret. From this the amount of hydrogen sulfide leaking through the filter was calculated after correction for the collection efficiency of the impinger.

The required amount of air—generally 1 m^3/h—was drawn through the apparatus by a pump regulated by a valve.

Determination of the stability of the retained hydrogen sulfide

A known amount of hydrogen sulfide was sampled on impregnated filters as described above, the amount sampled being about 150 μg. One series of filters was then kept dry over silica gel, and another was kept at 70% humidity over a saturated salt solution, both at a temperature of 22°. After some time the filters were analysed for sulfide by the methylene blue method[5] as follows. The filter was put in a 100-ml Nessler glass and 80 ml of distilled water was added. The water was agitated by moving a glass stamper up and down in the glass, the neutralizing amount of acid was added and the solution was agitated again. Ten ml of reagent (0.44 g of *p*-amino-dimethylaniline per liter of 2.4 *N* sulfuric acid) were added and mixed. After a few min, 4 ml of 10% ferric chloride was added. The optical density was measured after 15 min at a wavelength of 670 $m\mu$ in a 1-cm cuvet.

All determinations were carried out in duplicate.

RESULTS

As a check on the hydrogen sulfide concentration the quantity found in the impinger was determined without any filter in the holder. It was, on average, 95.2% of the calculated quantity, which compares well with the collection efficiency of 95.7% determined by the two-impinger method.

Figure 3 gives an example of the graphical representation of the amount of hydrogen sulfide titrated in the impinger as a function of the time. As in the case of hydrogen fluoride there was a certain time of breakthrough; in the example given, this was 15 min. After this point the collection efficiency as calculated from the slope of the curve decreased; as shown in Fig. 2 the decrease was from 95% at 15 min to 80% after 30 min.

Times of breakthrough at various concentrations, humidities and temperatures are given in Figs. 4 and 5. The breakthrough time generally increased with decreasing concentration and increasing temperature and humidity as expected.

Another method of representation is shown in Figs. 6 and 7, where the total amount of hydrogen sulfide collected before breakthrough is chosen as the dependent variable.

Though there is some uncertainty because of the variability of the filters it is clear that the amount sampled generally increased with increasing temperature and humidity. The relation with the concentration is not so simple.

In the case of hydrogen fluoride it was found that the collection efficiencies depended not so much on the concentration and air velocity alone as on their product, which

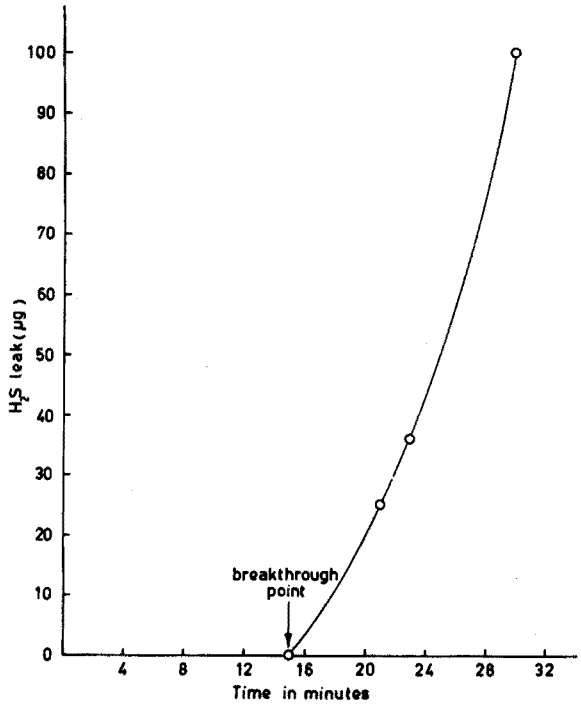

Fig. 3. Amount of H_2S leaked through the filter (concn., 4 mg/m³; humidity, 50%; temp., 20°; air-flow, 1 m³/h).

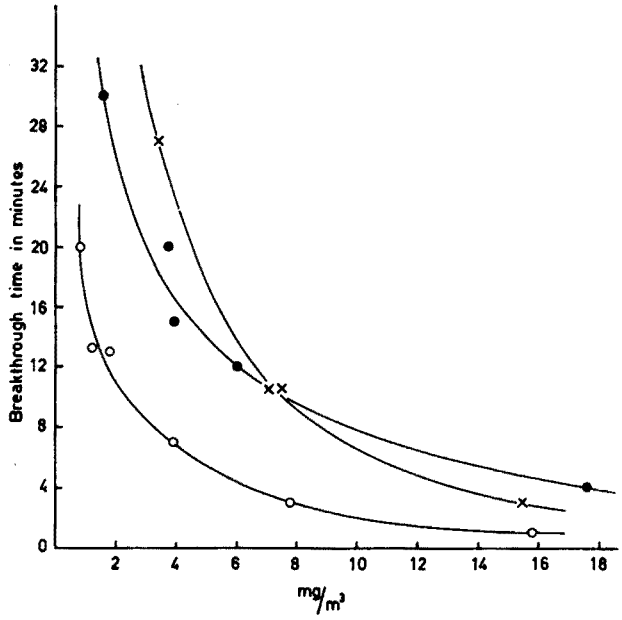

Fig. 4. Variation of time of breakthrough with increase of concn. at 20° (air-flow, 1 m³/h): ○, 25%; ●, 50%; ×, 90% humidity.

was called the rate of supply, *i.e.* the quantity arriving per unit of time at the filter. In Fig. 8 is shown the dependence of the time of breakthrough on the air velocity at constant rate of supply in the case of hydrogen sulfide.

In this case the breakthrough time and therefore the quantity sampled before

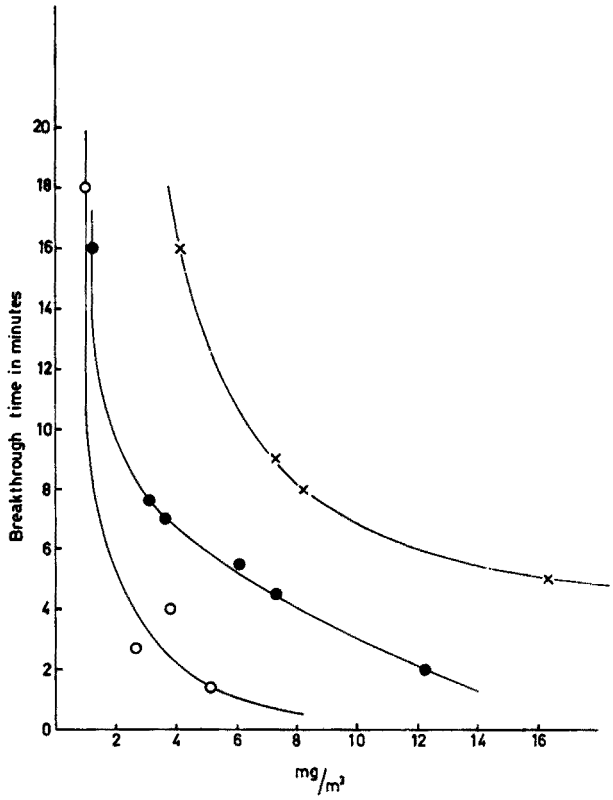

Fig. 5. Variation of time of breakthrough with increase of concn. at 0° (air-flow, 1 m³/h): ○, 25%; ●, 50%; ×, 90% humidity.

breakthrough depended strongly on the air velocity; they were in several cases almost inversely proportional to it. The curves shown in Fig. 8 were drawn as straight lines for lack of further experimental information, but they probably should have the form of hyperbolae, going asymptotic to both axes.

As found by titration to phenolphthalein and methyl orange indicators, the potassium hydroxide of the filters that were exposed to humid air for some time, changed to the extent of 60% into potassium bicarbonate and to the extent of 40% into potassium carbonate. The zinc was present as zinc carbonate. This will be the composition of the filters after a long sampling time. The collection efficiencies of the exposed filters were generally poor even at high humidities. The total quantity sampled before breakthrough was not more than a few tens of micrograms.

The stability of the sulfide collected on the filters may be gathered from Fig. 9.

There is hardly any loss in one month when the filters are kept dry. At 70% humidity the decrease is about 1.5% per day.

When the hydrogen sulfide was sampled on filters without zincate a loss of 85% in 2 h was found at 70% humidity.

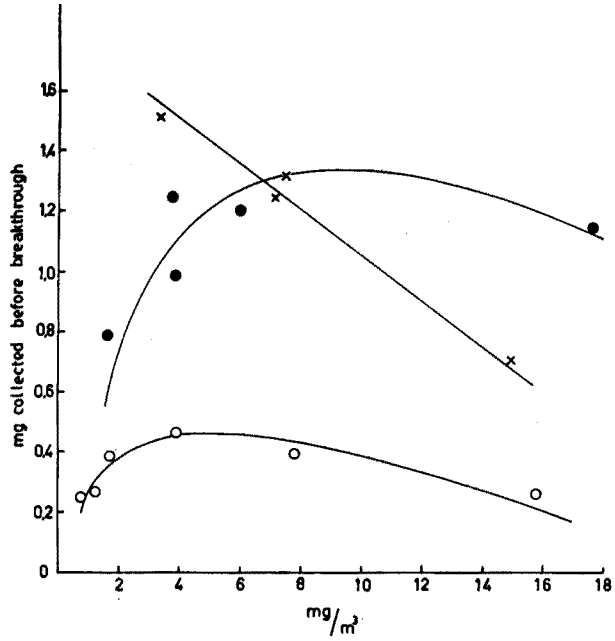

Fig. 6. Variation of quantity of H_2S collected before breakthrough, with increase of concn. at 20° (air-flow, 1 m³/h): ○, 25%; ●, 50%; ×, 90% humidity.

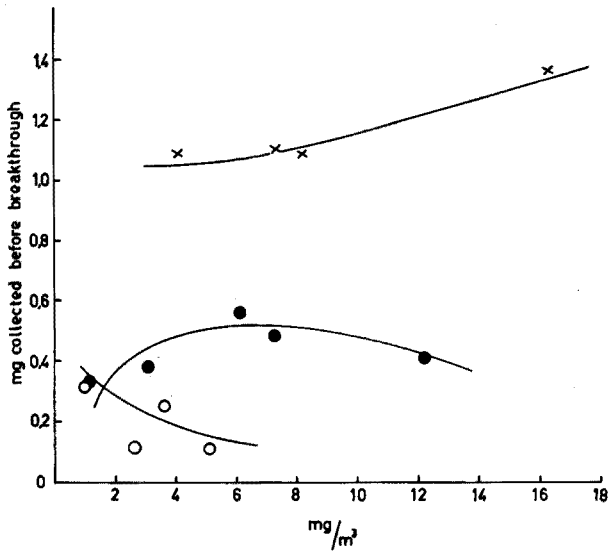

Fig. 7. Variation of quantity of H_2S collected before breakthrough, with increase of concn. at 0° (air-flow, 1 m³/h): ○, 25%; ●, 50%; ×, 90% humidity.

The quantity of hydrogen sulfide determined on the filters immediately after sampling was always a few per cent lower than the calculated value. Possibly the loss occurred during the determination, for a faint smell of hydrogen sulfide was detectable over the Nessler glasses, though the order of addition of the reagents and the form of the glasses were chosen so as to minimize losses.

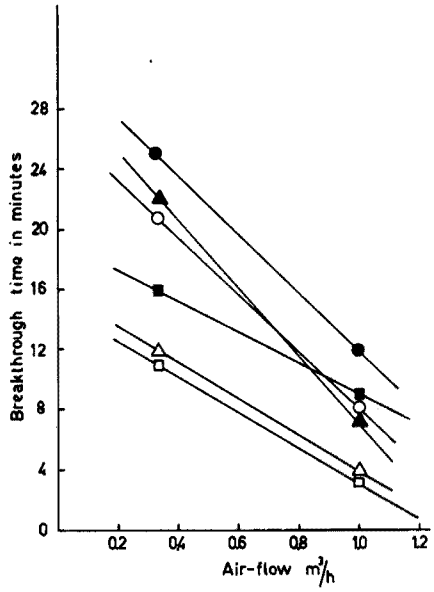

Fig. 8. Variation of time of breakthrough with increase in air velocity: ○, 20°, 25% humidity, 3.3 mg/h; ●, 20°, 50% humidity, 6.0 mg/h; ■, 20°, 90% humidity, 8.6 mg/h; △, 0°, 25% humidity, 3.7 mg/h; □, 0°, 50% humidity, 3.6 mg/h; ▲, 0°, 90% humidity, 7.3 mg/h.

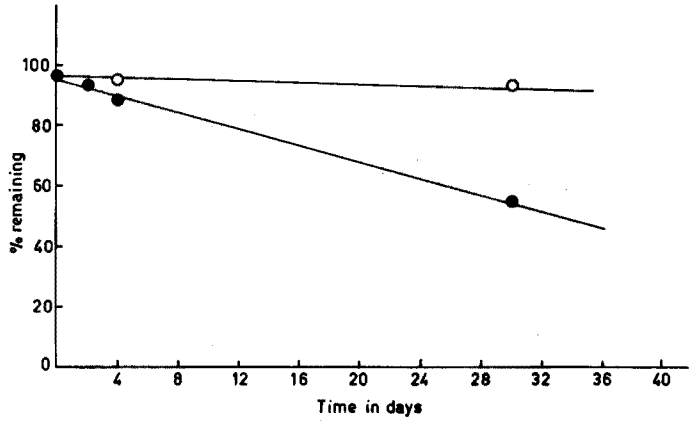

Fig. 9. Stability of collected H_2S: ○, kept dry; ●, kept at 70% humidity.

DISCUSSION

The picture of the mechanism of the collection of gases by impregnated filter paper, as suggested by the results on sulfur dioxide[1] and hydrogen fluoride[2] is that the diffusion in the gas phase is comparatively fast, the rate of uptake being determined in the first place by the diffusion in the liquid phase. The gases are taken up at the surface of the liquid and the surface is "renewed" by the diffusion process.

With this picture in mind one expects the collection to be improved by increasing the humidity, because the collecting surface area is enlarged and the viscosity of the liquid decreased. An increase in temperature which furthers the diffusion, should also improve the collection. Lower concentrations would be expected to be better sampled because more time would be available for the regeneration of the surface of the liquid.

The results in the case of hydrogen sulfide are not completely in accordance with this scheme. It is less easily collected than sulfur dioxide and hydrogen fluoride, possibly because hydrogen sulfide is a weaker acid, escaping more readily from the surface of the liquid. The manner in which the amount collected before breakthrough depends on the concentration (Figs. 6 and 7) is not so easy to explain. The complicated behaviour may be caused by the addition of zincate to the impregnating fluid, insofar as precipitates are formed with hydrogen sulfide and carbon dioxide that may hinder the diffusion.

That the zincate addition is necessary and effective was proved by the experiments on the stability of sulfide on filters with and without zincate.

In practical air pollution measurements, the efficiency of the impregnated filters should be generally satisfactory, because the concentrations of hydrogen sulfide should not be higher than a few mg/m^3, whereas the humidity would be higher than 25%. Moreover, the efficiency does not drop immediately to zero after breakthrough (Fig. 3). Nevertheless the margin of safety is somewhat lower than in the cases of sulfur dioxide and hydrogen fluoride.

From Figs. 6, 7 and 8, can be derived the following general rules. Sample not more than 250 μg of hydrogen sulfide per filter at an air-flow of not more than 1 m^3/h; or taking into account the area of the filters: sample not more than 20 $\mu g/cm^2$ at an air velocity of not more than 22 cm/sec. Higher amounts can be sampled at lower air-flows. However, 250 μg is about the maximum amount which can be conveniently determined by the colorimetric method described, giving an absorbance of about 1.1.

Another important rule concerns the maximum sampling time. Because of the loss of efficiency resulting from the uptake of carbon dioxide and the limited stability of sampled sulfide, it is advisable to use a sampling time of certainly not more than 2 days. For the same reasons filters must be used fresh if possible, and should be stored in a dry, and if possible carbon dioxide-free atmosphere before and after sampling.

Up to now, more than 100,000 samples of hydrogen sulfide in air have been taken and analysed by our laboratory by the proposed method; in part the automatic sampling and analysis described in the paper on sulfur dioxide[1] was applied.

SUMMARY

A method is proposed for the quantitative collection of hydrogen sulfide in air on impregnated filter paper. An aqueous solution of potassium hydroxide, potassium zincate and glycerol is used as impregnating fluid. The stability of the collected sulfide and the efficiency of collection at different humidities, temperatures, hydrogen sulfide concentrations and air velocities were determined.

RÉSUMÉ

Une méthode est proposée pour la collection quantitative de l'hydrogène sulfuré dans l'air sur papier filtre imprégné. On utilise comme réactif d'imprégnation, une solution aqueuse d'hydroxyde de potassium, de zincate de potassium et de glycérol. L'influence de divers facteurs (humidité, température, etc.) a été examinée.

ZUSAMMENFASSUNG

Es wird eine Methode vorgeschlagen, mit der sich Schwefelwasserstoff aus der Luft quantitativ mit imprägniertem Filtrierpapier sammeln lässt. Als Imprägnierungsflüssigkeit wird eine wässrige Lösung von Kaliumhydroxid, Kaliumzinkat und Glycerin benutzt. Es wurde die Stabilität der gesammelten Sulfide und die Wirksamkeit der Sammlung bei verschiedenen Feuchtigkeiten, Temperaturen, Schwefelwasserstoffkonzentrationen und Luftgeschwindigkeiten bestimmt.

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THE ULTRAVIOLET SPECTROPHOTOMETRIC DETERMINATION OF CADMIUM BY THE DIETHYLDITHIOCARBAMATE METHOD

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Colorimetric methods for determining cadmium are extremely limited in number, with dithizone[1-3] being the principal reagent. Other reagents which have been suggested include di-β-naphthylthiocarbazone[4], 4-hydroxy-3-nitrobenzenearsonic acid[5], Reinecke salt and thiourea[6], eriochrome grey BL[7], 1-(6-bromobenzothiazol-2-ylazo)-2-naphthol[8], "Cadion", a triazine dye[9], and Acid Chrome Dark Blue[10]. One differential ultraviolet spectrophotometric method has been devised using 1,10-phenanthroline, but sensitivity to pH and numerous interferences were found[11]. An ultraviolet spectrophotometric study of selected reagents for cadmium is in progress in our laboratory and this paper reports the results of the investigation using diethyldithiocarbamate.

EXPERIMENTAL

Reagents

Sodium diethyldithiocarbamate. Dissolve 1.00 g of sodium diethyldithiocarbamate trihydrate (Eastman No. 2596) in distilled water containing 1 ml of ammonia and dilute to 500 ml with a 1 : 100 ammonia solution. Store in an amber bottle.

Standard cadmium solution. Dissolve 0.4570 g of $3CdSO_4 \cdot 8H_2O$ in water and dilute to 2 l. This stock solution was standardized gravimetrically by the 8-quinolinol method and found to contain 0.101 mg of Cd per ml. Dilute standard cadmium solutions were prepared by a ten-fold dilution of this stock standard solution.

Citrate–tartrate masking solution. Dissolve 100 g of potassium tartrate and 100 g of potassium citrate in distilled water and dilute to 1 l.

Ammonia buffer solution. Add 3.0 g of ammonium chloride and 200 ml of ammonia (s.g. 0.90; 28% NH_3 by weight) to distilled water and dilute to 1 l.

Apparatus

Spectrophotometric measurements were made using a Cary 14 spectrophotometer. All measurements were made in 1.000-cm silica cells. A Leeds and Northrup Model 7664 pH meter equipped with glass and calomel electrodes was used for all pH measurements.

General procedure

Transfer a solution containing approximately 0.01 to 0.1 mg of cadmium to a

100-ml separatory funnel. Add 10 ml of the citrate–tartrate masking solution and 10 ml of the ammonia buffer solution. Add 5 ml of the 0.2% reagent solution by means of a transfer pipet, mix, and let stand for several min. Add 25 ml of reagent-grade chloroform to the separatory funnel and shake vigorously for about 1 min. Transfer the chloroform extract to a 50-ml volumetric flask. Repeat the extraction of the aqueous solution with 15 ml of chloroform. Add this chloroform extract to the volumetric flask and dilute to the mark with chloroform. Prepare a reference solution by repeating this procedure using a volume of distilled water equal to the volume of the sample solution. Measure the absorbance of the chloroform extract from the sample solution at 260 mμ against the extract of the reagent blank solution in the reference cell.

RESULTS

Ultraviolet absorption spectrum

The use of diethyldithiocarbamate as an extractant for metal ions has been studied by BODE[12] who observed that aqueous solutions of the reagent exhibited a characteristic ultraviolet absorption spectrum and that certain metal chelates exhibited ultraviolet absorptivity below 320 mμ. We found that the absorbance maximum for the cadmium diethyldithiocarbamate chelate was at 262 mμ when a reagent blank solution was used in the reference cell (Fig. 1). The chloroform extract of the reagent blank solution exhibits appreciable absorptivity, 0.01 to 0.1 absorbance unit, in the 240 mμ to 300 mμ region, so that either the extract of the reagent blank solution should be used in the reference cell, or a correction should be made for the absorbance of the blank solution if chloroform is used in the reference cell.

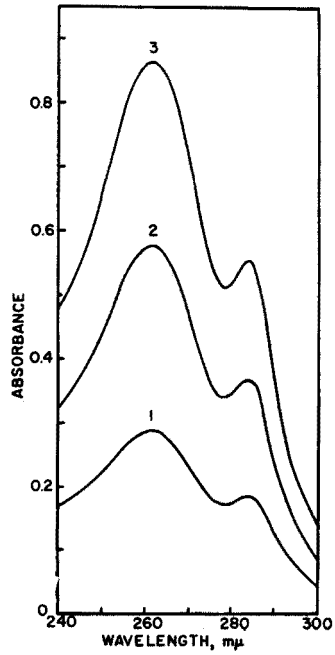

Fig. 1. Ultraviolet absorption spectra of cadmium(II) diethyldithiocarbamate system. 1, 1.01 p.p.m. of cadmium; 2, 2.02 p.p.m. of cadmium; 3, 3.03 p.p.m. of cadmium.

Cadmium concentration

The optimum concentration range using the general procedure is 0.5 to 3 p.p.m. of cadmium. Conformity to Beer's law was observed for this range. The molar absorptivity at 262 mμ was found to be $3.27 \cdot 10^4$ l/mole-cm.

Effect of pH

The amount of ammonia solution added to the solution before extraction is not critical, because of the buffer action of the citrate–tartrate solution. A pH of 11 ± 0.5 is recommended.

Reagent concentration

With 2 p.p.m. of cadmium and with volumes of 0.2% reagent varying from 0.1 to 5 ml, it was found that 0.3 ml was the minimum volume for maximum color formation; the absorbance was unchanged up to 5 ml of reagent. A volume of 5 ml is recommended to ensure sufficient reagent in case of larger amounts of cadmium or partial decomposition of the reagent. The reagent solution is unstable in neutral and acidic solutions[12]. The reagent solution should be prepared fresh each week.

Effect of diverse ions

Five hundred p.p.m. of the following ions did not cause interference: ammonium, sodium, potassium, aluminum, calcium, cerium(III), arsenate, chloride, bromide, perchlorate, citrate, and tartrate. Those ions which were found to cause interference are listed in Table I.

TABLE I

INTERFERING IONS

Ion	*Added as*	*Amount added (p.p.m.)*	% *Relative error*	*Permissible amount[a] (p.p.m.)*
Ag^+	Ag_2SO_4	2	+45	0
Au^{3+}	$AuCl_3$	1	+34	0
Bi^{3+}	$BiCl_3$	1	100	0
Cu^{2+}	$Cu(ClO_4)_2$	1	+69	0
Co^{2+}	$Co(ClO_4)_2$	1	+72	0
Cr^{3+}	$Cr(ClO_4)_3$	250	−10.3	50
Fe^{2+}	$Fe(ClO_4)_2$	10	+10.7	1
Fe^{3+}	$Fe(ClO_4)_3$	50	+ 5.2	10
Hg^{2+}	$Hg(ClO_4)_2$	1	+46	0
Mn^{2+}	$Mn(ClO_4)_2$	1	+ 4.3	0
Mg^{2+}	$Mg(ClO_4)_2$	500	− 4.8	250
Ni^{2+}	$Ni(ClO_4)_2$	1	+28	0
Pb^{2+}	$Pb(ClO_4)_2$	1	+31	0
Sn^{2+}	$SnCl_2$	2.5	+41	0
Sn^{4+}	$SnCl_4$	50	+ 1.4	50
Sb^{3+}	$KSbC_4H_4O_7$	10	+ 5.2	1
Zn^{2+}	$Zn(ClO_4)_2$	1	100	0
$EDTA^{4-}$	Na_2H_2EDTA	10	100	0

[a] Causes less than 2.5% relative error using 2 p.p.m. of cadmium.

Precision

An indication of the reproducibility of the diethyldithiocarbamate method was

obtained from a series of 5 determinations of solutions containing 2.02 p.p.m. of cadmium. Absorbance values of 0.590, 0.588, 0.589, 0.582 and 0.592 were obtained when the same reagent blank solution was used in the reference cell. A mean absorbance value of 0.588, a standard deviation of 0.004 absorbance unit, and a per cent relative standard deviation of 0.7% were obtained.

SUMMARY

An ultraviolet spectrophotometric method for the determination of small amounts of cadmium is proposed. The method is based on measuring the absorbance at 262 mμ of the cadmium diethyldithiocarbamate chelate which has been extracted from basic solution with chloroform. The effect of solution variables and numerous diverse ions has been investigated.

RÉSUMÉ

Une méthode est proposée pour le dosage de faibles quantités de cadmium, par spectrophotométrie dans l'ultra-violet. Elle est basée sur la mesure de l'absorption à 262 mμ du diéthyldithiocarbamate de cadmium, extrait d'une solution basique, au moyen de chloroforme. L'influence de divers facteurs et de nombreux ions a été examinée.

ZUSAMMENFASSUNG

Es wird eine spektralphotometrische Methode mit ultraviolettem Licht zur Bestimmung von kleinen Cadmiumgehalten vorgeschlagen. Die Methode beruht auf der Extraktion des Cadmiumdiäthyldithiocarbamatchelats aus basischer Lösung mit Chloroform und Messung der Extinktion. Der Einfluss zahlreicher Ionen wurde untersucht.

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SPECTROPHOTOMETRIC DETERMINATION OF THE DISSOCIATION CONSTANTS OF N,N'-BIS(2-HYDROXYETHYL)DITHIO-OXAMIDE

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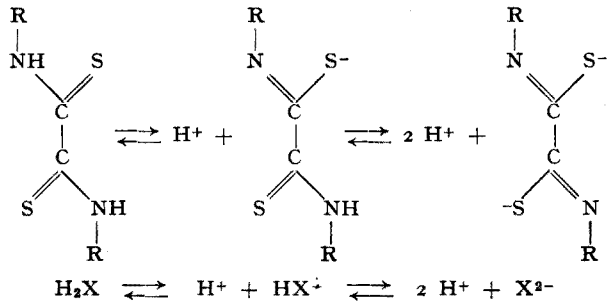
The dissociation constant of dithio-oxamide has been determined by YAFFE AND VOIGT[1]. These authors consider dithio-oxamide as a monobasic acid. However, while studying the silver(I) complexes of N,N'-bis(2-hydroxyethyl)dithio-oxamide, we found[2,3] that the ligand can react as a dibasic acid. In order to calculate the complex formation constants and solubility products of these complexes, the acidity constants of the ligand are required. In this communication we deal with the results of the determination by the spectrophotometric method of the dissociation constants of N,N'-bis(2-hydroxyethyl)dithio-oxamide.

According to RÂY AND XAVIER[4], the dissociation of N,N'-bis(2-hydroxyethyl)-dithio-oxamide can be given in the following manner (R = $-CH_2-CH_2OH$)



METHOD

Several workers[5,6] have used the basic relationship (1) between the optical absorbance E and the hydrogen ion concentration for the estimation of the dissociation constants of a dibasic acid

$$E = \frac{L \cdot C_{H_2X}\left[\varepsilon_1 + \frac{K_1}{[H^+]}\varepsilon_2 + \frac{K_1K_2}{[H^+]^2}\varepsilon_3\right]}{1 + \frac{K_1}{[H^+]} + \frac{K_1K_2}{[H^+]^2}} \qquad (1)$$

where $K_1 = (HX^-)[H^+]/(H_2X)$ and $K_2 = (X^{2-})[H^+]/(HX^-)$, L is the length of the

absorption cell and ε_1, ε_2 and ε_3 are the molar extinction coefficients of the species H_2X, HX^- and X^{2-}, respectively.

C_{H_2X} represents the total concentration of the dibasic acid

$$C_{H_2X} = (H_2X) + (HX^-) + (X^{2-})$$

After a few transformations the basic relationship (1) becomes

$$(\varepsilon_1 - \varepsilon_n)\frac{[H^+]^2}{K_1} + \varepsilon_2[H^+] + \varepsilon_3 K_2 - \varepsilon_n K_2 = \varepsilon_n[H^+] \qquad (2)$$

At any wavelength the so-called apparent extinction coefficient ε_n, is obtained from the measured absorbance E, as $E = \varepsilon_n L \cdot C_{H_2X}$. The values of ε_1 and ε_3 can usually be obtained by measurement of the optical absorbance E at low and high pH values, respectively. Indeed, at a low pH value $C_{H_2X} = (H_2X)$, and therefore $E = \varepsilon_1 \cdot C_{H_2X} \cdot L$. Hence it is possible to measure ε_1 directly. At a sufficient high pH value $C_{H_2X} = (X^{2-})$ and $E = \varepsilon_3 \cdot C_{H_2X} \cdot L$. However, N,N'-bis(2-hydroxyethyl)dithio-oxamide decomposes quickly in strong alkaline solutions and therefore the extinction coefficient ε_3 cannot be estimated accurately from the experimental data.

Accordingly, there are 4 unknown parameters in relationship (2): K_1, K_2, ε_2 and ε_3. If we write:

$$1/K_1 = a; \quad \varepsilon_2 = b; \quad \varepsilon_3 K_2 = c; \quad -K_2 = d$$
$$(\varepsilon_1 - \varepsilon_n)[H^+]^2 = x; \quad [H^+] = y; \quad \varepsilon_n = z; \quad \varepsilon_n[H^+] = u$$

we obtain a linear equation:

$$ax + by + c + dz = u \qquad (3)$$

with 4 unknown parameters: a, b, c and d.

By measuring E at different pH values we obtain a set of linear equations

$$f_i = ax_i + by_i + c + dz_i - u_i = o$$

The best estimates for a, b, c and d are obtained by means of the method of least squares.

Values of $[H^+]$ are obtained by potentiometric measurements. From considerations of the experimental procedure it follows that the functions f_i have different variances over the range of hydrogen ion concentrations studied. Therefore it becomes necessary to apply a weighting method such as those described by SULLIVAN, RYDBERG AND MILLER[7] and by ANDEREGG[8] in order to calculate complex formation constants from potentiometric measurements.

The weight w_i is found from the relationships (4) and (5).

$$w_i = 1/s_i^2 \qquad (4)$$

where s_i is the standard deviation of the function $f_i = f_i(\varepsilon_1, \varepsilon_n, [H^+])$ and is given by the relation:

$$s_i^2 = \left(\frac{\partial f_i}{\partial [H^+]}\right)^2 s_H^2 + \left(\frac{\partial f_i}{\partial \varepsilon_n}\right)^2 s_{\varepsilon_n}^2 + \left(\frac{\partial f_i}{\partial \varepsilon_1}\right)^2 s_{\varepsilon_1}^2 \qquad (5)$$

where s_H, s_{ε_n} and s_{ε_1} are the standard deviations of H^+, ε_n and ε_1, respectively.

It is accepted that $s_{\varepsilon_n} = s_{\varepsilon_1} = 50$ corresponds to an error of about 1% in the

extinction coefficients.

$$s_H = 2.302[H^+]s_{pH} \qquad \text{(ref. 8)}$$

where s_{pH} is the standard deviation of the pH values and is in the case of the glass electrode assumed to be 0.02.

Each function f_i must be multiplied by the square root of the weight w_i.

Since K_1 and K_2 must be known in order to calculate s_i^2 from eqn. (5), it is useful to calculate a set of approximate values for K_1 and K_2 without weighting coefficients.

If $K_1 \gg K_2$, then K_1 can be determined separately since the expression $K_2(\varepsilon_3 - \varepsilon_n)$ in the basic eqn. (2) may be neglected for a pH range where only the first stage of dissociation exists.

In this case, eqns. 2 and 3 become

$$(\varepsilon_1 - \varepsilon_n)\frac{[H^+]}{K_1} + \varepsilon_2 = \varepsilon_n \qquad (6)$$

$$f_i = ax_i' + b - y_i' \qquad (7)$$

where $x_i' = (\varepsilon_1 - \varepsilon_n)[H^+]$ and $y_i' = \varepsilon_n$.

The best estimates for a and b are found by the method of least squares. The calculation of the weights w_i is the same as in the case above.

EXPERIMENTAL

Apparatus

Absorbances at a definite wavelength were measured with a HILGER UVISPEK spectrophotometer. Matched silica cells of 1 cm light path were used.

The pH values were determined by means of a lithium glass electrode (Pye-Dynacap pH and millivoltmeter).

All measurements were made at 25°.

Reagents and solutions

N,N'-Bis(2-hydroxyethyl)dithio-oxamide was the same as used in a previous study[2].

C_{H_2X} was in all measurements $1 \cdot 10^{-4} M$.

Buffer solutions were obtained by mixing adequate amounts of 0.2 M glycine solution with 0.2 N sodium hydroxide solution.

To control the ionic strength, sodium perchlorate was used as an indifferent electrolyte.

In order to match each scale of the pH meter, glycine buffer solutions were used as described by KRATZ[9].

The measured optical absorbance was corrected for absorption of the solvent, buffer and indifferent electrolyte.

Determination of ε_1

The value of ε_n does not change in the pH range up to 9. Thus $\varepsilon_n = \varepsilon_1$ in this pH range. Table I shows ε_1 for different wavelengths.

Determination of the dissociation constants at ionic strength $\mu = 0.5$

The values of ε_n at different pH values and for wavelengths between 275 mμ and 271 mμ are given in Table II.

From these results it can be seen that the plot of the optical absorbance against pH gives a decided maximum (indicated by *). It means that 3 absorbing species of N,N'-bis(2-hydroxyethyl)dithio-oxamide are present, and so the assumption of a dibasic acid is proved.

The results obtained by the method outlined above are summarized in Table III.

TABLE I

CHANGE OF ε_1 AT 270–275 mμ

Wavelength (mμ)	ε_1
275	5950
274	5800
273	5680
272	5570
271	5460
270	5350

TABLE II

ε_n AT DIFFERENT PH AND WAVELENGTH VALUES

pH	ε_n *at different wavelengths*				
	275 mμ	*274 mμ*	*273 mμ*	*272 mμ*	*271 mμ*
10.44	6450	6420	6390	6360	6330
10.74	6810	6800	6780	6790	6800
10.92	6990	6980	7000	7020	7060
11.14	7140	7170	7220	7260	7330
11.33	7240	7290	7340	7430	7520
11.58	7320	7350	7420	7520	7630
11.73	7460	7560*	7640	7770	7880
12.16	7530*	7560*	7650	7800	7930
12.28	7530*	7540	7660*	7810*	7950*
12.83	7380	7400	7530	7690	7830
13.13	7210	7280	7410	7590	7770
13.31	6980	7050	7230	7400	7570
13.43	6830	6910	7090	7290	7470
13.53	6740	6850	7040	7230	7450

TABLE III

VALUES OF K_1 AND K_2

Wavelength (mμ)	K_1	pK_1	K_2	pK_2
275	$1.91 \cdot 10^{-11}$	10.72	$0.94 \cdot 10^{-14}$	14.03
274	$2.03 \cdot 10^{-11}$	10.69	$1.41 \cdot 10^{-14}$	13.85
273	$2.00 \cdot 10^{-11}$	10.70	$1.42 \cdot 10^{-14}$	13.85
272	$1.91 \cdot 10^{-11}$	10.72	$1.33 \cdot 10^{-14}$	13.88
271	$1.93 \cdot 10^{-11}$	10.71	$1.07 \cdot 10^{-14}$	13.96
Mean		10.71		13.92
Standard deviation		±0.01		±0.08

TABLE IV

VALUES OF K_1 AT DIFFERENT IONIC STRENGTHS

Ionic strength / *Wavelength (mμ)*	$\mu = 0.01$		$\mu = 0.1$		$\mu = 0.2$		$\mu = 0.3$		$\mu = 0.4$	
	K_1	pK_1	K_1	pK_1	K_1	pK_1	K_1	pK_1	K_1	pK_1
275			$1.19 \cdot 10^{-11}$	10.92						
274	$0.93 \cdot 10^{-11}$	11.03	$1.15 \cdot 10^{-11}$	10.94	$1.26 \cdot 10^{-11}$	10.90	$1.37 \cdot 10^{-11}$	10.86	$1.75 \cdot 10^{-11}$	10.76
273	$0.93 \cdot 10^{-11}$	11.03	$1.23 \cdot 10^{-11}$	10.91	$1.28 \cdot 10^{-11}$	10.89	$1.44 \cdot 10^{-11}$	10.84	$1.77 \cdot 10^{-11}$	10.75
272	$0.99 \cdot 10^{-11}$	11.00	$1.20 \cdot 10^{-11}$	10.92	$1.24 \cdot 10^{-11}$	10.91	$1.45 \cdot 10^{-11}$	10.84	$1.81 \cdot 10^{-11}$	10.74
271	$0.97 \cdot 10^{-11}$	11.01	$1.27 \cdot 10^{-11}$	10.90	$1.28 \cdot 10^{-11}$	10.89	$1.49 \cdot 10^{-11}$	10.83	$1.75 \cdot 10^{-11}$	10.76
270	$0.95 \cdot 10^{-11}$	11.02	$1.23 \cdot 10^{-11}$	10.91	$1.26 \cdot 10^{-11}$	10.90	$1.48 \cdot 10^{-11}$	10.83	$1.80 \cdot 10^{-11}$	10.76
Mean		11.02		10.92		10.90		10.84		10.75
Standard deviation		±0.01		±0.02		±0.01		±0.01		±0.01

Determination of K_1

Since $K_1/K_2 > 10^3$, K_1 may be calculated separately in the pH range 10 to 12. Table IV gives the results at different ionic strengths.

In order to determine the thermodynamic dissociation constant pK_1, the plot of pK_1 *vs.* μ was extrapolated to zero ionic strength. The value obtained was 11.04 (Fig. 1).

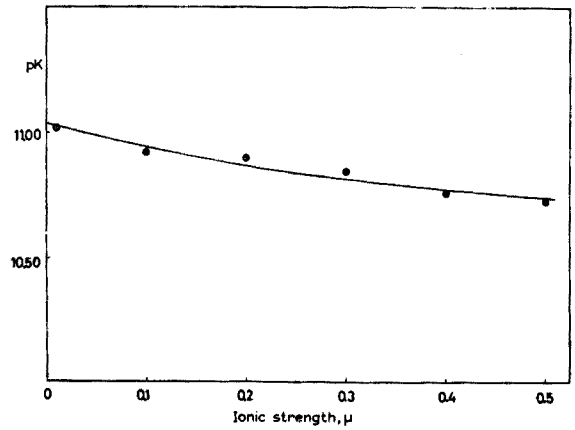


Fig. 1. Extrapolation of the plot of pK_1 *vs.* μ to $\mu = 0$.

From the pK_1 value at $\mu = 0.01$ and the Debye–Hückel equation $\log f_{HA^-} = -0.506\sqrt{\mu}$, a thermodynamic dissociation constant $pK_1 = 11.07$ was obtained.

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SUMMARY

The dissociation constants of N,N'-bis(2-hydroxyethyl)dithio-oxamide were determined by a spectrophotometric method. The dissociation constants were calculated by means of a weighted least squares technique. N,N'-Bis(2-hydroxyethyl)dithio-oxamide was found to be a dibasic acid with a thermodynamic dissociation constant $pK_1 = 11.04$. At ionic strength $\mu = 0.5$, $pK_1 = 10.71$, and $pK_2 = 13.92$.

RÉSUMÉ

Les constantes de dissociation du N,N'-bis(2-hydroxyéthyl)dithio-oxamide ont été déterminées par une méthode spectrophotométrique. Ces constantes ont été calculées par la méthode des moindres carrés pondérée. Il a été prouvé que le produit mentionné ci-dessus se comporte comme un acide dibasique dont la constante de dissociation thermodynamique est $pK_1 = 11.04$. Pour une force ionique $\mu = 0.5$, l'on trouve pour les pK_1 et pK_2 respectivement 10.71 et 13.92.

ZUSAMMENFASSUNG

Die Dissoziationskonstanten von N,N'-Bis(2-hydroxyäthyl)dithio-oxamid wurden mit Hilfe einer spektralphotometrischen Methode berechnet. Die Konstanten wurden nach der Methode der kleinsten Quadrate erhalten unter Berücksichtigung des Gewichtes eines jeden Messpunktes. Die Verbindung ist eine zweibasische Säure mit einer thermodynamischen Dissoziationskonstanten $pK_1 = 11.04$. Bei der Ionenstärke $\mu = 0.5$ betragen die Konstanten $pK_1 = 10.71$ und $pK_2 = 13.92$.

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SPECTROPHOTOMETRIC DETERMINATION OF SUBMICRO QUANTITIES OF AJMALINE

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Ajmaline was isolated by SIDDIQUI AND SIDDIQUI[1] from the roots of *Rauwolfia serpentina* Benth. The pharmacology of this alkaloid has been reported by many workers: ARORA AND MADAN[2] found it to be a strong antiarrhythmic agent and it is more active than quinidine in auricular fibrillation, auricular flutter and also in ventricular arrhythmias[3]. Ajmaline lowers the blood pressure of dogs in a dose of 2 mg/kg body weight[4] and was later used by several workers in human subjects for treatment of cardiac arrhythmias of various origins.

KLEINSÖRGE AND GAIDA[5] estimated ajmaline in pharmaceutical preparations by first extracting it with chloroform and then extracting the chloroform layer with sulphuric acid; the acid solution was mixed with a diazo reagent to give a red colour which was measured after 5 days. The same method was used for the estimation of ajmaline in urine[6]. This procedure is obviously time-consuming; moreover, the recovery was only 86% and the accuracy $\pm$ 5.0%. A turbidimetric method for the estimation of ajmaline in submicro quantities was recently described by SHAH AND NARGIS[7]; interference from other alkaloids (*e.g.* reserpine, ajmalicine, serpentine) was eliminated by electrophoresis or chromatographic methods, before the turbidity produced with sodium tetraphenylboron was measured.

SIDDIQUI AND SIDDIQUI[8] showed that ajmaline gives a fading red coloration with concentrated nitric acid forming trinitroajmaline. It was observed in the present study that with dilute nitric acid or even in the presence of very small amounts of water, the colour fades much more rapidly than with concentrated nitric acid. Hence, by using concentrated nitric acid and by eliminating water from the ajmaline-containing solution, the red colour was made sufficiently stable for an absorptiometric measurement to be made conveniently.

Figure 1 shows the absorption spectrum of ajmaline in concentrated nitric acid at different time intervals; the fading of colour with time is quite apparent. In another series of tests, 50 μg of ajmaline were dissolved in 5 ml of concentrated nitric acid and the absorbance was measured at 510 mμ in a 1-cm cell against a blank of concentrated nitric acid after different time intervals. The optical density decreased from 0.715 after 3 min, through 0.580 after 10 min, to 0.430 after 20 min, but the readings

at each time interval were quite reproducible. The reaction can therefore be used for the estimation of ajmaline. Concentrated nitric acid shows no absorbance at 510 mμ against a blank of distilled water.

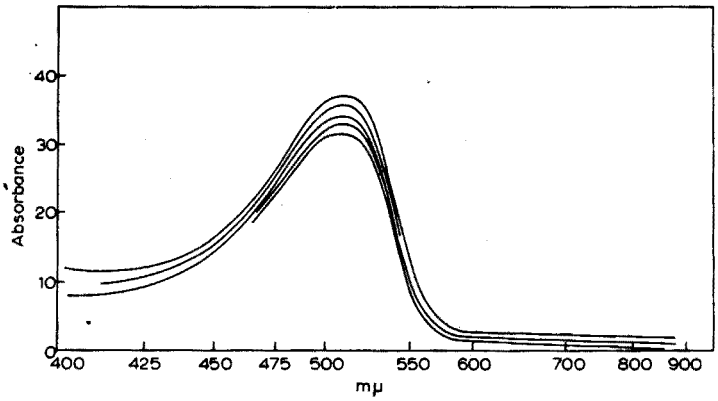

Fig. 1. Absorbance spectra of ajmaline in concentrated nitric acid after 3, 5, 10, 15 and 20 min.

EXPERIMENTAL

Apparatus and chemicals

Beckman Dk and Unicam SP 600 spectrophotometers were used with 1-cm cells.

Ajmaline solution. Dissolve 25 mg of ajmaline (dried at 100° under vacuum over P_2O_5 for 1 h) in 25 ml of ethanol. This solution is always freshly prepared.

Preparation of calibration curves

Transfer 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 μl aliquots of the ajmaline solution to 10-ml centrifuge cones by means of an "Agla" micrometer syringe. Evaporate to dryness under an infrared lamp or on a water bath. Add 5 ml of concentrated nitric acid (for curve I) or 10 ml (for curve II), from a pipet and stir with a glass rod for about 1 min. Start a stop-watch as soon as nitric acid begins to flow into the cone. Measure the absorbance after exactly 5 min at 510 mμ against a blank of concentrated nitric acid.

Both the curves were linear over the range examined. Curve II is convenient for 5–60 μg of ajmaline; for higher concentrations, *i.e.* 40–100 μg, curve I is preferable. The optical densities of curve II were exactly half of the optical densities of curve I for the same quantities of ajmaline. The curves did not pass exactly through the origin.

Procedures

Ajmaline (pure) or in combination with ajmalicine and serpentine. Weigh 40–60 μg of ajmaline into a centrifuge cone, or prepare a solution of higher concentration in ethanol and take a suitable volume for analysis. Evaporate to dryness over an infrared lamp or on a water bath. Add 5 ml of concentrated nitric acid and then follow the procedure described for the preparation of calibration curves.

Assay of intramuscular injections (50 mg of ajmaline in 2 ml). Transfer 1 ml of the solution from the ampoule to a 25-ml volumetric flask and dilute to 25 ml with

ethanol (1 μl of the solution contains 1 μg of ajmaline). Transfer 50 μl or any convenient volume of this solution with an "Agla" micrometer syringe to a 10-ml centrifuge cone, evaporate to dryness and proceed as described above.

Intravenous injection (50 mg of ajmaline in 10 ml) ampoule. Dilute 5 ml of the solution from the ampoule to 25 ml with ethanol and continue as described above.

Tablets of ajmaline (50 mg/tablet). Grind one tablet finely and transfer to a 50-ml volumetric flask. Add a mixture of 45 ml of ethanol and 5 ml of chloroform, stopper the flask and shake vigorously for 30 min. Centrifuge the resulting suspension and filter the clear liquid through a sintered glass funnel. Then evaporate 50 μl of the filtered solution to dryness on a water bath in a centrifuge cone and proceed further as described above.

RESULTS AND DISCUSSION

The procedures described were arrived at after investigation of the interference of other alkaloids and the factors which have bearing on the stability of the colour. The measurements of absorbance described were carried out at room temperature (29°). The effect of cooling on the stability of the colour developed after the addition of concentrated nitric acid was studied by cooling the nitric acid to 15° in ice; at lower temperatures, the surfaces of the cell clouded so that measurement was impossible. The results found at time intervals up to 20 min were exactly the same whether the solution was at 29° or 15°; there is thus no temperature effect within normal limits.

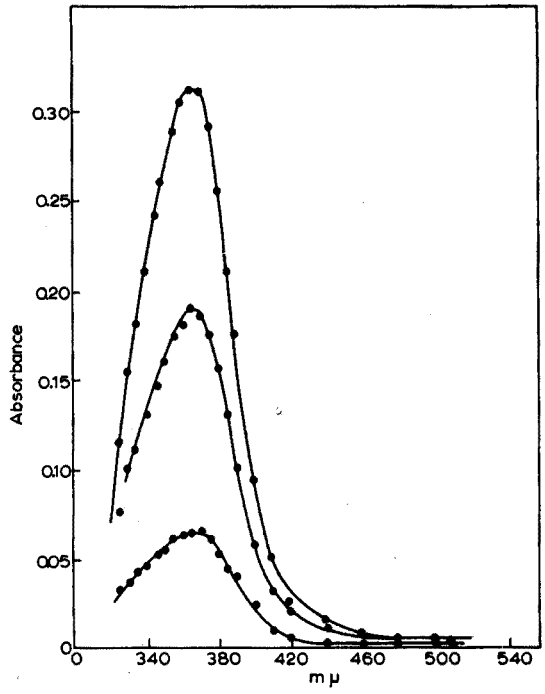

Fig. 2. Absorbance spectra of different concentrations (20, 40 and 100 μg) of serpentine in concentrated nitric acid.

The interference of other alkaloids found in *Rauwolfia serpentina* Benth was studied. Interference of this type was expected while determining ajmaline in some of the complexes, *e.g.*, serpajmaline, ajmalexine[9]. Figures 2 and 3 show the absorbance curves of serpentine and ajmalicine, in concentrated nitric acid. Neither of these bases absorbed at 510 mμ and therefore no interference in the determination of ajmaline should occur. In order to determine the limit up to which these bases will not interfere, increasing amounts of serpentine and ajmalicine were added to ajmaline. It was found that even 200 μg of serpentine or ajmalicine had no significant effect on the absorbance of 50 μg of ajmaline and that 25 μg had no effect on the absorbance of 5 μg of ajmaline. The method can therefore be applied directly for the estimation of ajmaline in the presence of serpentine and ajmalicine.

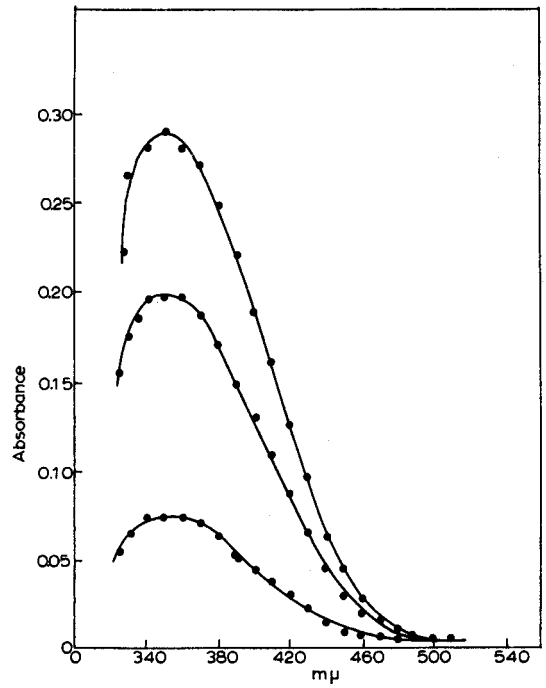

Fig. 3. Absorbance spectra of different concentrations (20, 60 and 100 μg) of ajmalicine in concentrated nitric acid.

The analysis of the pharmaceutical products was made directly without any preliminary extraction of the base; no interference of any kind was observed. However, in the case of tablets excipient material had to be removed by centrifuging after the powdered tablet had been dissolved in an ethanol–chloroform mixture.

Table I shows the results obtained for ajmaline in some of the pharmaceutical products. It can be seen that the results are accurate and reproducible. Replicate analyses of a single sample showed a variation in absorbance of only ±0.005; this corresponds to an error of ±1% at the 50 μg level. The method has the advantage of being rapid and is unaffected by the presence of the usual interfering substances.

TABLE I

ANALYSIS OF AJMALINE PREPARATIONS

Name of preparation	*Amount of ajmaline as indicated on the preparation (A)*	*Amount taken for analysis after dilution of (A) (μg)*	*Ampoule no.*	*Ajmaline found (μg)*	*Difference (μg)*
Ajmaline intramuscular injections	50 mg/2 ml	50	1	50.2	+ 0.2
				50.0	0.0
-do-	-do-	-do-	2	48.0	− 2.0
				48.0	− 2.0
-do-	-do-	25	2	24.0	− 1.0
				23.5	− 1.5
				24.0	− 1.0
-do-	-do-	50	3	47.5	− 2.5
				48.0	− 2.0
				48.0	− 2.0
-do-	-do-	25		24.0	− 1.0
				23.5	− 1.5
				24.0	− 1.0
Ajmaline intravenous injections	-do-	50	1	50.0	0.0
				50.0	0.0
-do-	-do-	50	2	49.5	− 0.5
				49.5	− 0.5
		25		24.0	− 1.0
				24.2	− 0.8
				24.0	− 1.0
Ajmaline tablets	50 mg/tablet	50	Tablet no. 1	50.0	0.0
		50	Tablet no. 2	50.0	0.0
		50	Tablet no. 3	52.0	+ 2.0
Ajmaline(pure)	×	50	×	50.0	0.0
		25		25.0	0.0

The time limit for the measurement of absorbance was fixed at 5 min only for working convenience and can be varied if desired. The method is being applied to the estimation of ajmaline in urine and blood plasma.

The authors wish to express their indebtedness to Dr. SALIMUZZAMAN SIDDIQUI, F.R.S. for helpful suggestions. Thanks are also due to Yahya & Sons, Karachi, for supplying ajmaline preparations from GEBR Giulini GMBH, Ludwigshafen, and to Dr. SARFRAZ SIDDIQUI for supplying literature on the pharmacological aspects of ajmaline.

SUMMARY

A spectrophotometric method for the determination of 5–100 μg samples of ajmaline is described. Ajmaline gives a red colour with concentrated nitric acid, which is measured at 510 mμ after a definite time interval. The limits of interference from serpentine and ajmalicine are given. The method can be applied directly for the estimation of ajmaline in pharmaceutical preparations.

RÉSUMÉ

Une méthode spectrophotométrique est décrite pour le dosage de l'ajmaline, en quantités allant de 5 à 100 μg. Cet alcaloïde donne avec l'acide nitrique concentré une coloration rouge, dont on mesure l'intensité à 510 mμ. Cette méthode de dosage peut être appliquée directement à des préparations pharmaceutiques.

ZUSAMMENFASSUNG

Eine spektralphotometrische Methode zur Bestimmung von 5–100 μg Ajmalin wird beschrieben. Ajmalin gibt eine rote Farbe mit konzentrierter Salpetersäure, welche nach einer bestimmten Zeit bei 510 mμ gemessen wird. Die Störgrenzen von Serpentin und Ajmalicin werden angegeben. Die Methode kann direkt zur Bestimmung von Ajmalin in pharmazeutischen Präparaten angewandt werden.

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THE SOLUBILITY OF AMMONIUM 12-MOLYBDOPHOSPHATE IN DILUTE ACIDS

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Ammonium 12-molybdophosphate was for long stated to be insoluble in water and aqueous electrolytes, although it was known to be soluble in alkali. The standard reference works[1-3] still show ammonium molybdophosphate as being insoluble in water, though no quantitative data are given. SEIDELL[4] gives data compiled by DI LUCHI in 1910. The solubility of ammonium molybdophosphate is given as 0.238 g/1000 g in water, 0.137 g/1000 g in 5% aqueous ammonium nitrate, and 0.203 g/1000 g in 1% nitric acid.

THISTLETHWAITE[5] studied the solubility of the substance in various recommended wash solutions. He obtained solubilities of 26.2 mg/100 ml in 1% aqueous nitric acid, 6.9 mg/100 ml in a 1% potassium nitrate–0.5% nitric acid mixture, 6.2 mg/100 ml in 0.8% ammonium nitrate, and 0.8 mg/100 ml in a 0.5% nitric acid–0.8% ammonium nitrate mixture.

STOCKDALE[6] noticed that the solubility increased to 400 mg/l after 5 days in 2% nitric acid. This suggested that hydrolysis was occurring. Earlier, JANDER AND DREWS[7] had suggested that 12-molybdophosphoric acid decomposed in aqueous acids with the formation of molybdenyl cations

$$H_3PO_4Mo_{12}O_{36} + 24\ H^+ \rightarrow H_3PO_4 + 12\ MoO_2^{2+} + 12\ H_2O$$

They further suggested that in dilute acid solution a 12-molybdophosphate was hydrolysed to hexamolybdate and 6-molybdophosphate

$$(PMo_{12}O_{40})^{3-} + 6\ H_2O \rightarrow (H_3Mo_6O_{21})^{3-} + (H_5PMo_6O_{25})^{4-} + 4\ H^+$$

SOUCHAY AND FAUCHERRE[8] claim to have shown the existence of 11, 9, 2.5 and 1-molybdophosphates.

The work reported in this paper is an attempt to clarify the position regarding the apparent solubility of ammonium molybdophosphate on prolonged standing with dilute acids. The investigation was prompted by certain anomalies which appeared with some wash solutions in a study of the conditions for the quantitative precipitation of ammonium molybdophosphate[9].

EXPERIMENTAL

Chemicals

A solution of A.R. potassium dihydrogen phosphate containing 1.28 g/l was prepared.

The nitric acid/ammonium molybdate reagent and the phosphorus-32 solution were prepared as described previously[9].

The radioisotope molybdenum-99 was obtained as sodium molybdate from The Radiochemical Centre, Amersham. A small amount of ammonium molybdate solution was added to 5 mC of active molybdate and the solution was diluted to 100 ml.

Wash solutions. (*a*) Acid ammonium nitrate. 20 g A.R. ammonium nitrate and 12.5 ml of nitric acid (s. g. 1.42) were made up to 2.5 l with distilled water.

(*b*) Nitric acid. A 1% solution was prepared.

Apparatus

Sintered glass crucibles. The filters were of porosity 4.

Counting equipment. Both the phosphorus-32 and the molybdenum-99 were counted using a Mullard MX 124 liquid counter or a Mullard MX 123 end-window counter and an Ecko automatic scaler, type N530F. Triplicate readings of all counts were taken.

Chromatographic equipment. A jar 45 cm × 5 cm was used. The butanol/nitric acid mixture was prepared by taking 25-ml portions of *n*-butanol and 2 *M* nitric acid and shaking them in a separatory funnel. The mixture was left for 24 h to equilibrate and the aqueous layer was discarded.

Preparation of ammonium 12-molybdophosphate

Ammonium molybdate reagent (20 ml) was added to aqueous potassium dihydrogen phosphate (10 ml) containing active phosphate (5 ml) at 70°. The solution was maintained at this temperature for 1 h, with constant stirring, then allowed to stand at room temperature for 1 h. The ammonium 12-molybdophosphate was filtered and washed with acid ammonium nitrate followed by 1% nitric acid. About 50 mg of the precipitate was dried at 200° for 2 h, weighed, dissolved in 50 ml of 2 *M* aqueous ammonia, and used as a standard. Ammonium 12-molybdophosphate labelled with molybdenum-99 was prepared as above, but with ammonium molybdate containing 5 ml of active molybdate in place of the 5 ml of active phosphate.

Determination of solubilities

Dried 50-mg samples of ammonium 12-molybdophosphate were placed with 50 ml of solvent in 100-ml conical flasks. The samples, maintained at the selected temperature in a water bath fitted with a Techne Tempunit, were stirred continuously; 10-ml aliquots of the solution were withdrawn at intervals, centrifuged and counted in a liquid counter. The solubility in mg/l was determined by comparison with the standard. The 10-ml sample was then returned to the bulk solution.

Solubility of ammonium molybdophosphate in nitric acid of different strengths

Ammonium 12-molybdophosphate samples, labelled with phosphorus-32 or molybdenum-99, were stirred with dilute nitric acid solutions of different strengths. Samples were withdrawn at intervals, counted and returned to the solution. The solubilities

were calculated from the corrected count-rates; the results obtained based on the two different activities are shown in Table I.

TABLE I

SOLUBILITY OF AMMONIUM MOLYBDOPHOSPHATE IN NITRIC ACID SOLUTIONS

Time (h)	*Solubility of ammonium molybdophosphate (mg/l) 25°*				
	Water	*0.10 M Nitric acid*	*0.20 M Nitric acid*	*0.40 M Nitric acid*	*0.60 M Nitric acid*
Based on ^{32}P dissolved					
1	8.7	3.0	2.9	8.0	98
2	10.8	5.9	5.9	16.9	190
3	13.3	8.1	8.3	29.6	290
4	14.9	9.9	10.4	40.0	350
5	15.9	11.9	12.3	64.6	432
6	18.0	13.3	13.6	110	501
24	27.5	38.0	42.1	631	598
26	28.0	42.0	48.0	642	600
28	29.0	47.0	56.0	655	600
48	34.1	130	195	710	602
Based on ^{99}Mo dissolved					
1	31.2	26.8	12.7	21.7	42.1
2	63.0	34.4	14.6	100	100
3	78.0	36.0	18.6	187	180
4	90.2	42.0	22.0	268	250
5	98.0	47.3	28.0	345	312
6	105	49.3	42.0	354	410
24	155	144	432	616	631
26	157	160	446	620	634
28	160	178	456	624	636
48	166	370	540	668	658

TABLE II

SOLUBILITY IN DIFFERENT ACIDS OF THE SAME MOLARITY (BASED ON MEASUREMENT OF ^{32}P)

Time (h)	*Solubility of ammonium molybdophosphate (mg/l) 25°*			
	Water	*0.60 M Nitric acid*	*0.60 M Perchloric acid*	*0.60 M Hydrochloric acid*
1	8.7	100	11.3	104
2	10.8	190	23.2	170
3	13.3	290	42.3	212
4	14.9	350	72.5	318
5	15.9	430	105	415
6	18.0	500	160	549
24	27.5	599	940	870
26	28.0	600	945	890
28	29.0	600	958	892
48	34.1	602	1100	990

Solubility of ammonium 12-molybdophosphate in different acids

Ammonium 12-molybdophosphate samples, labelled with molybdenum-99 or phosphorus-32, were stirred with dilute aqueous solutions of different acids of the same molarity. Samples were withdrawn at intervals, counted, and returned to the solution. The solubilities were calculated from the corrected count-rates (Table II).

Chromatography

A sample of ammonium molybdophosphate labelled with both phosphorus-32 and molybdenum-99 was prepared, and the sample was dried at 200° for 2 h and weighed. The sample was then hydrolysed with 0.6 *M* aqueous nitric acid until counts on 10-ml portions of the solution showed that all the ammonium molybdophosphate had dissolved. The solution was then concentrated to approximately 2.5 ml on a hot-plate at 60°, and 0.05 ml of it was transferred by a micro-pipet to a strip of Whatman No. 3 MM chromatography paper. The strip was eluted with butanol/nitric acid mixture for 4 h, dried at room temperature and counted 0.5 cm at a time, using a Mullard 123 MX end-window counter. A histogram (Fig. 1) was drawn from the corrected

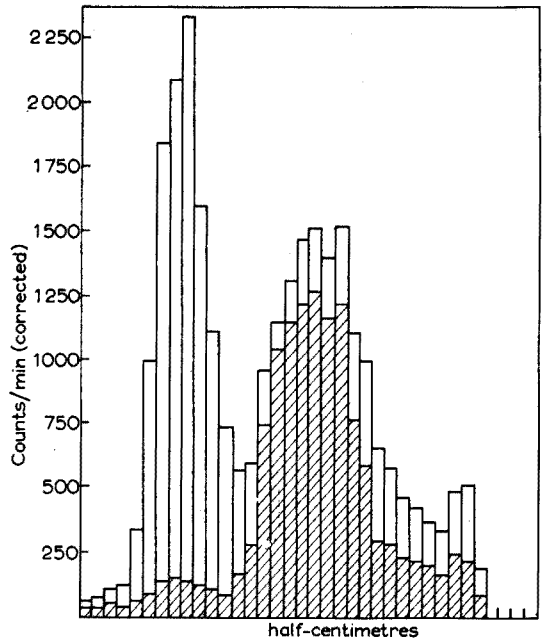

Fig. 1. Distribution of phosphorus and molybdenum in the hydrolysis products from ammonium molybdophosphate: blank, molybdenum; shaded phosphorus.

count-rates. The strip was retained and counted again 14.3 days later. During this time the count-rate due to the phosphorus had been halved, whereas that due to the molybdenum had become negligible, over 5 half-lives having been lost. The corrected count-rates were doubled and plotted on the original histogram. By subtracting this

second count from the original count for each 0.5 cm of the strip, the count-rate due to the molybdenum-99 was obtained.

The total count-rate due to the phosphorus-32 was obtained from the histogram and the percentage of phosphorus in each 0.5 cm of the strip was calculated. The same procedure was used to calculate the percentage of molybdenum in each 0.5 cm of the strip. Taking the total count-rate due to phosphorus and the total count-rate due to molybdenum to be in the atomic ratio 1 : 12, the ratio of phosphorus to molybdenum for each 0.5 cm of the strip was calculated.

A typical set of results is shown in Table III.

TABLE III

CHROMATOGRAPHIC RESULTS

Strip	*Immediate count-rate (counts/min corrected) (A)*	*Count-rate after 14.3 days (B)*	*Original count-rate due to phosphorus (2B)*	*Count-rate due to molybdenum (A-2B)*	*% P*	*% Mo*	*% Mo × 12*	*Mo/P ratio*
1a	57	10	20	37	0.16	0.24	2.88	18
1b	74	9	18	56	0.14	0.36	4.32	31
2a	107	19	38	69	0.30	0.45	5.40	18
2b	117	17	34	83	0.27	0.54	6.50	24
3a	338	29	58	280	0.47	1.8	21.6	46
3b	995	45	90	905	0.72	5.9	71.0	99
4a	1846	65	130	1716	1.05	11.1	133.0	127
4b	2098	68	136	1962	1.1	12.7	152.0	138
5a	2330	66	132	2198	1.1	14.2	170.0	155
5b	1601	62	124	1477	1.0	9.5	114.0	114
6a	1119	55	110	1009	0.88	6.5	78.0	89
6b	736	45	90	646	0.72	4.2	50.5	70
7a	564	85	170	394	1.37	2.5	30.0	22
7b	590	137	274	316	2.2	2.0	24.0	11
8a	948	372	744	204	6.0	1.3	15.6	2.6
8b	1143	520	1040	103	8.3	0.67	8.05	0.98
9a	1322	573	1146	176	9.2	1.1	13.2	1.4
9b	1475	608	1216	259	9.8	1.7	20.4	2.1
10a	1498	631	1262	236	10.2	1.5	18.0	1.8
10b	1400	563	1126	274	9.0	1.8	21.6	2.4
11a	1518	617	1234	284	9.9	1.8	21.6	2.2
11b	1105	385	770	335	6.2	2.2	26.4	4.2
12a	989	295	590	399	4.7	2.6	31.2	6.6
12b	634	152	304	330	2.4	2.1	25.2	10.5
13a	582	122	244	338	2.0	2.1	25.2	12.5
13b	465	106	212	253	1.7	1.6	19.2	11.3
14a	424	100	200	224	1.6	1.45	17.4	11.0
14b	375	86	172	203	1.4	1.3	15.6	11.1
15a	342	87	174	168	1.4	1.1	13.2	12.0
15b	501	131	262	239	2.1	1.55	18.6	8.8
16a	519	114	228	291	1.8	1.9	22.8	12.7
16b	199	45	90	109	0.72	0.7	8.4	11.7

The peaks on the histogram were compared with histograms of reference samples prepared by spotting active phosphate (Fig. 2) and molybdate (Fig. 3) on chromatographic paper, drying at room temperature and eluting for 4 h. The paper strips were

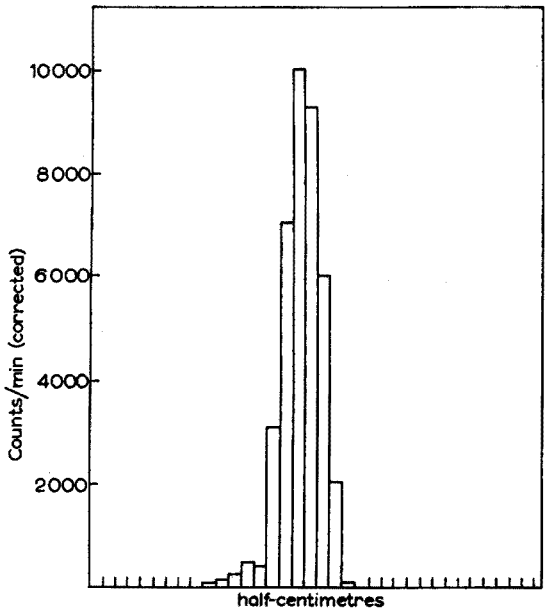

Fig. 2. Phosphate reference, $R_F = 0.51$.

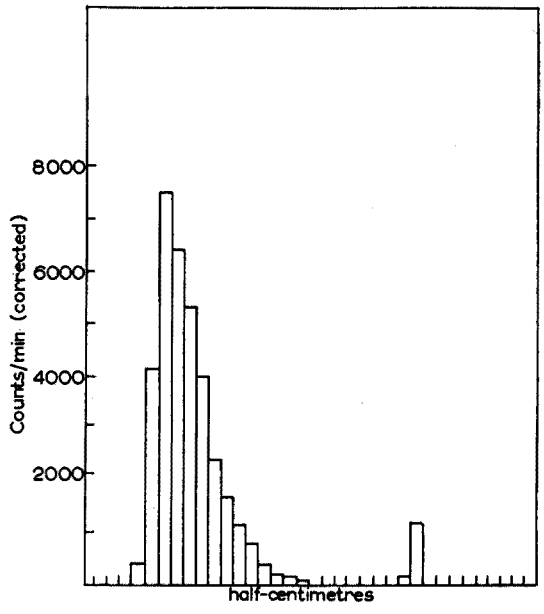

Fig. 3. Molybdate reference, $R_F = 0.17$ and 0.82.

allowed to dry at room temperature and counted. Histograms were prepared from the corrected count-rates, and R_F values for phosphate and molybdate were obtained. Further reference samples hydrolysed in nitric acid of different strengths gave similar histograms.

The following points are noteworthy.

(*a*) More than 60% of the molybdenum appears, with only traces of phosphorus, at R_F 0.15–0.40 which corresponds to the peak on the molybdate reference histogram.

(*b*) More than 60% of the phosphorus appears, with some molybdenum, at R_F 0.45–0.70, which corresponds to the peak on the phosphate reference histograms.

(*c*) About 14% of both the phosphorus and the molybdenum appear at R_F 0.75–0.95, in the proportion 1:12 suggesting that the 12-molybdophosphate anion is responsible. The slow dissolution of ammonium 12-molybdophosphate in aqueous nitric acid appears to be due to its hydrolysis mainly to phosphoric and molybdic acids, although the results do not rule out the possibility of a 2-molybdophosphate ion with roughly the same R_F value for the elution as the phosphate itself.

CONCLUSIONS

Dilute nitric acid, alone or mixed with ammonium nitrate, is the most frequently recommended wash solution for the ammonium 12-molybdophosphate precipitate. Although this compound has only slight immediate solubility in aqueous nitric acid weaker than 2.5%, stronger nitric acid dissolves it appreciably, and even in 0.6 *M* acid the solubility increases markedly on prolonged standing.

The precipitate dissolves very little faster in 0.6 *M* hydrochloric acid than in 0.6 *M* nitric acid, although the efficiency of precipitation of ammonium 12-molybdophosphate falls with hydrochloric acid concentration but is not affected by nitric acid concentrations up to 10 *M*[9]. Although perchloric acid and nitric acid do not inhibit the formation of an ammonium molybdophosphate precipitate whereas hydrochloric acid does, the three acids dissolve the precipitate about equally when they are stirred with it for a prolonged time.

But in 0.6 *M* perchloric acid the precipitate dissolves much more slowly initially than in the nitric acid or hydrochloric acid. Perchloric acid is evidently even more suitable as a wash solution than the nitric acid which is generally used.

We thank D.S.I.R. for a research studentship to D.W.A.

SUMMARY

An attempt has been made to clarify the position regarding the apparent solubility of ammonium molybdophosphate on prolonged standing with dilute acids. The solubility of ammonium molybdophosphate in nitric acid solutions of different concentrations was determined by radiochemical methods using ^{32}P and ^{99}Mo. On prolonged standing with aqueous solutions, ammonium molybdophosphate hydrolyses completely, mainly to phosphoric and molybdic acids. The precipitate dissolves in nitric acid at a much faster initial rate than in perchloric acid; the latter is suggested as an alternative wash solution.

RÉSUMÉ

Les auteurs ont examiné, à l'aide de méthodes radiochimiques (en utilisant ^{32}P et ^{99}Mo), la solubilité du phosphomolybdate d'ammonium dans des acides dilués, en particulier l'acide nitrique. En solutions aqueuses, le phosphomolybdate d'ammonium peut s'hydrolyser complètement à la longue, principalement en acides phosphorique et molybdique. Enfin, les auteurs proposent

l'utilisation de l'acide perchlorique pour les solutions de lavage, le précipité y étant moins soluble que dans l'acide nitrique.

ZUSAMMENFASSUNG

Die Löslichkeit von Ammonium-molybdato-phosphat in verdünnten Säuren, besonders aber in Salpetersäure wird mit Hilfe von radioaktiven ^{32}P und ^{99}Mo bestimmt. Die Hydrolyse des Ammonium-molybdato-phosphats in Wasser wird mit chromatographischen Methoden untersucht. Zum Waşchen wird 0.6 *M* Perchlorsäure vorgeschlagen, da sie bei kurzzeitigem Kontakt den Niederschlag weniger löst als Salpetersäure.

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Short Communications

The ion-exchange separation and determination of small amounts of magnesium and manganese

The determination of small quantities of magnesium and manganese was of particular interest in connection with the analysis of waters and boiler deposits which contained Fe, Al, Ti, Mn, Na, Mg, K, Ca, sulphate and phosphate. A separation of the ions could be effected with the exception of magnesium from manganese using ion-exchange methods[1]. Analysis of the manganese–magnesium effluent was attempted by a complexiметric method[2] which involved the simultaneous determination of manganese and magnesium, but this was unsuccessful. It was decided therefore to separate the manganese and magnesium by a simple procedure involving retention of the manganese on a strongly basic anion-exchange resin in the sulphide form at pH 9. The individual ions were then determined by EDTA titration.

Preparation of resin column

The ion-exchange column consisted of a glass tube 150 mm long of 10 mm internal diameter drawn out to 2 mm with a tap fitted. To the other end was attached a 50-ml capacity reservoir.

A 100-mm layer of New Amberlite CG400 (Cl) resin (100–200 mesh) was enclosed in the glass column between 2 glass wool plugs and converted to the sulphide form by washing with 50 ml of 2% sodium sulphide nonahydrate at 2 ml per min, then with 15 ml of water and finally with 5 ml of 10% ammonium acetate solution (adjusted to pH 9 with 1 : 4 ammonia).

Separation of manganese from magnesium

Dissolve the manganese and magnesium in diluted hydrochloric acid (or take the 1.0 *N* hydrochloric acid eluate previously described[1]) and evaporate just to dryness to remove hydrochloric acid. Redissolve in 1 ml of 5 *N* acetic acid and add 0.2 g of hydroxylamine hydrochloride (AnalaR) and 10 ml of water. Then adjust the pH of the solution to 9 with 1 : 4 ammonia solution. Transfer this solution to the top of the ion-exchange column by washing with water (the total volume of sample + washings must not exceed 20 ml). Mix the solution and run on to the column at 2 ml per min. Collect the effluent. Wash the sample completely on to the resin with 2 ml of a solution composed of 1 ml of 2% sodium sulphide solution, 4 ml of the above ammonium acetate solution and 15 ml of water. When this is complete, elute the column with the remainder of the solution at 2 ml per min. These combined effluents contain the magnesium.

Elute manganese from the resin with 20 ml of 1 *N* hydrochloric acid followed by a solution containing 3 ml of 1 *N* hydrochloric acid, 0.1 g of hydroxylamine hydrochloride and 12 ml of water at 2 ml per min. This effluent contains the manganese.

Determination of separated ions

Both manganese and magnesium can be determined by the following method. Evaporate the effluents to near dryness, redissolve in water and dilute to a suitable volume. Pipette a suitable aliquot of solution containing 2–10 mg of the required ion and neutralise to litmus paper with 1 : 4 ammonia. Add 2 ml of ammonium chloride–ammonia buffer solution (pH 10), 20 mg of potassium cyanide and 3 drops of solochrome black W.D.F.A. indicator solution. For the titration of manganese add 100 mg of hydroxylamine hydrochloride before adding the indicator. Titrate with 0.02 *M* EDTA until the pink colour changes to pure blue. In the vicinity of end-point add EDTA solution dropwise and wait for 10 sec between additions.

TABLE I

ANALYSES OF SYNTHETIC SAMPLES

Manganese		*Magnesium*	
Found (mg)	*Present (mg)*	*Found (mg)*	*Present (mg)*
4.6	4.6	9.8	9.8
4.5	4.6	10.0	9.8
3.7	4.6	9.8	9.8
4.6	4.6	9.7	9.8
4.6	4.6	9.8	9.8

Typical results are shown in Table I. Under the above conditions, the breakthrough capacity of the column was 5 mg of manganese.

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The sparingly soluble salts of lanthanides with benzenephosphonic and benzenephosphinic acid

Several reports about the existence of relatively insoluble salts of phosphonic and phosphinic acids are found in the literature beginning with the work of MICHAELIS[1]. Calcium, barium[2] and magnesium salts[3] have been widely used for the isolation and purification of phosphonic acids. Benzenephosphinic acid has been used for the determination and separation of iron(III)[4]. WENDLANDT[5] has reported the sensitivity of several organic reagents for the detection of lanthanide ions. A general survey of the sparingly soluble salts of benzenephosphonic acid was made[6] and preliminary in-

vestigations showed the possibility of using the reagent as a precipitant for the lanthanide ions.

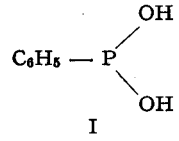
A modification of the method used by IRVING, BUTLER AND RING[7] was used to determine the reaction sensitivity. Tests were conducted in 1.5 × 10 cm tubes containing known amounts of lanthanide ions as the 0.01 to 0.001 *M* metal chloride solutions. To one drop of the metal ion solution, 2 drops of a sodium acetate buffer pH 5.5 and 2 drops of a 2% reagent solution were added. The total volume of the mixture was 0.25 ml. This was heated on a water bath for a few min, allowed to cool to room temperature and then observed visually for the appearance of precipitate. The results of sensitivity tests are given in Table I.

Benzenephosphonic acid ($C_6H_5PO_3H_2$) is a fairly strong dibasic ($pK_1 = 1.85$;

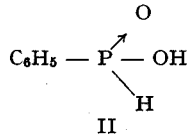
TABLE I

Metal ion	*Benzenephosphonic acid (μg/ml)*	*Benzenephosphinic acid (μg/ml)*
Y^{3+}	3.6	5.2
La^{3+}	4.8	2.5
Ce^{3+}	2.8	No precipitate
Pr^{3+}	10.2	140
Nd^{3+}	14.4	296
Sm^{3+}	8.8	84
Eu^{3+}	24	24
Gd^{3+}	6.4	44
Tb^{3+}	12.8	32
Dy^{3+}	9.6	16
Ho^{3+}	10	16
Er^{3+}	10	6.4
Tm^{3+}	13.6	9.6
Yb^{3+}	10.4	7.2
Lu^{3+}	12	12

$pK_2 = 7.2$) acid capable of forming both acidic and neutral salts. Benzenephosphinic acid ($C_6H_5PO_2H_2$) can be represented by the tautomeric structures I and II.



I



II

A characteristic feature of the lower acids of phosphorus is their tendency to assume whenever possible, a form in which phosphorus is tetravalent and to exhibit one less acidic function than might be expected. The monobasic character (II) is a better representation of the free acid ($pK = 1.47$). KOSOLAPOFF[8] has proposed that phosphonic and phosphinic acids are associated in long-chain intermolecular hydrogen bonding and that this association persists in aqueous solution. The hemi salt for sodium and potassium has been assigned a dimeric structure with a general formula

$RPO_3HM \cdot RPO_3H_2$, where M is the metal ion. It is possible that the lanthanide ions may be attached to the chain ends of the associated acids to form the insoluble salt. Further investigations are being carried out to elucidate the composition and structure of these salts. The sensitivity for the detection of the lanthanides does not show any order as is evident from Table I. Benzenephosphinic acid in general shows a lower order of sensitivity than benzenephosphonic acid. This could be due to differences in the basicity of the acids or the association of molecules or both.

Financial support by the Petroleum Research Fund of the American Chemical Society is gratefully acknowledged.

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(Received November 26th, 1963)

Anal. Chim. Acta, 30 (1964) 591–593

Potentiometric determination of mercury(II) with cyclohexanediaminotetraacetic acid
Analysis of binary mixtures

KHALIFA AND ALLAM[1] have shown that potentiometric determination of mercury with EDTA using silver amalgam as the indicator electrode is suitable for 0.2–100 mg of mercury; binary mixtures with several other metals could be analyzed when masking agents, selective pH and differential titrations were utilized. In the present work CDTA was examined for the same purpose; the influence of buffering materials on the magnitude of the potential jumps was investigated.

Direct and indirect potentiometric titrations of metal ions using the mercury electrode were investigated by REILLEY *et al.*[2]. They mentioned that oxygen reduced appreciably the magnitude of the end-point breaks as it imposed a negative potential limit on the mercury electrode varying between −30 and −100 mV *vs.* S.C.E. in the pH range 3–10; deaeration of the solutions was thus necessary. The present method eliminates this oxygen interference and proceeds with very satisfactory end-point breaks averaging 215 mV per 0.1 ml of 0.05 *M* titrant. The use of CDTA instead of EDTA has the advantage of higher sensitivity; 0.02 to 40 mg of mercury can be determined with high accuracy.

Reagents and chemicals

The water used was twice distilled. The chemicals were the purest available. Ni-

trates of mercury, ammonium, lead, cadmium, lanthanum and yttrium, and sulphates of zinc, copper, indium and thorium were used to prepare 0.025 *M* solutions which were standardised by the usual methods. Methyl thymol blue (M.T.B.), eriochrome black T, murexide and pyrocatechol violet served as indicators.

A 0.05 *M* CDTA solution (17.9 g/l) was prepared[3] from material dried in an air-oven at 80° ± 0.5° for 110 h, and standardised as recommended by BERMEJO[4]. Lower molarities down to 0.001 *M* were prepared by dilution.

A 0.05 *M* mercuric nitrate solution was standardised against the standard CDTA solution using M.T.B. as indicator, and using the silver amalgam as indicator electrode with standard potassium iodide solution as titrant. Lower molarities down to 0.001 *M* were prepared by dilution.

Ammoniacal buffer solutions (pH 8–11) were prepared from ammonia–ammonium nitrate mixtures as previously mentioned[1].

Urotropine buffer solutions (pH 5–12): the pH values 5, 6, 7 . . . 12 corresponded respectively to 200 ml of 10% urotropine solution plus 400, 100, 5 ml of 0.25 *M* nitric acid solution, 2, 4, 8, 16 and 40 ml of 0.5 *M* sodium hydroxide solution per liter.

Procedures

The titration cell consisted of a 200-ml beaker fitted with a rubber stopper with holes for the tip of the micro-buret, and the calomel and the silver amalgam electrodes. A pH meter of the Orion type (2518/S OP-201 ORION, Hungary) was used with glass and calomel electrodes.

Mercury alone was determined by titrating different volumes of buffered mercury solutions with CDTA solutions using the silver amalgam electrode to detect the end-points. Reverse titrations were also carried out.

For the analysis of mercury plus zinc, cadmium, yttrium, lanthanum or lead, mercury was determined potentiometrically with a standard potassium iodide solution using the silver amalgam as indicator electrode, and the total metal was determined with M.T.B. as indicator and CDTA solution as titrant. In the case of mercury plus thorium or indium, mercury was determined as mentioned above and the other cation was determined in another aliquot after mercury had been masked with cyanide. With copper a similar procedure was used, except that mercury was masked with iodide.

Results and discussion

The results obtained by titrating 0.05, 0.025 and 0.01 *M* mercury solutions with equivalent CDTA solutions or the reverse using ammoniacal buffers at pH values of 8, 9 and 10, were essentially the same as those obtained previously with EDTA. The potential breaks varied between 120 and 45 mV per 0.1 ml titrant and the percentage error averaged ± 0.6.

In an attempt to increase the magnitude of the potential breaks, other buffering materials were examined (acetate, hexamethylene tetramine and triethanolamine) and urotropine proved to be the best buffer.

In Table I are listed the results of titrating 0.05 *M* CDTA with 0.05 *M* mercury solutions at different pH values. These data indicate a stoichiometric reaction between mercury and CDTA and very satisfactory potential jumps occurring in the immediate vicinity of the theoretical end-points, which are conveniently detected

within 0.01 ml titrant with corresponding breaks varying between 50 and 150 mV at the pH range 5–10.5.

TABLE I

TITRATION OF 0.05 *M* CDTA WITH 0.05 *M* MERCURY

(2 or 3 ml of 0.05 *M* CDTA taken)

Hg solution found (ml)	*pH*	*mV per 0.1 ml titrant*	*Hg solution found (ml)*	*pH*	*mV per 0.1 ml titrant*
1.995	5.15	193	2.995	5.15	198
1.995	5.56	195	2.995	5.56	198
1.999	5.98	199	2.997	5.98	209
1.996	6.61	214	2.994	6.61	217
1.995	6.96	220	2.995	7.06	225
1.998	8.07	219	2.996	8.12	229
1.995	8.98	225	2.995	8.98	228
1.995	9.95	217	2.995	9.95	216
1.995	10.95	108	2.995	10.50	195
1.995	12.00	52	2.995	11.50	61

The results obtained with 0.025, 0.01 and 0.005 *M* CDTA and mercury solutions showed quite satisfactory potential breaks, amounting to average values of 220, 175 and 150 mV per 0.1 ml titrant, over the pH range 6–10. The relative percent error amounted to $\pm 1\%$ with 0.005 *M* solutions. With these very dilute solutions the buffer solutions were diluted with an equal volume of water before use.

Titrations with 0.001 *M* solutions gave satisfactory results (relative error of 2%) when a buffer solution containing 2.5 g of urotropine and 5 ml of 0.01 *M* sodium hydroxide per liter was used.

When extremely small amounts of mercury were determined, it was found best to add excess of 0.001 *M* CDTA solution and back-titrate with 0.001 *M* mercury solution. It was thus possible to determine a minimum amount of 0.02 mg of mercury, *i.e.* 10-fold less than the 0.2 mg which could be determined by the previous method[1].

When binary mixtures were analyzed as mentioned above, excellent results were obtained for both mercury and the other metal in mixtures containing 10–20 mg of mercury and zinc (3–7 mg), yttrium (4–6 mg), lanthanum (7–14 mg), indium (9–12 mg), thorium (12–24 mg), copper (3–5 mg), cadmium (7–10 mg) or lead (16–32 mg).

The author is indebted to Prof. E. SCHULEK for his interest in this work.

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(Received November 22nd, 1963)

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Book Reviews

N. D. CHERONIS AND J. B. ENTRIKIN, *Identification of Organic Compounds – A Student's Text using Semimicro Techniques*, John Wiley and Sons, New York–London, 1963, 477 pp., price 65 s.

This book is a shorter and completely revised version of the well-known text by the same authors — *Semi-micro Qualitative Organic Analysis*. Two particular points should be noted: firstly, the recent literature has been surveyed and, where appropriate, new tests and derivatives have been included along with literature references; secondly, surveys of newer techniques have been given.

It is good to see that mention is made of the use of infrared and chromatographic techniques for identification and separation respectively. Their inclusion will appeal especially to the teacher who argues that the classical approach by analysis for functional groups is outmoded for the identification of unknowns, for it bears no relation to the way in which a similar problem would be tackled by a research worker, whose first step would be to obtain an infrared spectrum. This is not to say that crystallization and derivative formation have had their day; in the description of these techniques, this text is sound, critical and detailed.

The book is well produced and indexed, but at the price it will not find its proper place on many students' bookshelves. However, it is likely to find widespread use as a laboratory reference work, for its derivative Tables are comprehensive and there are numerous references to the original literature.

E. J. FORBES (Birmingham)

Anal. Chim. Acta, 30 (1964) 596

H. J. M. BOWEN AND D. GIBBONS, *Radioactivation Analysis*, Oxford University Press, London, 1963, 295 pp., price 50 s.

In their book BOWEN AND GIBBONS give a practical survey of the methods and applications of activation analysis up to 1960. Knowing this, the reader will not be surprised that in general little attention is paid to the theory, whereas the practical side is extremely well elaborated. After a short introduction outlining the contents of the work, the first 3 chapters deal with the theory of growth and decay of active species, and the activation of samples with neutrons and with other particles. This section contains the following Tables:

(*1*) Neutron activation sensitivities using a flux of 10^{12} n/cm^2 sec;
(*2*) Thermal neutron outputs from various sources;
(*3*) Specific activities produced by (γ, n) reaction;
(*4*) Reaction products from proton bombardments of the light elements.

The next chapter gives a thorough discussion of scintillation spectrometric techniques and a comparison of the different modes of interpretation of the measured spectra. The authors include a Table of the common γ-ray emitters produced by

(n, γ) reactions in order of increasing γ-energy. Several chapters are devoted to the detection of radio-isotopes, the errors involved in sampling, irradiating and measuring and also the commonly used methods for fast separations of radionuclides.

The second part of the work deals with applications in geochemistry, biology and inorganic and pure chemistry. In each field numerous examples are discussed and references are made to the original literature.

Finally a list is given containing activation analysis data and a detailed radiochemical separation method for each element, while possible interferences are noted. This list is followed by an almost complete survey of the literature with over 600 references.

Chemists starting in activation analysis as well as experienced workers looking for a practical manual, will find this book of outstanding interest.

D. DE SOETE (Ghent)

Anal. Chim. Acta, 30 (1964) 596–597

Announcement

INTERNATIONAL INVESTIGATION INTO ERRORS IN ELEMENTARY ORGANIC MICROANALYSIS

conducted by

INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY

Division of Analytical Chemistry, Comission on Microchemical Techniques

The observations, findings, and effects governing the microanalytical errors encountered by every microanalyst in the course of his daily work only add to his special knowledge or that of his close collaborators. In order to go beyond the limits of the individual laboratory, the Commission on Microchemical Techniques has set up a Study Group on Errors in Elementary Organic Microanalysis, which is intended to centralize, classify, and publish these findings in generally workable form for the benefit of all.

The Study Group on Errors in Elementary Organic Microanalysis invites microanalysts of all countries, either personally or as representatives of their laboratories, to collaborate in this collective international task by communicating in the form of a report the results of their experiences.

In practice, it is desirable that each report conform to the outlines given below.

1. It must be drafted in either German, English, French, or Russian.
2. It must be divided into as many separate parts as the errors under examination.
3. Each part must follow the headings of the following outline.

* The present investigation does not include trace analysis.

OUTLINE

1. Element(s), amount(s).
2. Microanalytical range (milligram, decimilligram, centimilligram).
3. Microanalytical balance employed (principle, model, installation, with or without air-conditioning, etc.); standard deviation of weights.
4. Method employed—principle of mineralization (combustion), principle of the determination, apparatus, reagents, method of operation, etc.
5. Type of error studied—systematic or uncertain errors affecting the accuracy or precision of the results.
6. Order of algebraic magnitude of the systematic error, standard deviation or possible limits of the uncertain errors (errors of chance).
7. Sources of error, real or apparent. All of the factors of error must be retained, especially those which appear in the following unlimited list.

   Order of magnitude of the contents to be determined. Physical properties of the compounds analyzed (condition, vapor pressure, hygroscopicity, triboelectricity (static electricity) etc.). Structures of the compounds analyzed. Presence of interfering elements. Presence of inter-

fering functional groups. Principle of the method of mineralization (combustion) employed. Principle of determining the end product. Reagents employed (nature, degree of purity, source). Other chemical products employed. Small apparatus and various material employed (joints, stoppers, glass, silica, plastic materials, metals, etc.). Manual or automatic apparatus employed for the mineralization (combustion). Apparatus employed for the measurements (balances, burettes, colorimeters, spectrophotometers, potentiometers, coulometric, conductometric, or thermalconductometric set-ups, recorders, integrators, etc.). Possible influence of automation. Operative parameters (temperature, rate of gas flow, pressure, etc.). Environment parameters (temperature, relative humidity, atmospheric pressure, air pollution, vibrations, situation and exposure of the laboratory, place or position of installation in the laboratory, etc.). Subject parameters (manipulations, observations). Operative methods and techniques.

8. Means employed for characterizing the source of error (if possible).
9. Modifications eventually used with the intention of diminishing or eliminating the error.
10. Suggestions.
11. Specific questions.

The preceding outline must be considered as a helpful memorandum. The errors reported need not necessarily be "originals"; they may have been cited in publications from which they have been noted.

The present investigation is already in progress; consequently, the reports, depending upon the geographic location or the language used, can be addressed without delay to the following members of the Study Group on Errors in Elementary Organic Microanalysis:

a. American reports in the English language (U.S.A., Canada, etc.) to: Dr. AL STEYERMARK, Chairman, *Commission on Microchemical Techniques*, Hoffmann-La Roche Inc., Nutley 10, New Jersey, U.S.A.
b. European reports in the German or Slavic languages (with the exception of Russian) to: Dr. WOLFGANG SCHÖNIGER, Secretary, *Commission on Microchemical Techniques*, Microanalytical Laboratory, Department of Pharmaceutical Chemistry, Sandoz Ltd., Basle 13, Switzerland.
c. Reports originating in U.S.S.R. to: Dr. N. E. GEL'MAN, Associate Member, *Commission on Microchemical Techniques*, Institute on Elemento-Organic Compounds, Academy of Sciences, Moscow, U.S.S.R.
d. Reports in the English, French, or German languages which originate in countries other than those listed in a, b, and c, to: Dr. ROGER LÉVY, Titular Member, *Commission on Microchemical Techniques*, Chairman, Study Group on Errors in Elementary Organic Microanalysis, Central Department of Microanalysis of the National Center of Scientific Research, 39 bis, Rue de Dantzig, Paris 15, France.

In order to speed up the study, the Study Group would like to receive reports on errors which will eventually be part of a published report, even though the main portion is not ready for publication.

The deadline for this material will be August 1st, 1964.

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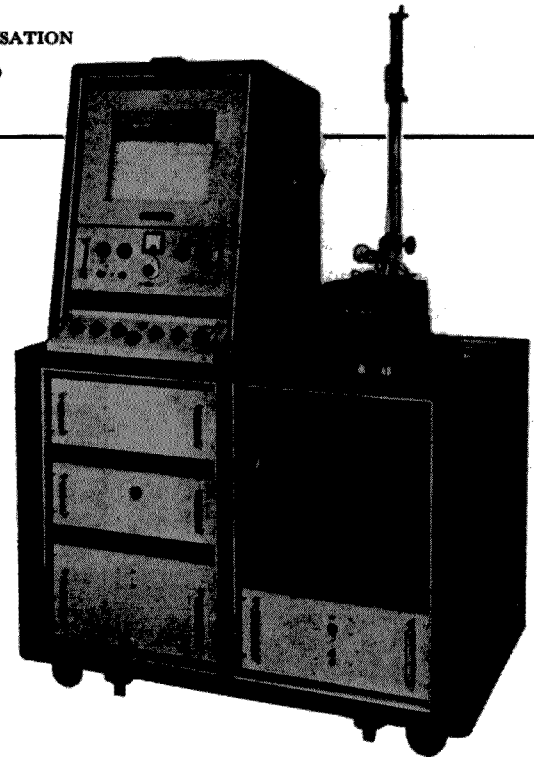

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