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devoted to the application of microtechniques in all branches of science

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Briefs

Spectrophotometric Determination of Phenylephrine. L. SZEKERES, R. E. HARMON, AND S. K. GUPTA, *Department of Chemistry, Western Michigan University, Kalamazoo, Michigan 49001.*

The determination of small amounts of phenylephrine in the presence of a variety of substances, including ascorbic acid, is described. The method is based on the formation of a blue indophenol derivative from the reaction of phenylephrine with *N*,2,6-trichloro-*p*-benzoquinoneimine.

Microchem. J. **18,** 583 (1973).

Determination of Carbon, Hydrogen, and Nitrogen in Inorganic Compounds by Means of an Automatic Organic Elemental Microanalyzer. A. COLOMBO AND R. VIVIAN, *Chemistry Department, Joint Nuclear Research Centre, Ispra Establishment, Commission of the European Communities, Italy.*

The F & M Model 185 CHN Analyzer is applied to the determination of carbon, hydrogen, and nitrogen in inorganic compounds. Nitrates, carbonates, and ammonium salts are included. Refractory nitrides, carbides, and carbonitrides give difficulties.

Microchem. J. **18,** 589 (1973).

Microdetermination of Calcium in Organic Compounds. J. PENIĆ, I. BREGOVEC, Z. ŠTEFANAC, AND Z. SLIEPČEVIĆ, *Institute for Medical Research, Yugoslav Academy of Sciences and Arts, Moše Pijade 158, 41000 Zagreb, Yugoslavia and Institut za istraživanja i razvoj INA Zagreb, Yugoslavia.*

A method is described in which combustion is done in an oxygen stream or oxygen flask with electrical ignition. Carbon dioxide is expelled and the interference of phosphate eliminated by adsorption on an anion exchanger prior to the spectrophotometric determination of calcium with glyoxal bis (2-hydroxyanil).

Microchem. J. **18,** 596 (1973).

Spectroscopic and Dielectric Examination of the Oleyl Alcohol–Oleic Acid System. JOZEF SLIWIOK AND TERESA KOWALSKA, *Institute of Chemistry, Silesian University, Katowice, Poland.*

The oleyl alcohol–acid systems composed of several molar ratios were studied. The infrared and dielectric constant measurements were applied as analytical techniques. The two compounds interact in a number of molar ratios giving hydrogen-bonded associates. In the range of temperatures between 30–60°C, the most stable hydrogen-bonded associate is formed in the ratio of alcohol to acid of 9 : 1.

Microchem. J. **18,** 605 (1973).

Convenient Technique for Microhydrogenation. THOMAS H. PARLIMENT, *General Foods Technical Center, White Plains, New York 10625.*

A technique is described for the batch-wise hydrogenation of samples in the range of 20–1000 μg. Palladium on carbon is used as the catalyst and analysis is accomplished by means of gas chromatography.

Microchem. J. **18,** 613 (1973).

Application of Fast Grey RA to the Spectrophotometric Determination of Copper in Soft Tissues of Egyptian Camels. H KHALIFA, M. T. FOAD, Y. L. AWAD, AND M. E. GEORGY, *Veterinary Research Laboratories, Dokki, Cairo, Egypt, U.A.R.*

Copper in different organs of Egyptian camels was determined spectrophotometrically using Fast Grey RA. Vanadium is the only element found to interfere.

Microchem. J. **18,** 617 (1973).

New Spot Test for Some Phosphorus Nerve Gases. S. S. M. HASSAN AND S. A. I. THORIA, *Research Microanalytical Laboratories, Department of Chemistry, Faculty of Science, Ain Shams University, Cairo, U.A.R.*

A spot test is described, which is based on peroxidation of the nerve gas with urea peroxide in alkaline solution followed by reaction with thallium(I) sulfate. A reaction with orthodianisidine is also described.

Microchem. J. **18,** 622 (1973).

Spectrophotometric Determination of Osmium Using Acenaphthenequinonemonoxime (AQM). S. K. SINDHWANI AND R. P. SINGH, *Department of Chemistry, University of Delhi, Delhi-7, India.*

A water soluble dark-brown complex is formed by osmium and acenaphthenequinoneoxime (metal:ligand, 1:2). The absorption maximum is at 430 nm and the pH range is 6.5–8.5.

Microchem. J. **18,** 627 (1973).

The Spectrophotometric Determination of Molybdenum and Uranium with Quinalizarin. SUNITA RANI AND SAMIR K. BANERJI, *Chemical Laboratories, Birla Institute of Technology and Science, Pilani (Rajasthan), India.*

Quinalizarin reacts with molybdenum and with uranium in molar ratios to form colored chelates which can be used for the determination of the respective metals.

Microchem. J. **18,** 636 (1973).

Extractive Photometric Determination of Palladium with *o*-Mercaptobenzoic Acid. M. M. L. KHOSLA, *Defence Laboratory, Jodhpur, India; and* S. P. RAO, *University of Jodhpur, Jodhpur, India.*

Palladium forms a yellow complex with *o*-mercaptobenzoic acid in the ratio of 1:2. Most of the cations do not interfere in the presence of ascorbic acid and EDTA.

Microchem. J. **18,** 640 (1973).

Determination of Stability Constants of Some Bivalent Metal Complexes with Thiovioluric and Diphenylthiovioluric Acids. R. S. CHAWLA AND R. P. SINGH, *Department of Chemistry, University of Delhi, Delhi-7, India.*

Stability constants of complexes of some bivalent metal ions—Cu(II), Ni(II), Zn(II), Pb(II), and Mg(II)—with thiovioluric and diphenylthiovioluric acids are determined potentiometrically.

Microchem. J. **18,** 646 (1973).

VI. Direct Titrimetric Microdetermination of L-Serine and L-Lysine. VII. Simultaneous Stepwise Microdetermination of Combinations of Amino Acids without Separating. O. C. SAXENA, *Chemical Laboratories, University of Allahabad, Allahabad, India.*

L-Serine and L-lysine have been quantitatively determined in microamounts separately and in presence of each by direct titrations against mercuric and zinc chlorides, respectively. These two amino acids in double and triple combinations with L-histidine and L-arginine have been titrated successfully in one solution without separation.

Microchem. J. **18,** 652 (1973).

Spectrophotometric Microdetermination of Cu(II) and Fe(III) Using 2,4-Dinitrosoresorcinol. S. E. ZAYAN, R. M. ISSA AND JAQUELINE Y. MAGHRABI, *Chemistry Department, Faculty of Science, Alexandria University, Egypt, U.A.R.*

The colored complexes formed by the reaction of dinitrosoresorcinol with Cu(II) and Fe(III) is utilized for the microdetermination of both metal ions either alone or in a binary mixtures. Satisfactory results are obtained when the proper media are utilized in the presence of an excess of the organic ligand. The interference of some ions is also investigated.

Microchem. J. **18,** 662 (1973).

Electroanalysis with Tungsten Electrodes. I. The Tungsten/Platinum Bimetallic Pair in Potentiometry at Zero Current. S. A. DARWISH AND R. SALIM, *Department of Chemistry, Faculty of Science, University of Cairo, Giza, U.A.R.*

The W/Pt bimetallic pair has been applied in various potentiometric titrations. Except for the neutralization of dicarboxylic acids, e.g., oxalic, the pair is suitable for neutralization titrations where $\Delta E/\Delta V$ values at the equivalence point are higher on W than on Pt. Tungsten oxides increase the inflexion pd.

Microchem. J. **18,** 670 (1973).

Spectrophotometric Determination of Rhodium Using Acenaphthenequinone Monoxime (AQM). S. K. SINDHWANI AND R. P. SINGH, *Department of Chemistry, University of Delhi, Delhi-7, India.*

Acenaphthenequinone monoxime forms a yellow colored complex which exhibits maximum absorption at 390 nm. The method described is very selective for rhodium.

Microchem. J. **18,** 686 (1973).

Titration of Aspartic Acid with Mercuric Chloride. KRISHNA BAHADUR AND PADAM SEN, *Chemical Laboratories, University of Allahabad, Allahabad, India.*

Aspartic acid was determined by direct complexometric titration with standard mercuric chloride using 1-(2-pyridyl-azo)-2-naphthol as indicator in a citrate buffer soluion at pH 4.

Microchem. J. **18,** 694 (1973).

Titration of L-Cysteine Hydrochloride with Standard Nickel Sulphate Solution Employing 1-(Pyridyl-2′-azo)-naphthol-(2) as Indicator. S. RANGANAYAKI AND BHARTI SRIVASTAVA, *Chemistry Department, University of Allahabad, Allahabad, India.*

Determination is carried out in a carbonate–bicarbonate buffer solution at pH 10.0. Other natural amino acids have no interference with the titration.

Microchem. J. **18,** 699 (1973).

MICROCHEMICAL JOURNAL 18, 583–588 (1973)

Spectrophotometric Determination of Phenylephrine[1]

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Received December 19, 1972

INTRODUCTION

Phenylephrine (1-*m*-hydroxy-α-[(methylamino)methyl] benzyl alcohol) is a commonly used adrenergic drug. Tablets which contain phenylephrine hydrochloride often contain several other compounds such as phenylpropanolamine hydrochloride, chlorpheniramine maleate, acetaminophen, caffeine, ascorbic acid, etc. Titration with perchloric acid in glacial acetic acid (*11*), a bromometric method (*2*) oxidation with HIO_4 (*3*) and a spectrophotometric method (*16*) are the frequently used methods for assaying phenylephrine. According to De Fabrizio (*5*) estimation of phenylephrine in mixtures can be accomplished by chromatographic separation followed by spectrophotometric determination. A photometric determination of phenylephrine (*4, 13*) is based on the formation of a colored azocompound from the reaction of phenylephrine with *p*-nitrophenyldiazonium chloride. This method was successfully used by Pohloudek-Fabini and Konig (*12*) after paper chromatographic separation of phenylephrine. Kelly and Auerbach (*8*) employed an ion-exchange resin to separate phenylephrine prior to its determination by the above method.

Since phenylephrine is a phenol with *para* position available for coupling with 4-aminoantipyrine, Hiskey and Levin (*6*) used this reaction for a colorimetric determination of phenylephrine. This method was widely used for sometime and was later modified by Tatsuzowa and Hashiba (*14*).

Murai (*9*) observed that phenylephrine forms a blue colored indophenol derivative with *p*-dimethylphenylenediamine. This derivative was stable enough for a photometric determination of phenylephrine. Modifications of this method (*10, 15*) as well as two other colorimetric methods of determining phenylephrine (*1, 7*) have been reported.

[1] Presented: International Symposium on Microchemical Techniques – 1973, held at The Pennsylvania State University, University Park, Pennsylvania, U.S.A., August 19–24, 1973.

In this paper we describe a new and convenient spectrophotometric method for the determination of phenylephrine in complex mixtures. This procedure is based on our observation that an isopropanol–water solution of phenylephrine at pH 8 forms a blue colored indophenol derivative with *N*,2,6-trichloro-*p*-benzoquinoneimine (chlorimine). This dye was found to be sufficiently stable for a photometric determination of phenylephrine.

MATERIALS AND METHODS

All chemicals and reagents used in this work were either USP or NF grade. *N*,2,6-Trichloro-*p*-benzoquinoneimine was purchased from Eastman Kodak Co. and used without further purification. The mixtures containing phenylephrine which were assayed using our new spectrophotometric method are listed in Table 1.

Preparation of Buffer Solutions

(1) A solution of 16 g of ammonium chloride (NH_4Cl) in water was added to a 100 ml measuring flask. After the addition of 16 ml of concn ammonium hydroxide (NH_4OH; sp gr 0.910) to the flask, the mixture was diluted with water to 100 ml. (2) A 20% solution of sodium acetate in water was prepared separately.

N,2,6-Trichloro-p-benzoquinomeimine (Chlorimine)

A solution of 10 mg of chlorimine in 25 ml of isopropanol was prepared. This solution must be prepared freshly before use.

Phenylephrine HCl Stock Solution

A solution of 50 mg of phenylephrine HCl in about 400 ml of water was prepared, and further diluted to 500 ml.

Phenylephrine HCl Standard Solution

The above stock solution (20 ml) was diluted to 100 ml with spectroscopy grade isopropanol.

Preparation of Phenylephrine HCl Mixture Solution

The phenylephrine HCl mixtures, 1, 2, and 3 (Table 1) containing about 50 mg of phenylephrine were ground into a fine powder and dissolved in about 400 ml of water. After stirring for 20–30 min (using a magnetic stirrer) each solution was diluted to 500 ml with water.

The above solution was filtered through a dry filter paper (Whatman No. 41) and the first 30 ml of the filtrate were discarded. Twenty milliliters of the clear filtrate were diluted to 100 ml with spectroscopy grade isopropanol.

TABLE I

COMPOSITION OF PHENYLEPHRINE MIXTURES ASSAYED SPECTROPHOTOMETRICALLY

Mixture no.	Name of chemical	Amount (g)
1	Phenylephrine HCl	1.88
	Pyrilamine maleate	4.45
	Acetaminophen	57.78
	Caffeine	5.78
	Ascorbic acid	10.00
	Starch	1.54
	Cellulose	12.99
	Ethylcellulose	0.38
	Stearic acid	2.89
	Magnesium stearate	0.98
	Silicium dioxide	1.33
2	Phenylephrine HCl	3.01
	Phenylpropanolamine HCl	5.04
	Chlorpheniramine maleate	0.81
	Acetaminophen	61.38
	Glaze-white	3.01
	Stearic acid	1.50
	Magnesium stearate	1.50
	Cellulose	19.45
	Starch	4.30
3	Phenylephrine HCl (USP)	0.90
	Acetaminophen	56.42
	Pyrilamine maleate	2.32
	Dextrometorphan HBr	1.74
	Lactose	15.56
	Cellulose derivatives	0.32
	Starch	18.04
	Stearic acid	2.71
	Silicium dioxide	0.09
	Talc	1.90

Estimation Procedure

The phenylephrine HCl solution (5 ml) to be assayed was placed in a flask fitted with a ground glass stopper. To this solution were added 1 ml of the NH_4Cl–NH_4OH buffer, 1 ml of sodium acetate and 1 ml of water and finally 1 ml of the chlorimine reagent solution. The mixture was shaken for about 10 sec.

The standard phenylephrine HCl solution (5 ml) was treated with the buffer solutions, water and chlorimine reagent in the same way. After a few minutes both the solutions developed blue-green color.

The color intensity was found to depend on the pH and the concentration and quality of the chlorimine reagent solution. The stability of color also depends on the quality of chlorimine reagent. Generally speaking the color was found to be stable from 25 min to several hours. Presence of impurities such as decomposed chlorimine causes the color to change faster than in the case of pure chlorimine.

The amount of phenylephrine HCl in each solution was determined using Beckman D. U. and Carey-14 uv spectrophotometers. Because of the blue-green color of the solution, the readings were taken starting at 650 nm. The solution showed a characteristic absorption maximum at 615 nm. However, readings at 650 nm were used in all the calculations. The results are given in Table 2. Figure 1 shows the absorption curve for the above dye.

DISCUSSION

The assay results reported in Table 2 indicate that the reaction between *N*,2,6-trichloro-*p*-benzoquinoneimine and phenylephrine in isopropanol–water solution at pH 8–8.5 can be used to determine even minute amounts (0.1 mg) of phenylephrine HCl in the presence of several foreign materials such as pyrilamine maleate, chlorpheniramine maleate, phenylpropanolamine HCl, acetaminophen, caffeine, ascorbic acid, etc. The percentage accuracy achieved by us using this method lies within ±3%.

One distinct advantage of using this method is that ascorbic acid, known to interfere with some of the colorimetric methods of estimating phenylephrine, does not interfere with this method.

According to our initial observations this procedure is suitable for the determination of many phenol derivatives.

SUMMARY

This paper describes a spectrophotometric assay procedure for the determination of small amounts of phenylephrine in the presence of a variety of foreign substances including ascorbic acid. This method is based on the formation of a blue colored in-

TABLE 2

ABSORPTION DATA OF PHENYLEPHRINE–INDOPHENOL DERIVATIVE AT 650 nm

Mixture no.:	I	I	II	II	III	III
Standard	0.410	0.280–0.285	0.275	0.280	0.260	0.260
Sample	0.405	0.270–0.275	0.285	0.285	0.260	0.265
Accuracy (%)	98.8	96.4–96.5	103.6	101.7	100	101.92

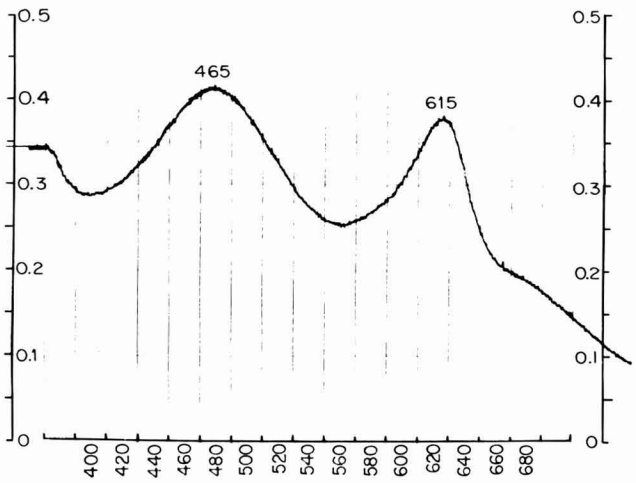

FIG. 1. Absorption curve for phenylephrine–indophenol derivative.

dophenol derivative from the reaction of phenylephrine with *N*,2,6-trichloro-*p*-benzoquinoneimine.

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MICROCHEMICAL JOURNAL 18, 589–595 (1973)

Determination of Carbon, Hydrogen and Nitrogen in Inorganic Compounds by Means of an Automatic Organic Elemental Microanalyzer[1]

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Received March 30, 1973

INTRODUCTION

During the past four years, in the frame of a support activity to biological programs, an extensive use has been made in our laboratories of the Hewlett Packard[2] (formerly F&M) Model 185 CHN Analyzer, for the determination of the carbon, hydrogen and nitrogen content of various planktons coming from different lakes. More recently the instrument has been in use in the field of environmental pollution, for the analysis of particulate samples collected from urban atmospheres.

Both these materials are likely to contain a large amount of inorganics: since the instrument is conceived essentially for the analysis of organic materials, though including "those that contain O, S, P, Cl, Br, I, F, As, Sb, and Sn" (*loc. cit.*), (1) it seemed necessary to test its ability for the inorganic ones. This paper summarizes the results obtained on a large variety of inorganic samples.

RESULTS AND DISCUSSION

Table 1 gives the average results obtained on a large variety of inorganic compounds run in duplicate. They have been analyzed in their usual form, i.e., as powders of various particle size. Two of these compounds are deliquescent (NaSCN and KSCN) and another two are hygroscopic materials (Na_2CO_3 and K_2CO_3). With the purpose of drying, they have been put before the analysis respectively in a vacuum oven at 70°C for 2 hr and in a stove at 250°C for 5 hr. No difficulties arose for the thiocyanates and for Na_2CO_3, but K_2CO_3

[1] During the elaboration of the manuscript, a paper on the carbon, hydrogen and nitrogen micro-analysis of rock and soil samples by means of the CHN 185 (2) came to our attention through the "Analytical Abstracts" review. From the abstract, this paper appears to be mainly devoted to the analysis of carbonates and of different types of carbon. It is hoped that our work, made independently, will be a good complement to this previous one.

[2] Hewlett Packard–F&M Scientific Division, Avondale, Pennsylvania, U. S. A.

TABLE 1
ANALYSIS OF INORGANIC COMPOUNDS

Compound	% C Theor.	% C Exp.	% C Error	% N Theor.	% N Exp.	% N Error	% H Theor.	% H Exp.	% H Error	Used standard[a]
$NaNO_3$				16.48	16.21	−0.27				Cys
$K NO_3$				13.85	13.67	−0.18				Cys
$Ba(NO_3)_2$				10.72	10.45	−0.27				Cys
$Ag NO_3$				8.24	7.86	−0.38				Cys
NH_4NO_3				35.00	35.02	+0.02	5.04	5.20	+0.16	C
$(NH_4)_2HPO_4$				21.21	20.70	−0.51	6.87	7.09	+0.22	C
$(NH_4)_2SO_4$				21.20	21.12	−0.08	6.10	6.36	+0.26	C
NH_4VO_3				11.97	11.87	−0.10	3.45	3.24	−0.21	Cys
NH_4Cl				26.18	26.23	+0.05	7.54	7.67	+0.13	C
NaN_3				64.64	63.82	−0.82				C
NaSCN	14.81	14.30	−0.51	17.28	16.91	−0.37				C
K SCN	12.36	11.93	−0.43	14.41	14.13	−0.28				C
$K_3Fe(CN)_6$	21.89	21.63	−0.26	25.53	25.79	+0.26				C
Li_2CO_3	16.25	16.44	+0.19							Cys
Na_2CO_3	11.33	10.97	−0.36							Cys
Na_2CO_3 wet	(11.08)	10.81	−0.27					0.25		Cys
K_2CO_3 wet	(7.70)	7.72	+0.02					1.27		Cys
$KHCO_3$	12.00	11.43	−0.57				1.01	0.84	−0.17	Cys
$CaCO_3$	12.00	12.13	+0.13							Cys
$SrCO_3$	8.13	8.41	+0.28							Cys
$BaCO_3$	6.08	5.77	−0.31							Cys
$COOK(CHOH)_2COOH$	25.53	25.35	−0.18				2.68	2.55	−0.13	Cys
H_3BO_3							4.89	4.79	−0.10	C
$Ca(OH)_2$							2.72	2.66	−0.06	C
$ZrH_{1.95}$							2.11	1.96	−0.15	C

[a] Cys = Cystine (% C = 29.99; % N = 11.66; % H = 5.03)—Standard NBS N. 143b; C = Cyclohexanone 2/4 –DinitroPhenylHydrazone (% C = 51.79; % N = 20.14; % H = 5.07)—Standard BDH.

readsorbed the atmospheric humidity so quickly that the analysis on the dry materials was impossible. Therefore, K_2CO_3 was analyzed wet and its theoretical carbon content was calculated assuming that its measured hydrogen content was due to absorbed water; for comparison purposes the same was made on wet Na_2CO_3. The table shows that more than 67% of the results are within the error of ± 0.3 absolute, and such a percentage is the accepted standard of accuracy of the instrument; the range, viz. the difference of magnitude between the single results of the duplicates, was in average 0.25 for carbon, 0.08 for nitrogen and 0.17 for hydrogen.

The data appearing on this table deserve some comments.

Nitrates

They break up quickly to give nitrogen (as nitrogen oxides) and no difficulties were encountered with the reduction tube packed with Cu in good conditions (hydrogen reduced, for instance). After long periods of use the Cu became relatively inactive and the results became erratic: this was attributed to incomplete conversion of the evolved nitrogen oxides to nitrogen and was evidentiated on the instrument by a distortion of the otherwise small sharp carbon dioxide peak given by the donor which is very well seen at attenuation $\times 2$; such a distortion indicated that something (probably N_2O) was eluting through the gas chromatograph together with the carbon dioxide. It is worthwhile to note that when the reduction tube was in these bad conditions it still worked for nitrates containing hydrogen or carbon like NH_4NO_3 or organic nitro-derivatives: clearly the reduction of the nitrogen oxides is performed by these atoms too, and the action of Cu become less important.

Carbonates

Despite the Smithells's (3) thermodynamic data which indicate the alkaline carbonates as the most stable in respect of thermal decomposition, difficulties in releasing carbon dioxide were encountered with $BaCO_3$ and $SrCO_3$ only. They were revealed by a distortion of the tail of the carbon dioxide peak and persisted even with the 50 sec combustion period. They were finally eliminated by a small modification of the analytical procedure: the $BaCO_3$ and $SrCO_3$ samples, prior to the addition of the donor to the capsule, were covered with a not-weighed equivalent amount of B_2O_3; the combustion period had to be increased to 50 sec, only in the case of $SrCO_3$.

The interpretation of these facts seems very clear; $CaCO_3$ gives no problems as it decomposes quickly at a temperature well below that of 1040–1080°C; alkaline carbonates, which should not decompose,

evolve wholly their carbon dioxide as they melt at the operating temperature and then react quickly with the donor to give intermediate manganates and/or tungstates: undoubtedly the fusion of the samples favors the kinetics of the reaction with the donor which is solid at the operating temperature; $BaCO_3$ and $SrCO_3$ being solid at 1040–1080°C react slowly to give manganates and tungstates and being relatively undecomposable give the greatest difficulties: the addition of a low melting point oxide like B_2O_3 (mp 450°C) achieves the goal of a fast release of the carbon dioxide through formation of borates.

Other Compounds

They gave no problems at all. Different types of carbon, namely activated, amorphous and graphitic, have also been examined, although they are not shown in Table 1.

In this case the type of material and the particles size appeared to play an important role: activated carbon (<250 μm) burnt easily within 50 sec, whilst amorphous and graphitic carbon (<37 μm) did not burn completely: a distortion of the tail of the carbon dioxide peak revealed the incompleteness of the combustion; it appeared anyway clear that the combustion becomes easier when the particles size decreases. Amorphous and graphitic carbon (<149 μm) were burnt completely with a manual combustion time of 100 seconds, but they gave rather inaccurate and imprecise results, which seemed to reflect both a strong difference in the combustion characteristics between the samples and the used standard (Acetanilide-Standard NBS no. 141 b–%C = 71.09) and an inherent imprecision of the instrument at combustion periods well beyond 50 sec (as an example of the last point, acetanilide gave on 6 determinations a relative standard deviation of 0.4% for carbon at 20 and 50 sec combustion time, whilst it gave a relative standard deviation of 0.8% at 100 sec).

So different is the reactivity of the many kinds of carbon, that to give rules for their analysis is out of the scope of this work: it seems safe to say in general that when the sample characteristics are such that a very long combustion period has to be used, the accuracy and precision of the determinations will be poorer than usual.

An examination of the chemical and physical properties of the other existing inorganic compounds show that, with few exceptions. they should be easily analyzable. The nitrates or carbonates of other elements are much less stable than those shown in Table 1. The nitrites are unstable too, and in particular the alkaline nitrites are the first product of the thermal decomposition of the nitrates.

All the other compounds containing hydrogen, carbon or nitrogen

either volatilize or break up into fragments and should be easily burnt by the oxygen atmosphere or by the residual oxidant, with the eventual addition of B_2O_3, when $BaCO_3$ or $SrCO_3$ formation is suspected. The formed oxidation products other than carbon dioxide, water vapor and nitrogen should in general not interfere with the determination as, apart from the eventual different chromatographic retention time, it seems that they can be blocked either by reaction with the donor or with the packing of the combustion and reduction tubes, or simply because they condense in some relatively cold part of the instrument.

The few exceptions should be represented by the series of the refractory nitrides, carbides and carbonitrides which although unlikely to be contained in environmental samples, are nevertheless interesting: some tests conducted on fine powders of BN, Mg_3N_2, Si_3N_4, Al_4C_3, SiC were in fact highly unsatisfactory even with a combustion period of 50 sec. (The recoveries were from about zero for SiC to about 30% for Al_4C_3). The problem appeared to be a kinetic one, and therefore, in order to increase the reaction rate, it was thought advisable to use a donor which is liquid at the temperature of 1040–1080°C: PbO_2 was chosen as, after decomposition, it gives PbO (mp 888°C), which being easily reductible, should perform well the oxidation of the samples. Although only few tests were made, indications were obtained of the ability of the new donor to burn within 50 sec the above-mentioned refractories and graphite. But, apart from the high and variable blank values of the PbO_2, the heavy corrosion of the sample injection rods due to the liquid oxide, created new problems which were not solved. As it seemed probable that other eventual liquid donors would have lead to the same difficulties, the research was not continued.

Nevertheless, the results of Table 1 show the remarkable aptitude of the instrument to broaden its original application field. In this context it is expected that the apparatus can be used, for instance, in the determination of crystallization water or in the rapid determination of percent weight composition of inorganic mixtures up to four components. The last point merits to be deepened as when analyzing solid mechanical mixtures, the problem of taking a representative sample is not negligible, in consideration of the small weights handled by the instrument. An example will make this point clear.

Let us assume an infinite amount of a thoroughly mixed mixture made by equal percentages of particles of A and B, all of the same weight and dimensions. A "correct" sample composed of a total of m particles, should contain $m/2$ particles of A and $m/2$ particles of B, whilst on the contrary there exist a definite probability given by the

binomial distribution, that it will contain n particles of A and $m - n$ particles of B. Such a probability is given by:

$$P = (0.5)^m \binom{m}{n}.$$

When m is large enough (let us say, larger than 50) this distribution is in practice equal to a Gaussian one given by:

$$P = (2/\pi\ m)^{1/2} e^{-(m-2n)^2/2m}$$

Remembering now that the Gaussian distribution of errors has a general form given by:

$$P = (1/\sigma\sqrt{2\pi}) e^{-1/2(X/\sigma)^2},$$

where X is the deviation of the single measurement from the mean value and σ is the standard deviation, it can be inferred after comparison of the equations, that the binomial distribution has with good approximation a standard deviation $\sigma = \sqrt{m}/2$.

In other words there is a probability of 68.3% that the given sample will contain $m/2 \pm \sqrt{m}/2$ particles of A; 95.5% of probability that these particles are $m/2 \pm \sqrt{m}$; 99.7% of probability that they are $m/2 \pm 3\sqrt{m}/2$; and about 100% probability that the sample will contain $m/2 \pm 2\sqrt{m}$ particles of A (the same applies obviously to B). This means that m must be very large, viz. the particles must be very small, in order to avoid big relative errors which may be represented as

$$E_{\text{rel}} = \sigma\ 100/(m/2) = 100/\sqrt{m}$$

TABLE 2
ANALYSIS OF MIXTURES

Run no.	% N		% H		Used standard
	Theor.	Exp.	Theor.	Exp.	
1		26.48		3.99	C
2		26.48		3.66	(see Table 1)
3		26.44		3.70	
4		26.55		3.86	
Av.	26.92	26.49	3.86	3.80	

	% Weight Theor.	% Weight Exp.
NH_4NO_3	52.34	49.78
KNO_3	31.41	33.10
NH_4Cl	16.25	17.12

Following this line, a mechanical mixture was prepared by grinding and sieving NH_4NO_3, KNO_3, and NH_4Cl to particle dimensions smaller than 37 μm. The components of the mixture, which was made on the basis of weight, were then thoroughly mixed and finally analyzed using the usual procedure; the samples, weighing about 0.7 mg, should have contained more than 12,000 particles. From the found averaged nitrogen and hydrogen results, the experimental percent weight composition of the mixture was calculated.

The results are shown in Table 2 and may be considered as satisfactory in view of the fact that, generally speaking, the instrumental results enter in the percent weight calculations as differences in respect of the theoretical nitrogen, hydrogen and carbon content of the components of the mixture: thus the small experimental errors usually encountered may bring to larger errors in the final results and the accuracy will depend to a great degree on the characteristics of the sample.

SUMMARY

The Hewlett Packard Model 185 CHN Analyzer, which is based on an automated modified Dumas combustion technique and was designed for the analysis of organic materials, has been applied to the determination of carbon, nitrogen and hydrogen in inorganic compounds.

With minor modifications to the procedure routinely used for the organic analysis, the instrument has proved to be able to broaden its original application field giving in general good performances in the inorganic domain.

A particular emphasis has been put in testing nitrates, carbonates, ammonium salts and different kinds of carbon. Till now it appears that the only materials really difficult to analyze are the refractory nitrides, carbides and carbonitrides.

Finally some considerations have been made in respect of the determination of the percent weight composition of mixtures through their carbon, nitrogen and hydrogen content.

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MICROCHEMICAL JOURNAL 18, 596–604 (1973)

Microdetermination of Calcium in Organic Compounds

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INTRODUCTION

Microdetermination of calcium in organic compounds was performed by a spectrophotometric final procedure after the oxygen flask or oxygen stream combustion of the sample.

Present even in very small amounts calcium forms a red-colored complex with glyoxal bis (2-hydroxyanil) [GBHA] (*1*, *2*). This complex-forming reaction provides for an emphasized sensitivity but not selectivity, because other alkaline earth cations also form red complexes with this reagent, differing only in their stabilities. This fact does not diminish the value of the spectrophotometric assay applied for calcium determination in organic samples as the simultaneous occurrence of more than one cation is not frequent. However, some other drawbacks had to be removed.

MATERIALS AND METHODS

Reagents

Glyoxal bis (2-hydroxyanil) was prepared and recrystallized following the original prescription (*1*); 3×10^{-4} *M* ethanolic solution was prepared daily.

Tetraborate buffer, pH 12.5, was prepared by dissolving 10 g of sodium tetraborate and 10 g of sodium hydroxide in 1000 ml of bidistilled water.

Anion exchanger Dowex-Al (50–100 mesh) in chloride form was used.

Apparatus

Beckman DU UV/VIS spectrophotometer with ground stoppered cells was used.

Procedure

Combustion of the sample. About 1 mg of substance is pyrolyzed

in an oxygen stream or an oxygen flask equipped for electrical ignition of samples in a porcelain crucible.

For such small samples ignition is accomplished in a short time. The crucible is left for a while in 0.02 *M* HCl solution, which is then neutralized and filled up to a volume of 25 ml.

Spectrophotometric determination of calcium. A 5-ml portion is transferred with a pipet into another volumetric flask, 4 ml of water, 1 ml of tetraborate buffer, and 2 ml of ethanolic 3×10^{-4} *M* GBHA added, mixed through, and made up to 20 ml with ethanol. The absorbance at 520 nm is read out after 30 min or more against blank prepared analogously with 5-ml portion of water.

Elimination of phosphate. In the presence of phosphate the neutralized solution is sucked through a 5-cm layer of anion exchanger (in chloride form) in a Gooch filter with sintered disc, 1 cm in diameter. The calcium determination follows as described before.

RESULTS

Complexes of Alkaline Earth Cations with GBHA

Schiff's base GBHA is used as reagent or indicator for the detection and determination of calcium (*2–4, 6, 13*). Strontium and barium also form colored complexes while magnesium and beryllium bind with the reagent without development of color (*12*).

Experiments performed in this work have shown that all alkaline earth cations form red-colored GBHA complexes under certain conditions. In alkaline ethanolic medium beryllium and magnesium complexes are considerably less stable than those of strontium and barium, while calcium complex is preferentially formed in aqueous ethanolic solutions (*9*).

The composition of the complexes determined by Job's continuous variation method found to be 1 : 1 is in agreement with the results obtained for calcium complex (*8, 12*). The conditional formation constants determined by the method of Likussar and Boltz (*5*) given in Table 1 indicate that in ethanolic medium the complexes of strontium and barium are more stable than those of calcium. The value for the latter increases with the addition of water congruent with the log $B_{Ca} = 4.3$ value in 50% ethanol reported earlier (*12*). Elemental analysis of prepared and recrystallized calcium complex corresponds also to 1 : 1 complex in dihydrate form as proposed by Bayer (*1*). Owing to low solubilities and easy decomposition, the prepared complexes of barium, strontium, and magnesium could not be satisfactorily purified for elemental analyses. The infrared spectra indicate in all cases the participation of the imino group in complex formation (*9*).

TABLE 1
LOGARITHMIC VALUES OF CONDITIONAL FORMATION CONSTANTS (5) OF GBHA COMPLEXES WITH ALKALINE EARTH CATIONS[a]

Metal	Ca		Sr		Ba	
$k \times 10^4$	2.5	3.0	2.5	3.0	2.5	3.0
Y_{max}	0.3949	0.450	0.6206	0.6355	0.5978	0.6753
log K_f	3.9359	3.9964	4.5376	4.5036	4.4707	4.6304

[a] k . . . . . .sum of molar concentrations of metal and ligand;
Y_{max} . . . . . D/D_{max} ratio at maximum.

Anomalous absorbances obtained in calcium-containing samples by addition of increasing amounts of magnesium, strontium, or barium illustrate the competition in the formation of colorless and colored GBHA complexes with two cations present at the same time in variable ratios (Fig. 1).

Spectrophotometric Determination of Calcium

The calibration diagram for the spectrophotometric calcium determination via the GBHA complex is linear in the concentration range 0–2 μg of Ca/ml with good reproducibility (Fig. 2). The constancy of absorbance readings for more than 1 hr was achieved by addition of

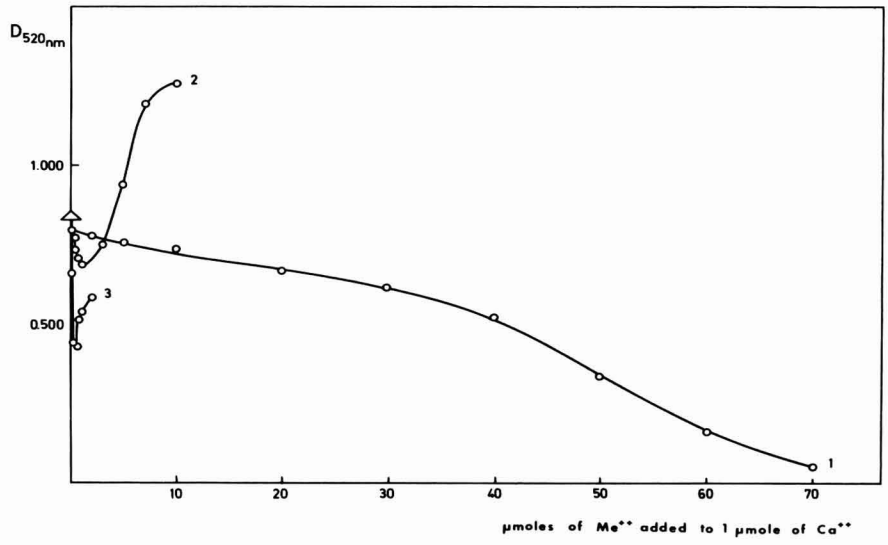

FIG. 1. The effect of other alkaline earth cations on the absorbance at 520 nm of GBHA–calcium complex. To 1 μmole of calcium increasing amounts of: (1) magnesium, (2) strontium, and (3) barium were added.

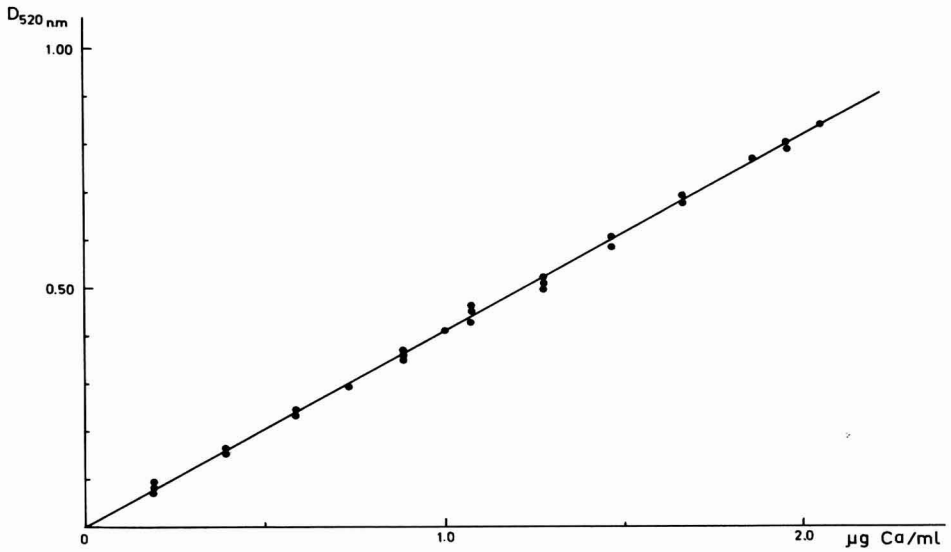

FIG. 2. Calibration diagram for the spectrophotometric calcium determination. Least-square method calculation gave $b = 0.4076$, $s_b = 0.0020$, $s^2 = 1.6 \times 10^{-4}$.

tetraborate buffer (*4*) (Fig. 3). The application of this procedure for the determination of calcium after flask combustion of organic sample (*7*) was disappointing: the color of calcium complex could not be detected at all. It was the presence of carbonate frustrating the color. In Fig. 4 is shown the effect of carbonate on the absorbance of calcium complex. The expellation of carbon dioxide by boiling the acidic absorption solution eliminated this interference. However, pyrolytic products of samples wrapped in filter paper influenced to a small extent the results for calcium too (Fig. 5). This was the reason for choosing the oxygen-stream combustion or oxygen flask combustion

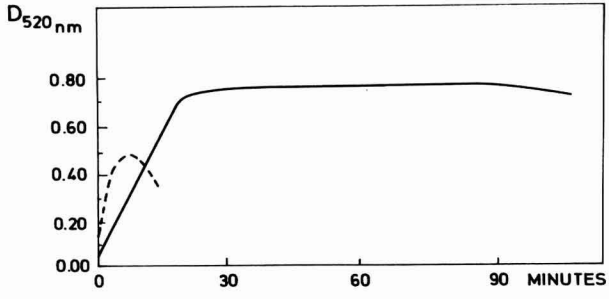

FIG. 3. The time dependence of absorbance at 520 nm of GBHA–calcium complex. Hatched line represents the absorbance recorded without tetraborate and full line with tetraborate present.

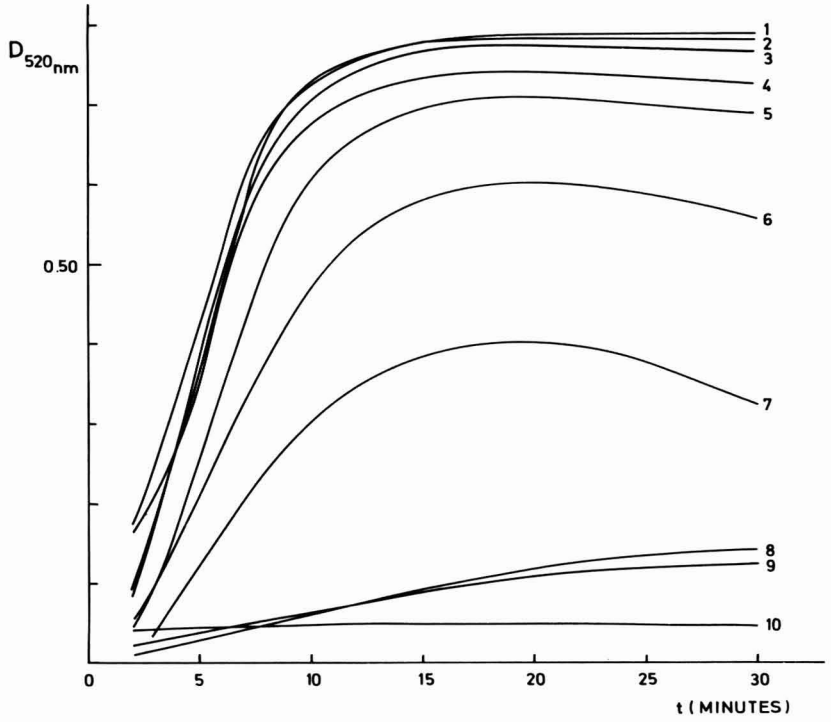

FIG. 4. The effect of carbonate on the stability of GBHA–calcium complex. To 50% ethanolic solution with tetraborate present carbonate was added in the following concentrations: (1) 0.00 μg/ml; (2) 0.5 μg/ml; (3) 1.0 μg/ml; (4) 10.0 μg/ml; (5) 50.0 μg/ml; (6) 100.0 μg/ml; (7) 200.0 μg/ml; (8) 500.0 μg/ml; (9) 700.0 μg/ml; and (10) 2.0 mg/ml.

omitting the paper. The results of a series of determinations are shown in Table 2. The phosphate present in the solution hindered also the development of color (Fig. 6), but could effectively be removed by adsorption on an anion-exchanger layer (Fig. 7). Results for calcium in some phosphorus-containing compounds have shown standard deviation ±0.58% and standard error ±0.20%.

DISCUSSION

The oxygen-flask combustion method performed in usual way i.e., with samples wrapped in filter paper (*11*) could not be successfully used if certain indicators were applied in the final titrimetric step.[1] In addition to the fact that this combustion is in many instances incomplete (*10*), in our case there was noted a disadvantageous influence on the spectrophotometric final determination of calcium. The

[1] Unpublished observations of the authors.

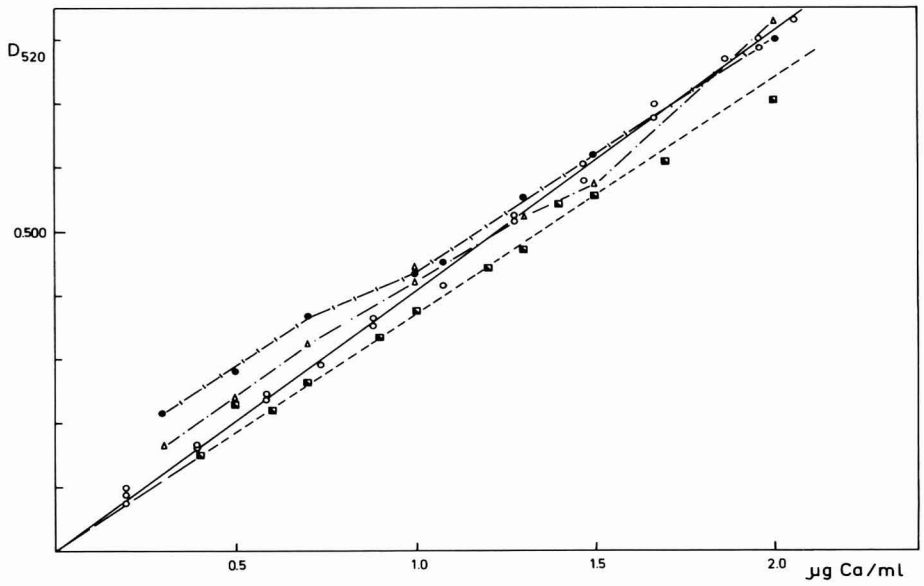

FIG. 5. Calibration diagram for the spectrophotometric calcium determination. In pure calcium solution: ○; in absorption solution after pyrolysis of filter paper: ◪; in absorption solution after pyrolysis of benzoic acid wrapped in filter paper: △; in absorption solution after pyrolysis of acetanilide wrapped in filter paper: ●.

sensitivity of this determination enabled the work with 1-mg samples so that the combustion in a stream of oxygen could be performed rapidly. Another suitable way was found to be the pyrolysis in an oxygen-filled flask with equipment for electrical ignition of the sample in a porcelain crucible.[2] Both procedures might be used equally well as initial step in cases when the nature of the sample or the solvent used causes trouble in direct calcium determination by atomic absorption spectroscopy and a previous ashing procedure is necessary.

TABLE 2
RESULTS OF CALCIUM DETERMINATIONS

Compound	Number of determinations	Ca% Calcd	Ca% Found (Mean value)	SD	SE
Ca–metacycline	25	6.07	5.94	0.45	0.09
Ca–lactate × H_2O	10	16.96	16.89	0.54	0.17
Ca–gluconate × H_2O	16	9.27	8.73	0.24	0.06
$CaNa_2EDTA \times 2.5H_2O$	19	9.56	9.03	0.44	0.10

[2] The details are being prepared for publication.

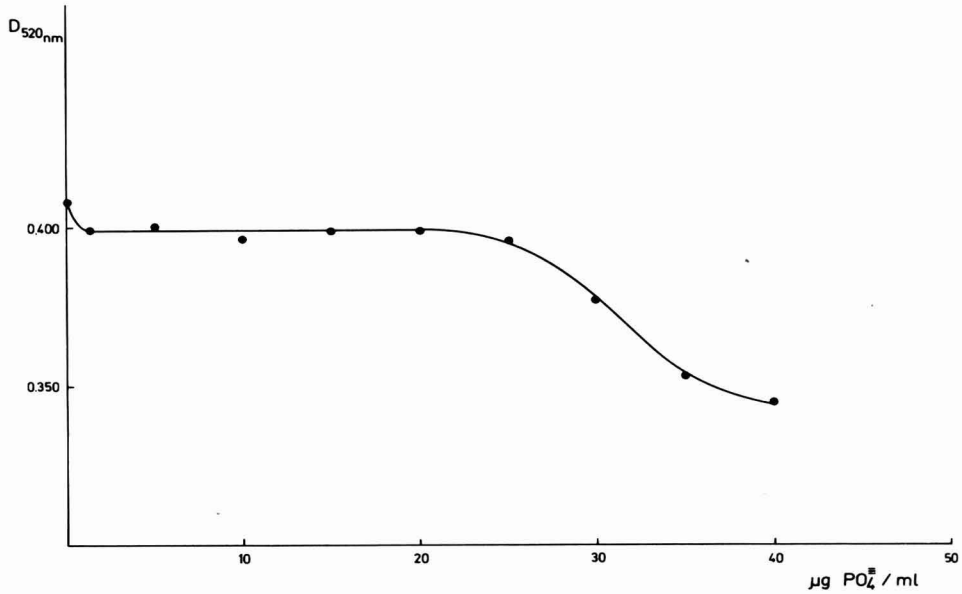

FIG. 6. The effect of phosphate on the absorbance at 520 nm of GBHA–calcium complex.

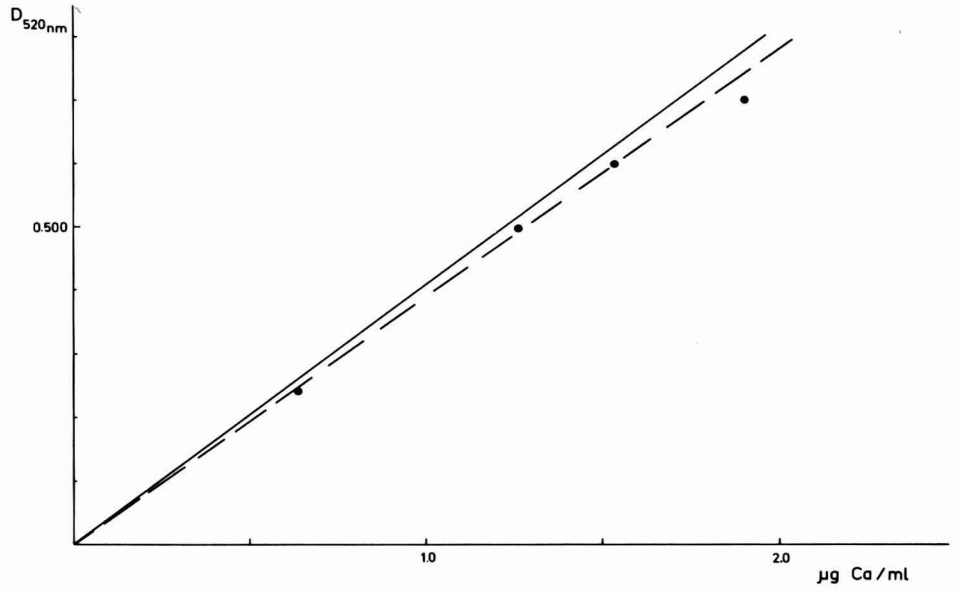

FIG. 7. The aberrance of the calibration diagram after elimination of phosphate by adsorption on anion exchanger.

Further on, the procedure is suitable for the work on ultramicro scale while on the other side with samples of usual size even traces of calcium could be determined by reducing the volumes of solutions.

The method was foreseen for the determination of calcium in some biologically active complexes containing phosphate. As the presence of phosphate causes the declining of measured absorbance, a way was found to eliminate this error in binding the phosphate with an anion exchanger prior to the spectrophotometric determination of calcium. The inserted sucking through a layer of exchanger diminished but to a negligible extent the reliability of the method.

SUMMARY

A sensitive method for microdetermination of calcium in organic compounds is described. The combustion in an oxygen stream or oxygen flask with electrical ignition of the sample in porcelain crucible was found advisable. Carbon dioxide has to be expelled and the erroneous effect of phosphate eliminated by adsorption on an anion exchanger prior to the spectrophotometric determination of calcium with glyoxal bis (2-hydroxyanil).

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MICROCHEMICAL JOURNAL **18,** 605–612 (1973)

Spectroscopic and Dielectric Examination of the Oleyl Alcohol–Oleic Acid System

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Problems of molecular interactions are closely connected with changes of dielectric constant values for examined systems. It was stated (*2*), that oleyl alcohol and oleic acid essentially differ with regard to the hydrogen-bond association. This fact was the starting point for our present work dealing with the investigations of oleyl alcohol–oleic acid system in the range of dielectric constant changes. The applied techniques were ir absorption spectroscopy and dielectric constant measurements.

EXPERIMENTAL METHODS

Oleyl alcohol (T. Schuchardt, West Germany) and oleic acid (R.C.B., Belgium) used in our experiment were of a high purity (98–100%).

For spectroscopic purposes mixtures of oleyl alcohol and oleic acid were soluted in carbon tetrachloride (Lachema, Czechoslovakia; for ir) and *n*-hexane (R.C.B., Belgium; for ir). These solutions were prepared in a special way: the total concentration of alcohol and acid in every sample was 0.043 mol/l, and the molar fraction of alcohol successively changed as follows: 1.0, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1, 0.

The ir spectra were registered at 22°C using a double-beam UR-20 spectrophotometer (Carl Zeiss, Jena, East Germany), employing an LiF prism and NaCl trays (thickness 2 mm). The registering conditions: speed, 64 cm^{-1}/min; width, 100 cm^{-1}/40 mm. The recorded region of spectra contained valency vibration band of nonassociated hydroxyl group in oleyl alcohol.

For dielectric investigations one prepared mixtures of oleyl alcohol and oleic acid, in which molar fraction of oleyl alcohol changed as follows: 1.0, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1, 0. Measurements were conducted at 22, 30, 40, 50 and 60°C with the help of DK-60 GK decameter together with its thermostatic chamber (East Germany).

DISCUSSION

The results of spectroscopic investigations are shown in Fig. 1. As it proceeds from these data, the initial diminution of molar fraction of oleyl alcohol in the oleyl alcohol–oleic acid system influences the lowering of maximum molar extinction coefficient for the valency vibration band of free O–H group in alcohol. This fact gives evidence of presence of new types of associates, namely alcohol–acid associates, which are coupled with the stronger hydrogen bonds and thus though the concentration of alcohol becomes lower, the greater number of alcohol molecules is involved in these structures. From the molar fraction value of 0.6 one observes the slow increase of the discussed coefficient. It is influenced with the smaller number of associates, in which alcohol participates.

Further information dealing with our dual system was obtained with the help of dielectric measurements. Their results are shown in Figs. 2–11 and it proceeds from them that the low values of dielectric constant for pure oleyl alcohol and oleic acid are evoked with a considerable association of these compounds. The analysis of dielectric

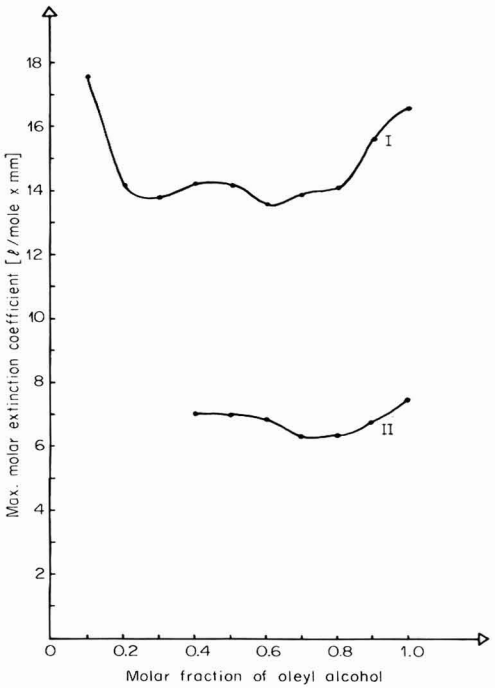

FIG. 1. Changes of maximum molar extinction coefficient for valency vibration band of nonassociated hydroxyl group of oleyl alcohol in the oleyl alcohol–oleic acid system [solutions in carbon tetrachloride (I) and *n*-hexane (II)].

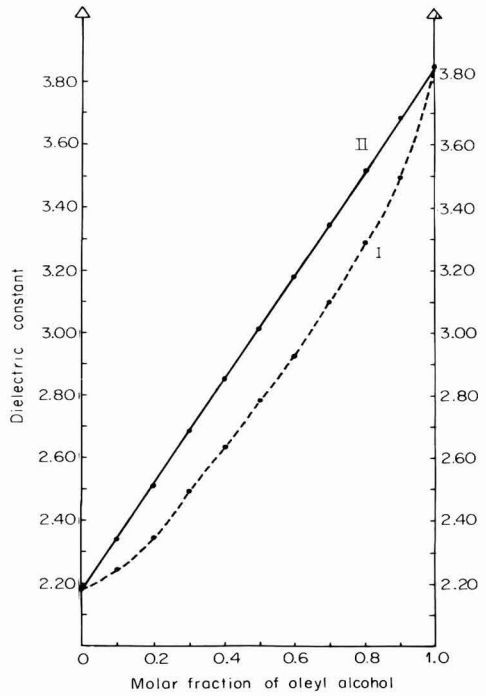

FIG. 2. Experimental (I) and additive (II) dielectric constant values vs molar fraction of oleyl alcohol in the oleyl alcohol–oleic acid system at 22°C.

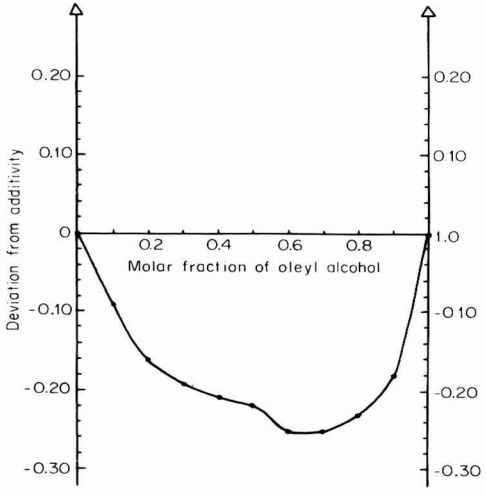

FIG. 3. Deviation from additivity of dielectric constant values vs molar fraction of oleyl alcohol in the oleyl alcohol–oleic acid system at 22°C.

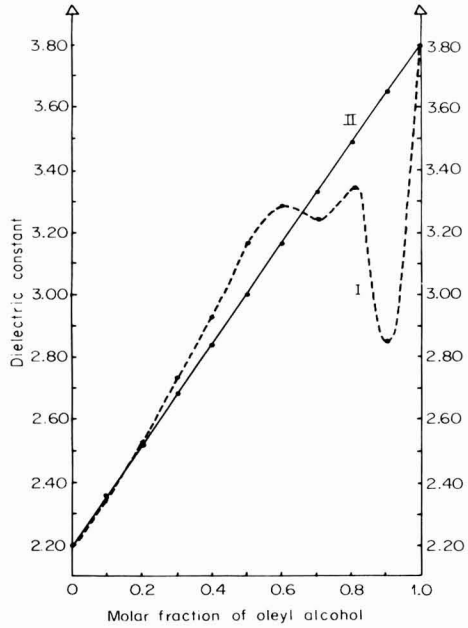

FIG. 4. Experimental (I) and additive (II) dielectric constant values vs molar fraction of oleyl alcohol in the oleyl alcohol–oleic acid system at 30°C.

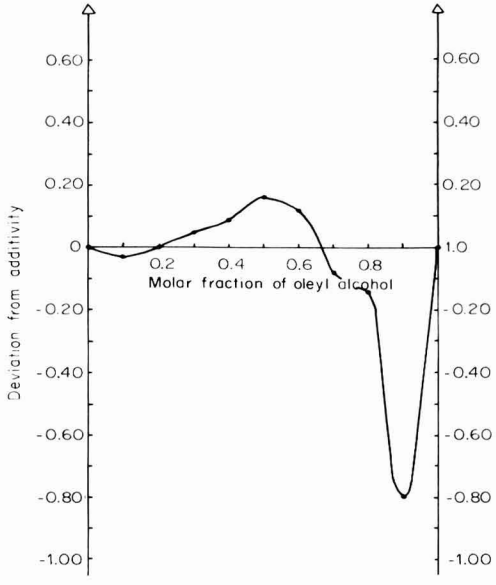

FIG. 5. Deviation from additivity of dielectric constant values vs molar fraction of oleyl alcohol in the oleyl alcohol–oleic acid system at 30°C.

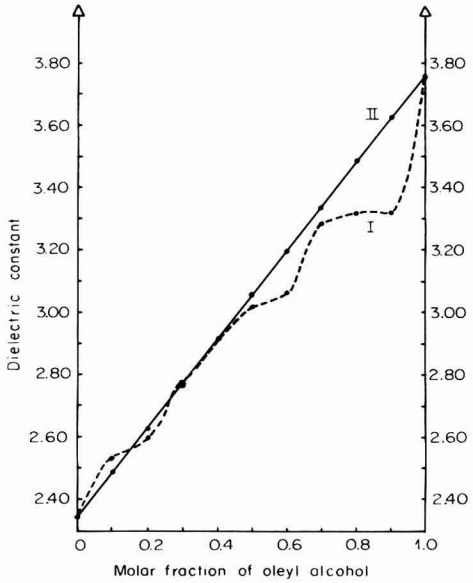

FIG. 6. Experimental (I) and additive (II) dielectric constant values vs molar fraction of oleyl alcohol in the oleyl alcohol–oleic acid system at 40°C.

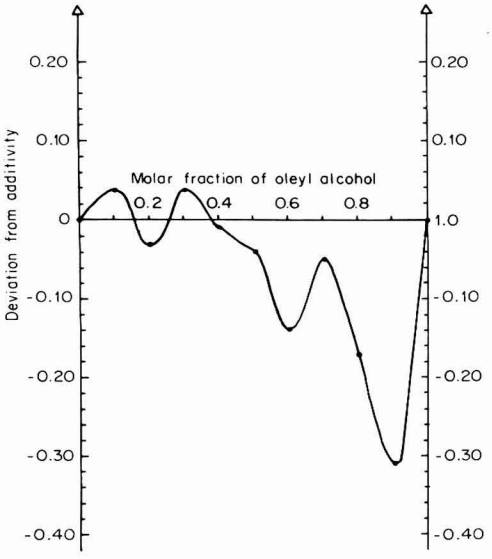

FIG. 7. Deviation from additivity of dielectric constant values vs molar fraction of oleyl alcohol in the oleyl alcohol–oleic acid system at 40°C.

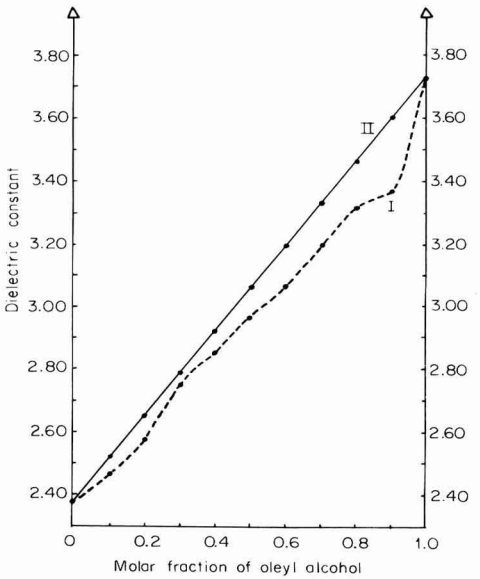

FIG. 8. Experimental (I) and additive (II) dielectric constant values vs molar fraction of oleyl alcohol in the oleyl alcohol–oleic acid system at 50°C.

constant values for the alcohol–acid systems composed in different ratios allows a conclusion, that experimental values essentially differ from those calculated according to the equation (*1*):

$$\epsilon_{ad} = \epsilon_A \cdot x_A + \epsilon_K \cdot x_K,$$

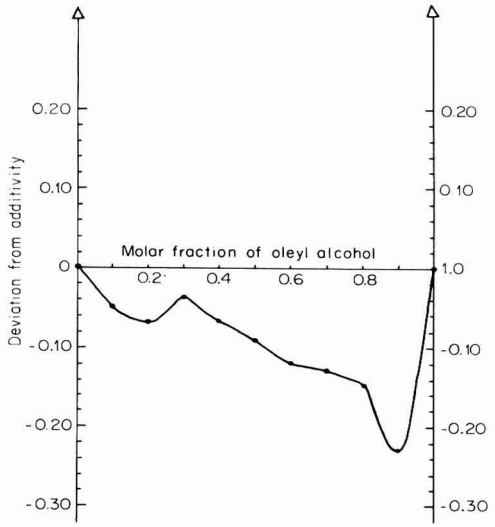

FIG. 9. Deviation from additivity of dielectric constant values vs molar fraction of oleyl alcohol in the oleyl alcohol–oleic acid system at 50°C.

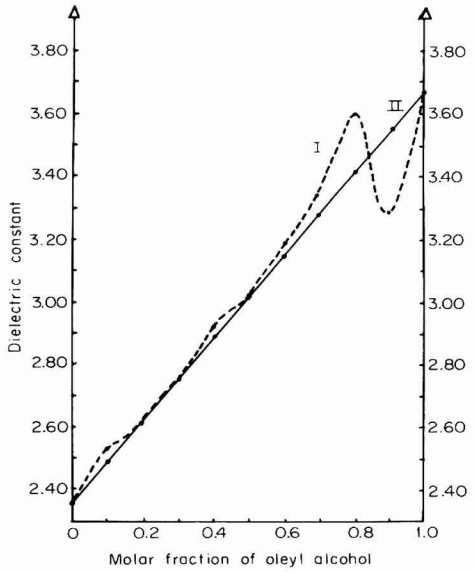

FIG. 10. Experimental (I) and additive (II) dielectric constant values vs molar fraction of oleyl alcohol in the oleyl alcohol–oleic acid system at 60°C.

where ϵ_{ad} = additive dielectric constant, ϵ_A = dielectric constant of oleyl alcohol, ϵ_K = dielectric constant of oleic acid, x_A = molar fraction of oleyl alcohol, x_K = molar fraction of oleic acid.

Isotherms of experimental dielectric constant values in the examined range of temperatures show the complexity of molecular interac-

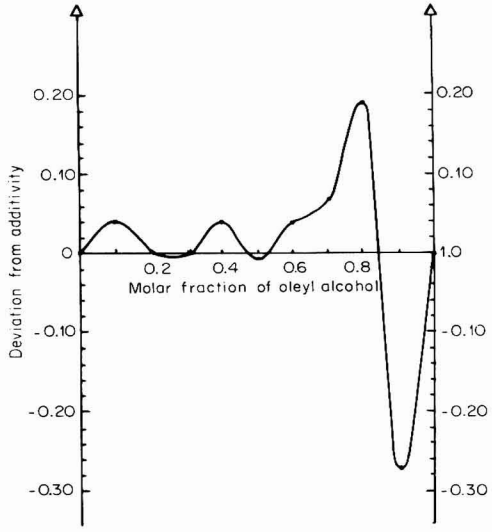

FIG. 11. Deviation from additivity of dielectric constant values vs molar fraction of oleyl alcohol in the oleyl alcohol–oleic acid system at 60°C.

tions between oleyl alcohol and oleic acid (*1*). On their base one may conclude that dielectric constant for mixtures of alcohol and acid represents nonadditive values and deviations from additivity are both positive and negative. Presumably the *a*/*m* interactions depend mainly on the hydrogen-bonded complexes.

From the curves of deviation from additivity vs molar fraction of oleyl alcohol (Figs. 3, 5, 7, 9 and 11) one may conclude, that the examined system gives characteristic complex for molar ratio of alcohol to acid of 9 : 1. The sharp extreme for this molar ratio of components is observed in the range of temperatures from 30 to 60°C and reflects lowering of polarity typical for polyassociates. Both spectroscopic and dielectric measurements suggest that the molar ratio of 9 : 1 enables the strongest association of components. At the lowest temperature (22°C) the shape of experimental dielectric constant isotherm makes evident some passivity of alcohol and acid.

The alcohol–acid mixtures composed in other molar ratios show a number of weaker positive and negative extremes, which depend on the molar fractions of alcohol and temperature of measurement. Apparently they represent a number of weaker associates.

On the basis of dielectric experiment it may be stated that the examined system shows the greatest activity of its components at 60°C. A proof for this supposition is the greatest number of extremes of the isotherm at 60°C, which reflects several possibilities of interactions between alcohol and acid.

SUMMARY

The comparison of association was made for the oleyl alcohol–oleic acid systems composed in several molar ratios. The ir spectroscopy and dielectric constant measurements were applied as analytical techniques. It was stated that oleyl alcohol and oleic acid interact in a number of molar ratios giving the hydrogen-bonded associates. In the range of temparatures 30–60°C the most stable hydrogen-bonded associate is formed in a ratio of alcohol to acid of 9 : 1.

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MICROCHEMICAL JOURNAL 18, 613–616 (1973)

Convenient Technique for Microhydrogenation

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In the analysis of complex materials such as flavor isolates, unsaturated compounds frequently are encountered which cannot be identified by gas chromatographic (GC) retention times or combined gas chromatography mass spectrometry (GC/MS). In such cases, microhydrogenation techniques have been used to prepare the saturated compound, followed by analysis of the reduced material. Stanley and Murray (*2*) discussed various procedures which have been suggested for the hydrogenation of natural materials. They recommended collecting the resolved peak from the GC followed by external hydrogenation and presented a reasonably complex heated trap reactor which is applicable to both hydrogenation and hydrogenolysis.

The following technique offers a more convenient procedure for the external hydrogenation of unsaturated compounds which is useful both as an adjunct to structural elucidation and as a simple micropreparative technique.

EXPERIMENTAL

Hydrogenation Technique

Hydrogenation is performed in the apparatus shown in Fig. 1. The reaction vessel, 1, is a piece of glass tubing, 8 mm o.d. × 4 cm long, drawn to a conical end. In this is placed the sample to be hydrogenated, 0.1 ml methanol and about 0.5 mg of 10% palladium on carbon catalyst (Engelhard Industries, Newark, New Jersey). Palladium was chosen as the catalyst in this study since this is considered by Augustine (*1*) to be the best catalyst for the hydrogenation of double bonds. The Orsat bag, 3, is filled with hydrogen and this is allowed to flush out the reaction vessel through the 20-gauge syringe needle, 2, which has been cut off to a flat end. The needle is then immersed to the bottom of the vessel and hydrogen is bubbled in at a rate of one bubble per second, thereby agitating the solvent/catalyst mixture. After an appropriate hydrogenation time, the apparatus is disconnected, the vessel centrifuged and the reaction mixture analyzed by GC.

Preliminary work indicated that an appropriate hydrogenation

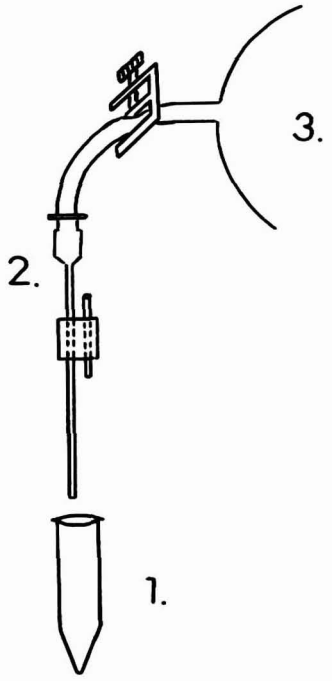

FIG. 1. Microhydrogenation apparatus.

period for samples of less than 100 μg was 5 min or less, while 10 min were required for 1 mg samples.

Reaction of Known Materials

To determine the utility of the technique, a number of chemicals were treated in the above fashion and the results are indicated in Table 1. In all cases hydrogenation was performed on 20, 100 and 1000 μg samples to demonstrate the adaptability of the method to various sample sizes.

Comparison of the material before and after hydrogenation was performed by analysis of an appropriate aliquot in a Perkin–Elmer Model 900 gas chromatograph equipped with a flame ionization detector. Separation was performed on a $\frac{1}{8}$ in. $\times$ 6 ft column packed with 10% DEGS on 80–90 Anakrom ABS with a carrier gas flow rate of 30 ml/min under appropriate isothermal conditions. The approximate yield of hydrogenated products was calculated by comparing peak area (by triangulation) of the starting material and of the hydrogenated product.

Reduction of Isolated Materials

The most convenient technique is to trap the compound of interest

TABLE 1

COMPOUNDS REDUCED BY MICROHYDROGENATION TECHNIQUE

Type	Compound	Number Double Bond Reduced	Reduction Product	Yield, %
Ketone	6-Methyl-5-heptene-2-one	1	6-Methyl-5-heptan-2-one	90
Alcohol	cis-3-Hexenol	1	Hexanol	80
	trans-2-Nonenol	1	Nonanol	90
	Cinnamic alcohol	1	Phenylpropylalcohol	85
	Linalool	2	3,7-Dimethyloctanol	85
Aldehyde	2-Decenal	1	Decanal	95
	2,4-Decadienal	2	Decanal	90
Ester	Ethyl crotonate	1	Ethyl butyrate	95
	trans-2-Hexenyl butyrate	1	Hexyl butyrate	90
	cis-3-Hexenyl butyrate	1	Hexyl butyrate	90
	Ethyl 2,4-hexadienoate	2	Ethyl hexanoate	85
	Ethyl cinnamate	1	Ethyl hydrocinnamate	85
	Methyl oleate	1	Methyl stearate	85
	Methyl linoleate	2	Methyl stearate	85
	Methyl linolenate	3	Methyl stearate	85
Other	2-Nonynol	one $C\equiv C$	Nonanol	75

at the exit port of the GC by condensation in a dry-ice cooled melting point capillary. The trapped material is transferred to a reaction vessel with 100 μl methanol and treated as above.

Caution

The catalyst should not be allowed to dry out after hydrogenation.

RESULTS AND DISCUSSION

The results obtained are summarized in Table 1. From the table, it is evident that this technique will yield high recoveries of the expected reduction products with sample sizes ranging from 20 μg to 1 mg. Subsequent work demonstrated that samples as small as 3 μg could be reduced by this technique. The utility of the indicated procedure is twofold. Hydrogenation will reduce a double bond to yield the saturated analog, and the latter can then be identified more readily by normal spectrometric analysis, by GC retention times, or by combined GC/MS. Secondly, this technique is convenient as a preparative procedure when micro or milligram quantities of the appropriate material are required.

The advantages of this technique are that it is rapid, simple and gives the expected product in high yield. Unexpected hydrogenolysis side reactions are minimized by the gentle reaction conditions. In addition, hazards are reduced since only a few milliters of hydrogen are required for a number of reductions.

SUMMARY

A convenient technique is described for the batch-wise hydrogenation of microgram and milligram quantities of unsaturated compounds. These samples are reduced catalytically in the presence of palladium in a rapid and highly efficient manner. The procedure is particularly useful in aiding in the identification of unsaturated compounds trapped from a gas chromatograph.

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MICROCHEMICAL JOURNAL 18, 617–621 (1973)

Application of Fast Grey RA to the Spectrophotometric Determination of Copper in Soft Tissues of Egyptian Camels

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INTRODUCTION

Copper is distributed all over the body tissue cells in organic combination with proteins or enzymes. However, it is stored in large amounts in certain organs such as the liver, kidney, spleen, and the blood, and appears to be very rare in other organs such as the brain and fat deposits. Underwood (*9*) mentioned that livers of normal adults of most species contain 10–50 ppm on dry basis. These levels apply to man, rats, rabbits, cats, dogs, foxes, pigs, kangaroos, whales, snakes, crocodiles, domestic fowls, turkeys, sharks, and herring. However, much higher levels (100–400 ppm) are exhibited by sheep, cows, ducks, frogs, and certain fish. It appears that species differences reflect physiological differences in ability to store copper in its cells. Although copper in body tissues was estimated in bovine and ovine fetuses (*7*), no available literature could be traced recording copper values in camels.

Recently Khalifa (*4, 5*) used the ortho-orthohydroxy azo dye 15690, Solochromate Fast Grey RA CI mordant 19 (reddish navy-blue black) for the spectrophotometric determination of copper in serum and liver of Egyptian camels. Organs and tissues in the same individual differ in the amount of other elements which may hinder or interfere with the formation of copper fast-grey complex.

The present work is a contribution to the spectrophotometric microdetermination of copper in various organs of Egyptian camels using Fast Grey RA.

EXPERIMENTAL METHODS

The water, chemicals, solutions, and equipment used were essentially the same as those described before (*5*).

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Procedure

A Sampling: From 15 apparently clinically healthy, mature, male camels that proved to be free from parasitic or bacterial infestation by postmortem examination at time of slaughter at Cairo Abbattoir, take the blood (citrated), spleen, liver, kidney, muscle, adrenal and lymph glands, integument, large and small intestines, heart, hump and brain samples (8–25 g); wash, except blood, thoroughly with twice distilled water, mince to small pieces, dry to constant weight at 110–120°C and keep in Mckartney bottles in a desiccator.

B. Standard curve: Use the same calibration curve obtained by the repeated evaporation technique described before (*5*).

C. Application: To determine copper, digest 1 g of dry tissue sample (1ml in case of citrated blood) and (5 g fat in case of hump) with 5 ml each of 50% v/v sulfuric and concd nitric acids, followed by 3 ml hydrogen peroxide (Heat the fat sample to the appearance of white fumes, continue heating until the volume reduces to one-tenth of the original one, and use twice the above volume of sulfuric acid. Get rid of excess acid by repeated evaporation and washing, and transfer quantitatively to 100-ml flask, and make with water up to volume. To 1 ml add 2 ml 0.05 *N* nitric acid, 2 ml 0.2 *M* ascorbic acid, 3 ml 0.005% FG, make up with water to 10 ml. Measure extinction at 555.5 mμ.

RESULTS AND DISCUSSION

Table 1 and Fig. 1 demonstrate the amounts of copper determined using FG and applying the described technique. Although no recorded data for the copper content in camels' organs can be traced, except those reported by Moustafa (*6*), who estimated copper in camels blood iodometrically and found it to range from 325 to 982 μg copper per 100 ml whole blood. Our results ranged from 348.8 to 1130.4 with a mean of 696.6 $\pm$ 63.7 μg per 100 ml, exhibiting good concordance with the reported values.

Hair is found to contain the largest copper content, which may be due to the presence of vanadium, reported to form similar colored FG complexes (*3*), as well as to melanin and pigments already found in its follicles which may have also caused the higher level recorded in the integument. Underwood (*9*) reviewed the copper content of hair in animals and mentioned that its value in pigmented hair of several species is significantly higher than that of white hair. Spector studies concerning this element (*8*) showed that its content is considerably high in hair of dogs in comparison with other organs. Liver is described to be the main storage organ of the body for copper, and hence its content in liver is a

TABLE 1

COPPER IN MICROGRAMS PER GRAM DRY MATTER OF CAMELS ORGANS

	Min value	Max value	Mean	Error ±
Hair	125.40	325.90	200.8	11.4
Liver	59.80	286.30	155.8	16.1
Spleen	109.09	190.91	142.3	6.8
Lung	86.00	166.70	128.3	6.9
Heart	98.50	152.20	117.6	5.1
Integument	75.00	143.30	113.0	5.3
Muscle	62.70	107.50	86.3	3.2
Brain	50.00	114.30	79.0	5.2
Lymph gland	59.10	98.50	76.3	3.7
Small intestine	41.20	107.50	66.3	4.3
Large intestine	50.20	71.60	59.8	1.7
Kidney	35.80	80.60	57.6	3.0
Adrenal gland	41.20	77.00	53.0	2.2
4th Stomach	23.30	59.10	36.3	2.3
Blood (1 ml)	3.488	11.304	6.966	0.637
Hump	1.33	5.33	3.03	0.32

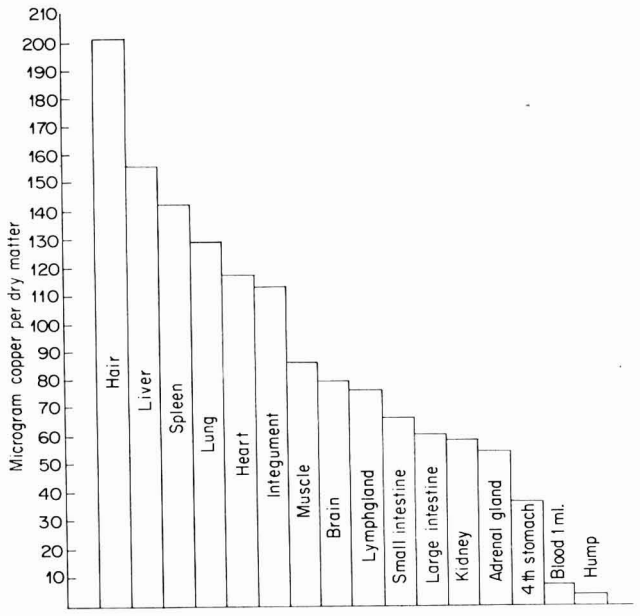

FIG. 1. Copper in micrograms per g dry matter of camel's organs using Fast Grey RA.

useful index for the copper status of the animal. The high spleen copper content of 142.3 ± 6.8 in camels (Table 1) can be explained, because the camel spleen is known to contain large amounts of blood, which is reported to contain higher amounts of copper than the spleen of other ruminants (*6*).

The fact that the copper content in body muscle, lung and heart appears to be higher than that which is found in muscle, lung, and heart of other ruminants is attributed to the high blood vascularity as evidenced by the rose-pink coloration of these organs.

Bowen (*1*) mentioned that copper constitutes 0.19% of cerebrocuprein and 0.05–0.32% of metalloprotein enzymes that are known to be high in the brain, lymph, and adrenal glands, a fact which explains the relatively high copper content of these organs.

From the intestinal tract, the fourth stomach contained the lowest amount in comparison with the relatively higher values of the large and small intestine, as copper is known to be absorbed in the latter.

The hump, composed of fat deposits, is known to be very low in copper content as shown by the value of 3.03 (Table 1).

The normal biological higher level of Al, As, I, S, in hair; Co, Fe, Mn, Mo, Rb, in liver; Cd, Hg, Zn, in kidney; P in brain; Ti in lung, and Sn in intestine did not interfere with the estimation of copper using the present technique. Such observation was previously verified experimentally (*2*).

That vanadium is the only element reported to form similarly colored FG complex, leads to the conclusion that liver, with the estimated copper content of 155.8 ± 16.1 (Table 1), is the main copper reservoir in the Egyptian camel.

SUMMARY

Copper in different organs of Egyptian camels was successfully determined spectrophotometrically using Fast Grey RA. Its values amounted to: 200.8 ± 11.4, 155.8 ± 16.1, 142 ± 6.8, 128.3 ± 6.9, 117.6 ± 5.1, 113.0 ± 5.3, 86.3 ± 3.2, 79.0 ± 5.2, 76.3 ± 3.7, 66.3 ± 4.3, 59.8 ± 1.7, 57.6 ± 3.0, 53.0 ± 2.2, 36.3 ± 2.3 and 3.03 ± 0.32 μg/g dry matter of hair, liver, spleen, lung, heart, integument, muscle, brain, lymph gland, small intestine, large intestine, kidney, adrenal, fourth stomach, and hump, respectively, while 1 ml citrated blood contained 6.97 ± 0.64 μg. Of 16 elements mentioned, only vanadium interferes with the determination of copper in hair by the present method.

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MICROCHEMICAL JOURNAL 18, 622–626 (1973)

New Spot Test for Some Phosphorus Nerve Gases

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INTRODUCTION

Organic pentavalent phosphorus compounds having structures either of the type $\diagdown$POF or $\diagdown$POCN are highly toxic and of a rapid physiological action *(5)*. Most of these are used in military purposes as nerve gases. However, few reactions have been proposed for detection of some of these compounds.

Schönemann described a method involving the use of an alkaline peroxide to react with the nerve gases to form perphosphorate which develops a colored azo dye on a reaction with *o*-tolidine *(8)*. This reaction is greatly improved by Gehauf *et al. (2)* who used *o*-dianisidine in place of *o*-tolidine. Diisonitrosoacetone reacts with some nerve gases to give an intense magenta color *(6)*.

A method based on the formation of a fluorescent solution of indoxyl by the reaction of the nerve gas with indole and sodium perborate has been described *(3)*.

The chemiluminescence method, based on a reaction with 5-amino-2,3-dihydro-1,4-phthalazinedione (luminol), has been also advocated *(4)*. The enzymatic method, based on the inhibition of esteratic enzymes found in horse serum, which in the absence of the nerve gas hydrolyze naphthol (deep red), is employed *(1)*.

The present work describes a new spot test reaction for the nerve gases by their reaction with thallium(I) sulfate in alkaline peroxide. Thallium(I) solutions are more stable than the oxidizable amines which are widely used. In addition the reaction of *o*-dianisidine *(2)* is critically studied, and a spot test procedure of higher sensitivity and rapid response is developed.

EXPERIMENTAL

Reagents and Samples Used

All the reagents are of analytical grade:

3% aqueous thallium(I) sulfate;

3% aqueous potassium sodium carbonate;

5% urea peroxide;

o Dianisidine reagent: Dissolve 20 mg of o-dianisidine dihydrochloride in 10 ml methyl alcohol and 15 ml of water. Add 5 drops of 5*N* sulfuric acid and 4 ml of 25% hydrogen peroxide. This reagent is stable for 12 hours.

Stock isopropyl alcoholic solutions of the following compounds are tested: Methyl, isopropyl phosphofluoridate (Sarin), Diisopropyl phosphofluoridate (DFP), and Dimethyl diamino phosphofluoridate.

PROCEDURE

Test with Thallium(I) Sulfate

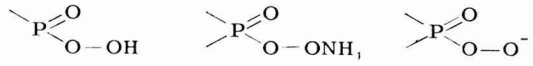
A drop of 3% thallium(I) sulfate solution is placed on a spot plate. Add one drop of 3% potassium sodium carbonate solution and a drop of 5% urea peroxide. Add one drop of the organic phosphofluoridate test solution. An immediate brown precipitate or color indicates a positive response. Carry out a blank. The lower limit of identification is 5–15 μg.

Test with o-Dianisidine Dihydrochloride

One drop of the *o*-dianisidine reagent *(vide supra)* is mixed with one drop of 3% potassium–sodium carbonate solution on either a spot plate or filter paper. Add one drop of the test sample solution to the reagent mixture. A red color is developed. Carry out a blank. The lower limit of identification is 0.1–5 μg.

RESULTS AND DISCUSSION

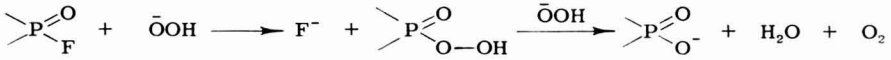
Organophosphorus fluoridates immediately react with hydrogen peroxide, in ammoniacal or alkaline media, to give the corresponding peracid salts:



(a) (b) (c)

The solution of the peracid (a), or its salt (b), and anion (c) have a far grater oxidizing action than the equivalent quantity of hydrogen peroxide. This indicates that the oxidizing action of hydrogen peroxide in

basic media is enhanced by the addition of the organophosphorus fluoridate:

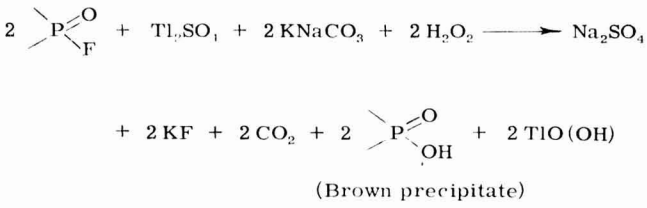


$$Tl_2SO_4 + KNaCO_3 + H_2O_2 \longrightarrow \text{No reaction}$$

The catalytic effect effectuated by the formation, action, and regeneration of perphosphorus compounds has been made the basis of a convenient spot test procedure for detection of some phosphorus nerve gases.

Reaction with Thallium (I) Sulfate

It was found that a solution of thallium(I) sulfate which remains unchanged on treatment with peroxides, in basic media, immediately precipitate brown thallium(III) oxyhydrate when the organophosphorus fluoridate compounds are added. The reaction can be represented in accordance with the equation:



(Brown precipitate)

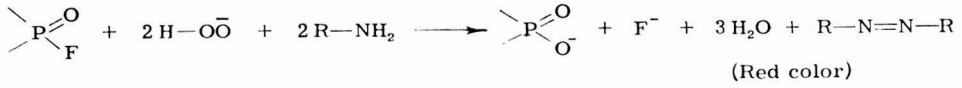
Various concentrations of sodium perborate, urea peroxide, and hydrogen peroxide are used as peroxidants in basic media. An adequate excess of peroxide during the reaction is required to increase the rate of peroxidation and to decrease the rate of hydrolysis of the phosphorus fluoridate. Urea peroxide (5%) is recommended as a suitable peroxidant reagent.

Peroxidation with urea peroxide is carried in 3% solutions of potassium hydroxide, ammonium hydroxide, potassium bicarbonate, and potassium sodium carbonate. In caustic alkali solution, the peroxide *per se* precipitates the brown TlO(OH). In ammoniacal and bicarbonate solutions, the rate of color development is slow. However, potassium sodium carbonate is the best medium to attain a suitable basicity for the peroxidation reaction. In general a pH of 9–10 is required for the reaction.

Using the procedure outlined in the experimental section, 5–15 μg of sarin, DFP, and dimethyl diamino phosphofluoridate are detected.

Reaction with o-Dianisidine

Although the reaction of perphosphorus fluoridate compounds with *o*-dianisidine is recommended as a reaction for detection and determination of some nerve gases, the color is developed slowly.



Studies of the factors that might influence the time of maximum color development and the degree of sensitivity show that the composition of the reagent has a great effect. Figure 1 shows a comparison of the intensity, stability, and the rate of color development of 100 μg DFP, using the procedure described by Gehauf *et al. (2)* and that of the present authors. The color is measured at 440 mμ using spectrophotometer SP. 800 (UNICAM). The slit width is 0.01 cm and the path length is 2.0 cm.

The results shows that 2 minutes are sufficient, with the present procedure, to produce a color of higher intensity than that obtained after 20 minutes on applying Gehauf *et al.* procedure *(2)*.

Apply the procedure described in the experimental part, 0.1–5 μg of sarin, DFP, and dimethyl diamino phosphofluoridate are detected.

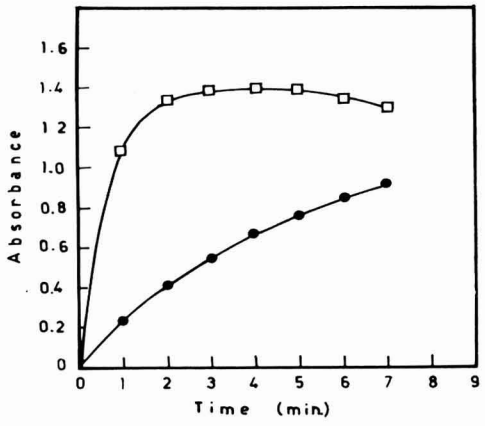


FIG. 1. Intensity, stability and rate of color development produced by the reaction of DFP (100 μg) with *o*-dianisidine. ● According to Gehauf *et al.* procedure. □ According to the present procedure.

SUMMARY

A new spot test reaction is described for the identification of some phosphorus nerve gases. The reaction is based on peroxidation of the nerve gas with 5% urea peroxide in 3% potassium–sodium carbonate followed by a reaction with thallium(I) sulfate to form a yellow color or precipitate of thallium(III) oxyhydrate. A rapid and sensitive reaction with *o*-dianisidine is also described. Some nerve gases (0.1–15 μg) are detected by both procedures.

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MICROCHEMICAL JOURNAL 18, 627–635 (1973)

Spectrophotometric Determination of Osmium Using Acenaphthenequinonemonoxime (AQM)

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Numerous organic reagents have been tried for colorimetric determination of osmium. Practically all the methods for the determination of this metal deal with the problem of interfering constituents. The methods available for the spectrophotometric determination of osmium are dependent on factors such as temperature, acidity, concentration of reagents, and certain anion and cation impurities, which affect the rate of color change. Hence the methods require strict control of working conditions. Different methods for spectrophotometric determination have been critically reviewed (*3, 4*). Recently reported reagents are oximidobenzotetronic acid (*10*), and 2-aminocyclopentene-1-dithiocarboxylic acid (*7*).

The present study was undertaken to explore the possibility of using acenaphthenequinonemonoxime (AQM) as a spectrophotometric reagent for osmium. AQM reacts with ruthenium(III), rhodium(III), palladium(II), osmium(VIII), iridium(III), and platinum(IV) on heating. The complexes are extractable into chloroform and carbon tetrachloride except that of osmium.

EXPERIMENTAL METHODS

Reagents and Equipment

AQM solution. The solution of AQM was prepared in dimethylformamide (DMF) and methanol in the ratio of 30:70. Reason for this choice is given below.

Dimethylformamide. (A.R)Dimethylformamide was distilled before use.

Standard osmium solution. A 1-g ampoule of osmium tetroxide (B.D.H.) was broken beneath the surface of about 100 ml of 0.2 *N* sodium hydroxide contained in a glass stoppered flask as described by Ayres and Wells (*2*). The red orange solution was washed, made up to

1 liter and standardized iodometrically by the method of Klobbie (*9*). The reported difficulty of determining the end point on account of green color of the reduced osmium was circumvented by taking iodine into benzene and titrating with sodium thiosulfate untill colorless layer of benzene was obtained. Triplicate titrations gave the same results.

The oxidation state of osmium in the solution is $8(K_2OsO_4\ (OH)_2)$ and not $6(K_2OsO_4)$ as was formerly believed (*6*).

Buffer solutions. Phthlate buffers (Clark and Lubs) of pH values varying from 5.0 to 11.0 were prepared.

Instruments. A Unicam spectrophotometer, SP 600, was used for taking absorbance. Measurements of pH were carried out on a Metrohn pH meter, type E-350.

Preliminary investigations. Addition of AQM solution in methanol to the osmium solution results in the formation of a reddish-brown color. The reaction is slow at room temperature but is accelerated on heating. However, on cooling, the complex was found to precipitate partially. The chelate could not be extracted into usual water-immiscible organic solvents. AQM dissolves readily in dimethylformamide but its solution in DMF does not exhibit any change of color with osmium solution even on heating. The presence of methanol is necessary for color development which apparently reduces osmium(VIII). In the presence of DMF, the osmium complex does not precipitate. In view of this, mixtures of methanol and DMF in different proportions were tried. It was found that 30:70 (v/v) ratio of DMF and methanol gives reproducible results.

It was also observed that in the absence of a buffer, pH of the solutions was lowered on heating. This was apparently due to hydrolysis of DMF. To overcome this difficulty, phthalate buffers have been used in subsequent studies.

Procedure for Development of Color

As a result of preliminary investigations described above, it was possible to establish certain boundry conditions for the development of color. To a 50-ml stoppered bottle (Pyrex) was added 1 ml of osmium solution, 2.0 ml of buffer of requisite pH of high ionic strength and 2 ml of a reagent solution in DMF–methanol (30:70). Total volume was made up to 10 ml by addition of methanol and the contents were heated on a water bath for about 1.5 hours under reflux for full color development.

Absorption spectra. The absorption spectra of the reagent and osmium chelate against reagent blank are recorded in Fig. 1. The complex exhibits maximum absorption at 430 nm, where there is insignifi-

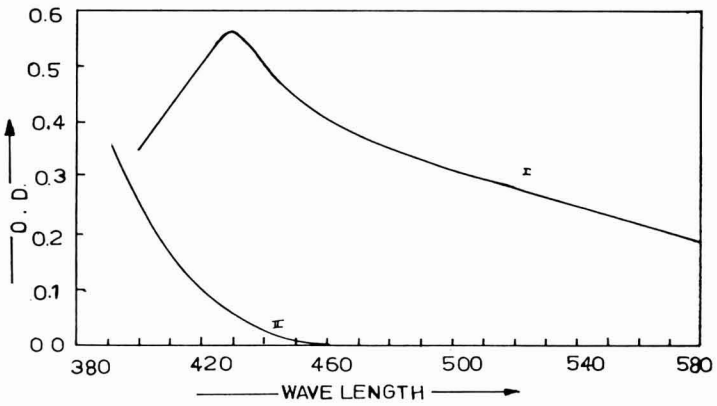

FIG. 1. Absorption spectra of reagent and its osmium complex: (I) osmium complex vs reagent blank; (II) reagent vs water, osmium, 1×10^{-4} *M*; reagent, 2×10^{-3} *M*.

cant absorption due to the reagent. Subsequent studies were carried out at this wavelength and absorbances were measured against reagent blanks.

Rate of color development. At room temperature, very little color is developed but at elevated temperatures the color devlops slowly. A study of rate of color development was made after heating the reaction mixtures for different intervals of time on a steam bath. The absorbance was found to become constant after heating for about 1.5 hours.

Effect of methanol. Keeping the percentage of DMF constant, per centage of methanol was varied to study its effect on the formation of osmium–AQM complex. A series of solutions containing 1 ml of osmium (1×10^{-3} *M*), 2 ml of buffer of pH 6.8 and 2 ml of ligand in 30:70 (v/v) DMF–methanol were prepared. Varying amounts of methanol were added to these solutions and total volume was raised to 10 ml in each case by addition of requisite amounts of water. After heating for 90 minutes, the absorbance values were recorded. Above 60% concentration of methanol, the absorbances were found to become maximum and constant.

Effect of pH. The pH dependence of the chelate was investigated by preparing a series of solutions buffered at various pH values. 1.0 ml of 1×10^{-3} *M* osmium(VIII), 2 ml of 1×10^{-2} *M* reagent solution in 30:70 DMF–CH_3OH, and 2 ml of the buffer were added to stoppered bottles. Total volume was raised to 10 ml by addition of more of methanol. The contents were heated on a water bath for 1.5 hours, cooled to room temperature and made up 10 ml again by addition of methanol. The absorbances were measured at 430 nm against reagent blanks.

A constancy in absorbance was observed between pH 6.5–8.5. Subsequent studies were made at pH 6.80.

Effect of excess reagent. The effect of excess reagent on the absorbance of osmium chelate was investigated by preparing solutions containing 1.0 ml of 1×10^{-3} *M* osmium(VIII) and varying amounts of reagent. Absorbances were measured at 430 nm, a plot of absorbance in moles of reagent revealed that maximum color develops when the molar concentration of the reagent is at least 15 times that of osmium (Fig. 2). During subsequent studies, however, 20-fold molar excess of the reagent was employed.

Adherence to Beer's Law, Sensitivity, and Stability

Samples containing different amounts of osmium and a large excess of the reagent were taken. The procedure for the development of color was followed. The plot of absorbance against varying amounts of osmium shows that the system adheres to Beer's law up to 22.80 ppm of osmium. The sensitivity of the color reaction, in terms of Sandell's definition, is 0.0323 μg of Os/cm^2 for log $I0/I = 0.001$. The optimum range for determination of osmium, as evaluated from Ringbom plot given in Fig. (3), is 3.80–22.80 ppm Measurements of absorbance of a solution after various time intervals showed no change in absorbance for 24 hours, after which the measurements were discontinued.

Recommended Procedure for Determination of Osmium

A suitable aliquot containing 38–228 μg of osmium is taken in a Pyrex stoppered bottle and 2.0 ml of (1×10^{-2} *M*) reagent solution in DMF–methanol (30:70) is added. The contents are buffered in the pH range 6.5–8.5 by addition of 2.0 ml of buffer and the total volume is made to 10 ml by addition of methanol. The mixture is heated for 1.5 hour on a water bath for full color development. After cooling to room temperature, the volume is again made to 10 ml by adding methanol. A blank containing all components except osmium is prepared under iden-

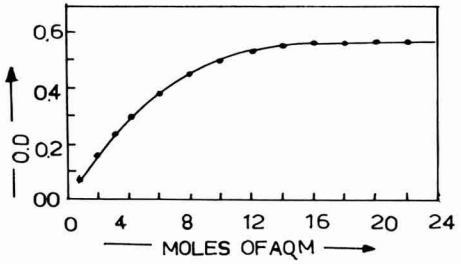

FIG. 2. Effect of ligand concentration: Os, 1×10^{-4} *M*.

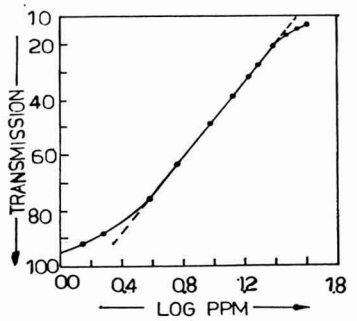

FIG. 3. Ringbom plot for Os–AQM complex.

tical conditions, and the absorbance is read against reagent blank at 430 nm.

Absorbance Deviation and Accuracy of the Method

Measured under the experimental conditions the absorbance of the solution containing 9.50 ppm of osmium gave an average deviation value = ±0.001 and maximum deviation value ±0.003. The average relative % error = ±0.37 and maximum relative % error = 1.11. Accuracy of the method for determination of osmium is found from a number of measurements, the average percentage error being 0.12.

Composition of the complex. The stochiometry of the chelate was determined by the method of continuous variations (*8*) and further confirmed by logarithmic (*5*) and Gerade method of Asmus (*1*).

In the case of method of continuous variations, solutions were prepared in different proportions keeping the total molarity constant. The curve obtained (Fig. 4) indicates the formation of a complex of osmium in which metal:ligand ratio is 1:3. With excess of metal, oxide of os-

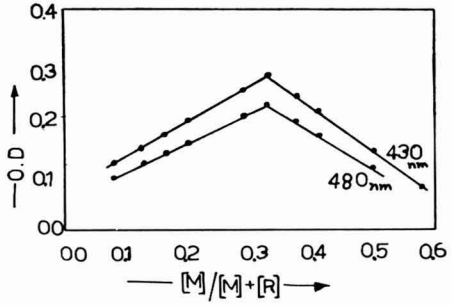

FIG. 4. Composition of Os–AQM complex by Job's method (*8*) at pH 6.8; total molarity, 4.8×10^{-4} *M*.

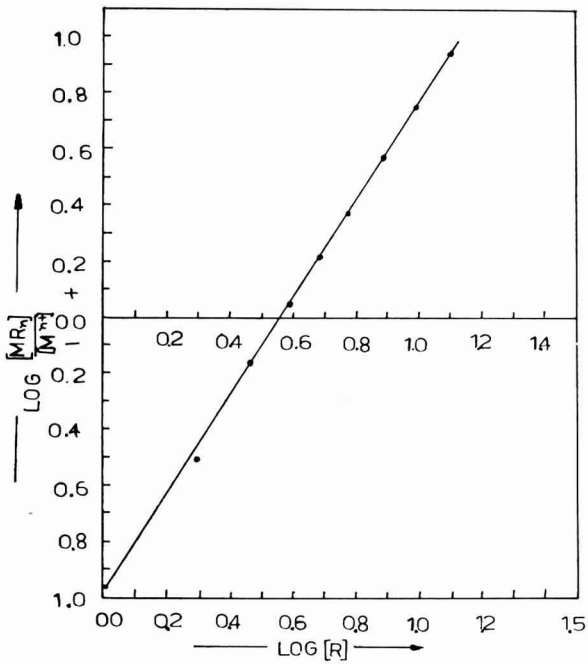

FIG. 5. Bent and French (5) method.

mium was precipitated, hence solutions containing large amount of metal were discarded. The conclusions arrived at by the logarithmic method of Bent and French (5) (Fig. 5) and Gerade method of Asmus (Fig. 6) from the study of exces reagent are in agreement with it.

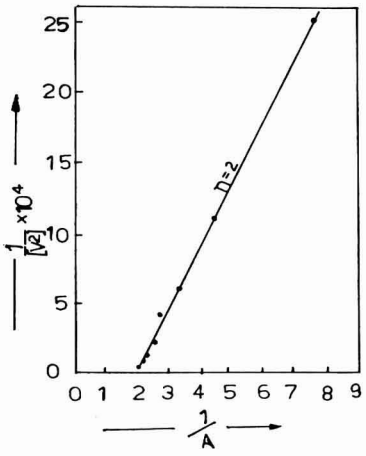

FIG. 6. Gerade method of Asmus.

Effect of diverse ions

Solutions containing known amounts of osmium (9.50 ppm) and varying amounts of diverse ions and excess of reagent were prepared. The results of the study of interfering ions and the amount which did not cause any interference are shown in Table 1. Serious interference was caused by EDTA, nitrite, iodate, bromate, and tartrate. Among the cations, iridium was found to interfere. The interference due to associated platinum metals was removed by suitable masking agents.

DISCUSSION

1,5-Diphenylcarbohydrizide has been claimed to be the most sensitive

TABLE 1

EFFECT OF FOREIGN IONS

Amount of osmium = 9.50 ppm; wavelength = 430 nm; pH = 6.80; absorbance in absence of foreign ions = 0.275.

Foreign ion	Masked by	Amount tolerated (ppm)
Acetate	—	200
Bromide	—	800
Chloride	—	400
Iodide	—	400
Nitrate	—	6200
Thiocyanate	—	700
Borate	—	300
Oxlate	—	1000
Phosphate	—	400
Pyrophosphate	—	300
Thiosulfate	—	100
Sulfate	—	400
Fluoride	—	2000
Silver(I) [a]	— [a]	3
Cobalt(II)	Thiosulfate	20
Copper(II)	Thiosulfate + iodide	10
Mercury(II)	Iodide	20
Beryllium(II)		1
Palladium(II)	Iodate	1
Rhodium(III)	Citrate	7
Ruthenium(III)	Citrate	1
Platinum(IV)	Iodide	2
Iron(III)	Fluoride	5
Antimony(III)	Iodide	4
Thorium(IV)	Fluoride	10
Molybdenum(VI)	Thiocyanate	80
Uranium(VI)	Oxalate	4

[a] Removed by precipitation as chloride.

reagent for osmium. The sensitivity being 0.00127 μg of Os/cm^2, but commonly associated platinum metals interfere. Tetraphenyl arsonium chloride has been used for osmium and extractive study has been done to minimize the interferences. The drawback with this method is that the partition coefficient is not favorable; and complete recovery is only accomplished by a number of extractions. Thiourea remains one of the most widely used reagents for osmium. The drawback with this method is that weight of thiourea required for a fixed amount of osmium depends on the volume of the solution. Anthranilic acid, *o*-amino-*p*-sulfonic acid and *m*-aminobenzoic acid have been used for spectrophotometric determination of osmium. The sensitivities of various methods are 0.008, 0.012, and 0.012 μg/cm^2, respectively. Quinisatin oxime has been suggested for determination of osmium spectrophotometrically. The method is simple and it suffers selectivity. In the case of AQM, the principal advantage is that platinum metal can be masked by the use of suitable masking agents up to certain limits. The sensitivity, however is less as compared to other reagents. The method is simple and color is stable for more than 24 hours.

SUMMARY

A water-soluble dark-brown complex formed by osmium with acenaphthenequinonemonoxime exhibits an absorption maximum at 430 nm. The composition of the complex comes out to 1:2 ((metal:ligand) as revealed by Job's method of continuous variations, logarithmic method, and Gerade method of Asmus. The pH range for complete formation of the complex is 6.5–8.5. Its sensitvitiy has been found to be 0.0323 μg/cm^2. The effect of diverse ions has also been investigated.

ACKNOWLEDGMENT

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The Spectrophotometric Determination of Molybdenum and Uranium with Quinalizarin

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In a search for sensitive spectrophotometric reagents for the determination of small amounts of hexavalent metals, quinalizarin was tried. Recently Srivastava (6) has reported the analytical application of quinalizarin for the determination of tungsten. Present communication deals with the use of the quinalizarin for spectrophotometric determination of very small quantities of molybdenum and uranium. The method is based on the highly sensitive color reaction of quinalizarin with molybdenum and uranium.

EXPERIMENTAL METHODS

Spectrophotometric measurements were made with a Hitachi Perkin-Elmer Uv-Vis spectrophotometer and pH was measured with a Beckman (H2) pH meter.

BDH reagent grade quinalizarin, after purification, was used for preparing the standard solution of quinalizarin in absolute alcohol. Sodium molybdate and uranyl nitrate (BDH AnalaR) were used in preparing the standard solutions of molybdenum and uranium. All other reagents were of analytical grade. pH was adjusted with dilute hydrochloric acid and dilute sodium hydroxide.

Standard Procedure

An aliquot containing small quantities of molybdenum and uranium was added to more than 5-fold excess of quinalizarian solution in a 25-ml volumetric flask. The pH of the molybdenum solution was adjusted to 5.0 $\pm$ 0.1; and of uranium, to 5.5 $\pm$ 0.1. The volume was made up to 25 ml; keeping volume of alcohol, 12.5 ml. The mixtures were allowed to stand for 30 minutes for equilibration and the absorbances were measured at 540 nm for molybdenum and at 630 nm for uranium against a reagent blank. The concentrations of the unknown

solutions were obtained by comparing the absorbance values with a calibration curve.

RESULTS AND DISCUSSION

Nature of the complexes formed

As reported earlier, composition of quinalizarin chelates of molybdenum and uranium (2, 3) was determined to be 1:1 (metal:reagent) in both the cases. The λ_{max} of molybdenum was found in the spectral region of 520 nm whereas the λ_{max} of uranium chelate was found at 630 nm.

Effect of pH

A series of solutions of each of the metal ions was prepared with an excess of quinalizarin solution at different pH values. The optimum pH values for these complexes for spectrophotometric determination was determined by plotting the absorbance of the complexes against different pH values at 540 nm in case of molybdenum and at 630 nm in case of uraninum. The pH range for the molybdenum quinalizarin chelate was found between 3.0–6.5; whereas for uranium quinalizarin chelate was between 4.0–6.5. Suitable pH for the determination of molybdenum was 5.0 $\pm$ 01; and for uranium, 5.5 $\pm$ 0.1.

Effect of Time and Temperature

The color of the molybdenum complex develops instantaneously and remains constant for an appreciable time; whereas that of uranium complex takes 15 minutes to attain maximum color development and then absorbance remains constant for at least 3 hours. Temperature has an effect on the absorbance; with an increase in temperature, the absorbance of molybdenum complex decreases while that of uranium complex increases.

Effect of Reagent Concentration

The absorbance of different mixtures of metal solutions with varying ratios of the excess of quinalizarin at 540 nm and pH 5.0 $\pm$ 0.1 for molybdenum chelate and at 630 nm and pH 5.5 $\pm$ 0.1, for uranium complex, show that the maximum color formation was attained only when mixtures contain more than 5-fold excess of the reagent with respect to the metal solutions.

CALIBRATION CURVE, SENSITIVITY, AND PRECISION

Calibration curves for the determination of molybdenum and uranium were prepared by the standard procedures. The experimental find-

TABLE 1

SUMMARY OF THE EXPERIMENTAL FINDINGS FOR EFFECTIVE PHOTOMETRIC DETERMINATION OF MOLYBDENUM AND URANIUM

Metal ion	Wavelength (nm)	Range (ppm) for: Adherence to Beer's law	Range (ppm) for: Effective photometric determination	Molar extinction coefficient	Sensitivity (Sandell's) ($\mu g/cm^2$)
Mo	540	1–10	1.6–9.5	12400	0.0077
U	630	3.5–21	4–20	9800	0.0242

ings for the effective spectrophotometric determination of metal ions are listed in Table 1.

Figure 1 shows calibration curve for molybdenum and uranium. A standard solution containing 5 ppm of molybdenum and 10 ppm of uranium was analyzed by the standard procedure and the results were within $\pm 0.5\%$.

Effect of Diverse Ions

The effect of diverse ions on the determination of molybdenum and uranium was examined under the conditions of the standard procedure. The tolerance limit was taken as the amounts that caused absorbance error not exceeding 3%. It was seen that Be(II), Pb(II), Cu(II), Mg(II), Al(III), Ga(III), In(III), Tl(III, I), La(III), Th(IV), Zr(IV), Ti(IV), V(V), W(VI), Cr(VI), rare earths, and uranium in case of molybdenum; and molybdenum in case of uranium interfere seriously when present even in traces.

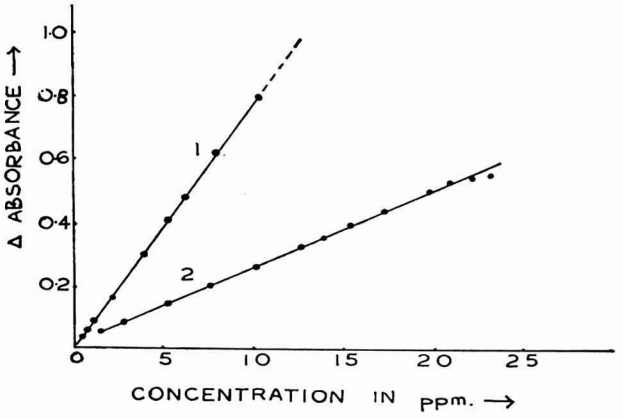

FIG. 1. Calibration curve for (1) molybdenum; (2) uranium.

Anions such as CI $-$, NO_3 $-$, SO_4^{2} $-$, do not interfere. Borate, tartrate, acetate, and citrate interfere considerably, Fluoride, oxalate, NTA, and EDTA bleach the complex. These interfering ions must be removed or masked before the determination in the usual way (1, 4, 5).

Several sensitive organic reagents have been used for the determination of molybdenum and uranium. The proposed method is simple, rapid, and sensitive but not very selective.

SUMMARY

Quinalizarin reacts very sensitively with molybdenum and uranium to form colored chelates having λ_{max} at 520 and 630 nm, respectively. The molar ratio for both the chelates is 1:1 (metal:reagent). Optimum conditions including the range for adherence to Beer's law, effect of pH on color intensity, effect of excess of the reagent, sensitvity, and interference of the foreign ions has been reported for the photometric determination of these metal ions using quinalizarin, in 50% ethanolic medium and at 30°C.

ACKNOWLEDGMENT

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MICROCHEMICAL JOURNAL 18, 640–645 (1973)

Extractive Photometric Determination of Palladium with o-Mercaptobenzoic Acid

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INTRODUCTION

Several reagents containing mercapto group have been used for the spectrophotometric determination of palladium (*3, 4*). These include the useful reagents such as 1-mercaptopropionic-*p*-aniside and the corresponding *p*-toluidide (*5*), 8-mercaptoquinoline (*2*), 2-mercaptobenzimidazole and 2-mercaptobenzothiazole (*16*), quinoxaline-2,3-dithiol (*1*), thiosalicylic acid (*10*), phenythiosemicarbazide (*15*), dibenzyldithioxamide (*17*), didodecyl derivatives of dithioxamide (*12*), *N,N*-bis(3-dimethylaminopropyl)-dithioxamide (*13*), thiomalic acid (*18*), bis-(allylthiocarbamyl) hydrazine (*11*), thioanilide (*6*), thioglycollic acid (*19*), β-mercaptohydrocinnamic acid (*7*), β-mercapto-β-phenylpropiophenone (*8*), and 4-4-bis(dimethylamino) thiobenzophenone (*9*). These methods, however, are not selective for the spectrophotometric determination of palladium. This paper reports a simple and rapid method for the selective determination of palladium (*11*) with *o*-mercaptobenzoic acid. The reagent forms a yellow complex with palladium (*11*) in pyridine at pH 5.2–7.2, having maximum absorption at 365–370 mμ. The formation and extraction of the latter complex at pH 5.2–7.2 with chloroform is rapid; and this makes for easy removal of the interferences of most of the base metals and the associated platinum metals, platinum(II, IV), rhodium(III), iridium(IV) and osmium(IV, VI) in the presence of ascorbic acid and EDTA. Gold(III) and silver are masked with thiocyanate before the addition of ascorbic acid and EDTA. Most of the anions do not interfere. The complex is stable at room temperature; and the sensitivity of the proposed method is comparable to most of the methods reported in the literature (*3, 4*).

EXPERIMENTAL METHODS

Reagents

All the materials used were of analytical reagent grade. Twice-distilled water was used throughout.

o-Mercaptobenzoic acid (Fluka); a 2% (w/v) solution was prepared by dissolving it in redistilled pyridine and it was stored in the refrigerator.

Stock aqueous solution of palladium(II) was prepared by dissolving about 1 g of palladium(II) chloride in 1 liter of distilled water containing sufficient hydrochloric acid to give a final concentration 1 *M* in the acid. The palladium content was determined gravimetrically to be 0.5858 mg/ml by precipitation of palladium dimethylglyoximate from homogenous solution (*14*). More dilute solutions were prepared from this standard.

EDTA solution. A 0.1 *M* solution was prepared by dissolving the required amount of disodium salt of EDTA in water.

Solvents. B.D.H. pure chloroform boiling in the range of 60–62°C was used. Pyridine (B.D.H.) was redistilled and the fraction (bp 114–116°C) was used.

Apparatus

Absorbance measurements were made with Bausch and Lomb spectroconic 20. A Beckmann pocket pH meter, model 180 was used for pH measurements. For general purpose, the narrow-range indicator papers (B.D.H. Ltd;) were used to adjust the pH.

Procedure

To an aliquot of weakly acidic solution containing 3.7 to 58.6 μg of palladium(II) in a 50-ml beaker, add sufficient ascorbic acid and 5 ml of 0.1 *M* EDTA to obviate the interferences of diverse ions. Then add 1 ml of the reagent and adjust the pH to 5.2 to 7.2 with dilute hydrochloric acid and pyridine. The volume of the solution is adjusted to about 10 ml with water. Transfer the colored product into a 50-ml separatory funnel and shake with 9 ml of chloroform for 1 minute. Allow the layers to separate and withdraw the organic phase in a 10-ml volumetric flask and dilute to the mark with chloroform. Measure the absorbance at 365–370 mμ against the reagent blank. Determine the palladium concentration from a previously prepared calibrating graph.

RESULTS AND DISCUSSION

Formation, Stability, and Extractability of the Complex

The reagent forms a yellow precipitate with palladium(II) at pH 2–4. The precipitate is insoluble in water, sparingly soluble in acetone and highly soluble in pyridine. The yellow complex in acetone medium is not extractable with chloroform. However, in pyridine medium, the yellow complex is readily extracted with chloroform, having maximum absorption at 365–370 mμ. The complex is very stable at room tempera-

ture. In the optimum pH range, a single extraction with 5 to 10 ml of chloroform leads to quantitative recovery of the complex.

Effect of pH

Quantitative recovery of palladium from the aqueous phase is achieved in the pH range from 5.2 to 7.2. Below pH 5.2, the complex separates out as a yellow precipitate. However, beyond pH 7.2, the extraction is incomplete.

Calibration, Range, and Sensitivity

The Palladium(II)-*o*-Mercaptobenzoic acid system conforms to Beer's law over the range 0.37–5.86 ppm of palladium. The molar absorptivity at 365 mμ is 16.7×10^3 and the Sandell sensitivity is 0.0065 $\mu g/cm^2$. The sensitivity of this method is comparable to those of many methods for palladium (*3,4*).

Stability and Effect of Reagent Concentration

The reagent solution in pyridine was found to be stable if it was stored in a refrigerator. One ml of 2% reagent in pyridine was sufficient for the full development of color. A large excess of reagent had no deleterious effect on the system.

Effect of Diverse Ions

Several ions were added to the system, 2.929 ppm of palladium, to study interferences. Most of the cations did not interfere in the presence of ascorbic acid and EDTA. There was no interference from the associated platinum metals, platinum(II, IV), rhodium(III), iridium(IV), and osmium(IV) in proportions of 1000:1. Cobalt(II), chromium(III, VI), nickel, manganese(II), copper(I, II), vanadium(V), molybdenum(VI), tungsten(VI), iron(II, III), bismuth(III), thorium(IV), thallium(I), lead(II), mercury(II), aluminum(III), arsenic(III), cadmium, zinc, and tin(II) did not interfere in milligram quantities. Gold and silver were effectively masked with excess of potassium thiocyanate prior to the addition of ascorbic acid and EDTA. Hydroxylamine hydrochloride can also be used in lieu of ascorbic acid. However, chromium(III) interferes forming an incomplete blue-violet complex with EDTA at room temperature in the presence of pyridine at pH 6.1–6.9. This interference was eliminated by adding excess of hydroxylamine hydrochloride and EDTA to the weakly acidic solution of palladium. The pH of the solution was then adjusted to 6.1–6.9 with 1–2 ml of pyridine, and the solution was swirled for 2 minutes. This was followed

by the addition of 1 ml of 2% *o*-mercaptobenzoic acid solution; and the palladium complex was extracted as outlined in the Procedure. The chromium–EDTA complex was not extracted with chloroform. Pyridine accelerates the formation of chromium(III)–EDTA complex at room temperature. Ascorbic acid, however, masks chromium, in the presence of EDTA. Ruthenium(III) interferes seriously and should be removed. The interference of ruthenium could not be studied due to its anamolous behavior in aqueous solutions. When kept for some time, the intensity of the color of ruthenium solution increased gradually, which subsequently made study of its interference difficult. Uranium(VI) forms a deep red complex even in the presence of ascorbic acid and EDTA. But the complex was not extracted with chloroform. Excess of reagent should be added in the presence of uranium(VI). Most of the common anions such as phosphate, sulfate, nitrate, oxalate, tartarate, citrate, thiocyanate, and arsenate did not interfere. Excess of ascorbic acid did not cause any interference. Five ml of 0.1 *M* EDTA did not interfere.

Composition of the Complex

The yellow-red precipitate was obtained by treatment of concentrated solution of palladium(II) chloride at pH 3.0 with excess of the reagent. The precipitate was filtered, and washed repeatedly with ether to remove the excess of reagent, and dried at 100°C. The precipitate was highly soluble in pyridine. Exactly 9.5 mg of the complex was dissolved in aqua regia, evaporated almost to dryness, and the mass was taken up with 10 ml of 1:1 hydrochloric acid. The evaporation and hydrochloric acid treatment was repeated three times to remove all the nitric acid. Finally, the residue was taken up with 10 ml of 1 *N* hydrochloric acid and the solution was diluted to 50 ml with water. To the neutralized solution, was added 5 ml of 0.1 *N* HCl, 10 ml of 2.5% potassium tetracyanonickelate solution, and 10 ml of ammonium chloride–ammonium hydroxide buffer of pH 10.0. Then was added excess of 0.01 *M* EDTA and 0.1 g of ascorbic acid. The excess of EDTA was back titrated with 0.01 *M* manganese(II) sulfate solution using Eriochrome black T indicator until the blue color changed to red. The percentage of palladium was found to be 25.51 as against theoretical value of 25.77 in 1:2 complex of palladium with *o*-mercaptobenzoic acid.

Further work is in progress on the spectrophotometric determination of uranium(VI) and rhodium(III) with *o*-mercaptobenzoic acid. Uranium(VI) forms a deep red complex with this reagent and is extractable with chloroform in the presence of *sym*-diphenylguanidine. Rhodium (III) forms a yellow complex in acidic medium at 100°C and is extractable with *n*-butanol.

SUMMARY

A procedure is described for the extractive photometric determination of palladium(II) with *o*-mercaptobenzoic acid. The reagent forms a yellow complex having maximum absorption at 365–370 mμ. The complex is quantitatively extractable with chloroform in the presence of pyridine at pH 5.2–7.2. The color develops immediately at room temperature and is very stable. Beer's law conforms over the range of 0.37–5.86 ppm of palladium. Most of the cations do not interfere in the presence of ascorbic acid and EDTA. Gold and silver are effectively masked with excess of thiocyanate prior to the addition of ascorbic acid and EDTA. Many common anions do not interfere. The molar absorptivity and Sandell sensitivity are 16.7×10^3 and 0.0065 $\mu g/cm^2$. The reagent forms a 2:1 complex with palladium. The proposed method is simple, rapid, and selective for the determination of palladium(II).

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Determination of Stability Constants of Some Bivalent Metal Complexes with Thiovioluric and Diphenylthiovioluric Acids

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Considerable amount of work has been carried out on thiovioluric acid diphenylthiovioluric acid as analytical reagents. Recently, thiovioluric acid has been utilized for the spectrophotometric determination of cobalt (*1*). However, no work has been reported on the determination of the stability constants of metal complexes with thiovioluric and diphenylthiovioluric acids. In the present study, the stability constants of some bivalent metal complexes with these ligands have been determined in aqueous dioxan medium, potentiometrically.

EXPERIMENTAL METHODS

Thiovioluric acid was synthesized by the method of Lal and Dutt (*4*) and purified by repeated crystallization from absolute alcohol (mp 210°, with decomposition), while diphenylthiovioluric acid was prepared by the method of Das and Dutt (2) and purified by repeated crystallization from glacial acetic acid when diphenylthiovioluric acid gets separated in the form of needles of mp 245°C.

A Metrohm pH meter, model E-350, with glass electrode EA 107 Ux (0–14 pH), was used for pH measurements.

Solutions of bivalent metal ions, *viz,* Cu(II), Ni(II) and Pb(II) were prepared by dissolving the corresponding nitrates, while those of Zn(II), Mn(II), and Mg(II) were prepared from the corresponding sulfates by dissolving analytical grade samples in double-distilled water and were standardized by gravimetric methods, wherever necessary.

Dioxan (AnalaR, B.D.H.) was freed from peroxide by refluxing it with sodium metal for 24 hours and was distilled over sodium before use.

Sodium perchlorate (Riedel) was used to keep ionic strength constant (0.1 *M*). Tetramethylammonium hydroxide (E. Merck, A.G., Darmstadt) was used as titrant. All measurements were carried out at 30 −

1°C. Presaturated nitrogen (with 50 or 75% aqueous dioxan) was passed through the solutions during titrations.

The following solutions were titrated potentiometrically against standard tetramethylammonium hydroxide in 50 and 75% aqueous dioxan medium, respectively, for thiovioluric and diphenylthiovioluric acids to determine n and pL values of bivalent metal complexes:

For Thiovioluric Acid

1. 1.0 ml of $HClO_4$ (0.02 *M*) + 1.0 ml of $NaClO_4$ (2.0 *M*) + 8 ml of H_2O + 10 ml of dioxan.
2. 1.0 ml of $HClO_4$ (0.02 *M*) + 1.0 ml of $NaClO_4$ (2.0 *M*) + 8 ml of H_2O + 10 ml of ligand (0.01 *M*) in dioxan.
3. 1.0 ml of $HClO_4$ (0.02 *M*) + 1.0 ml of $NaClO_4$ (2.0 *M*) + 0.5 ml of metal nitrate or sulfate (0.02 *M*) + 7.5 ml of H_2O + 10 ml of ligand (0.01 *M*) in dioxan.

For Diphenylthiovioluric Acid

1. 0.8 ml of $HClO_4$ (0.02 *M*) + 1.0 ml of $NaClO_4$ (2.0 *M*) + 3.20 ml of H_2O + 15 ml of dioxan.
2. 0.8 ml of $HClO_4$ (0.02 *M*) + 1.0 ml of $NaClO_4$ (2.0 *M*) + 3.20 ml of H_2O + 5 ml of ligand (0.01 *M*) in dioxan + 10 ml of dioxan.
3. 0.8 ml of $HClO_4$ (0.02 *M*) + 1.0 ml of $NaClO_4$ (2.0 *M*) + 0.25 ml of metal nitrate or sulfate (0.02 *M*) + 2.95 ml of H_2O + 5 ml of ligand (0.01 *M*) in dioxan + 10 ml of dioxan.

The experimental method Bjerrum-Calvin as modified by Irving and Rossotti (*3*) has been used to determine the values of $\bar{n}$ and pL. In all cases, correction for change in volume on mixing dioxan and aqueous solutions, as well as for changes in volume, which take place during the course of titrations, have been made.

Calculation of Stability Constants

In all the ligands used for the study, it is the grouping –C(NOH)–CO– which takes part in complex formation and the protons are replaced from it by metal ions during complexation reaction. Since only one proton per ligand molecule is liberated on complexation, $Y = 1$ in all the cases.

From the titration curves of acid alone and in the presence of ligand, $\bar{n}$H values of the ligands at various pH values were calculated and the pK_a values of the ligands were found by plotting log $[\bar{n}H/(1 - \bar{n}H)]$ vs pH, when a straight line of intercept equal to pK_a and slope equal to 1 was obtained (Fig. 1). These are the practical pK_a values of thiovioluric acid (pK_a 4.93) in 50% aqueous dioxan and diphenyl-

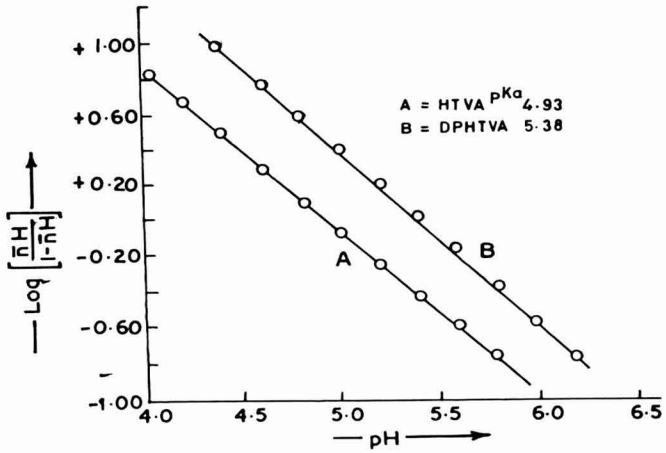

FIG. 1. Determination of dissociation constants of thiovioluric and diphenylthiovioluric acids.

thiovioluric acid (pK_a 5.38) in 75% aqueous dioxan medium which have been used in the calculations of stability constants.

From the data so obtained, $\bar{n}$ values of the metal complexes were determined at various pH values. From a knowledge of practical pK_a was then calculated. The $\bar{n}$ values are plotted against the corresponding value and $\bar{n}$ value at a particular pH, the corresponding value of pL pL values to give the formation curves of the metal complexation equilibria (Figs. 2 and 3). In certain cases $\bar{n}$ values greater than one were not obtained due to hydrolysis. The values of log K_1 and log K_2 were calculated by using the "correction term method" of Irving and Rossotti (*3*). The values of stability constants are tabulated in Table 1.

DISCUSSION

The order of stability of complexes with metals of the first transition series has been found to be the same in the case of all the ligands. The order is

$$\text{Mn} < \text{Ni} < \text{Cu} > \text{Zn},$$

which is identical with the order found by Irving and Rossotti. The stability of lead complexes has been found to be intermediate between Mn(II) while magnesium forms the least stable complexes.

The acid dissociation constants (pK_a) of the ligands decrease in the order:

diphenylthiovioluric acid > thiovioluric acid.

The pK_a value of thiovioluric acid has been determined in 50%

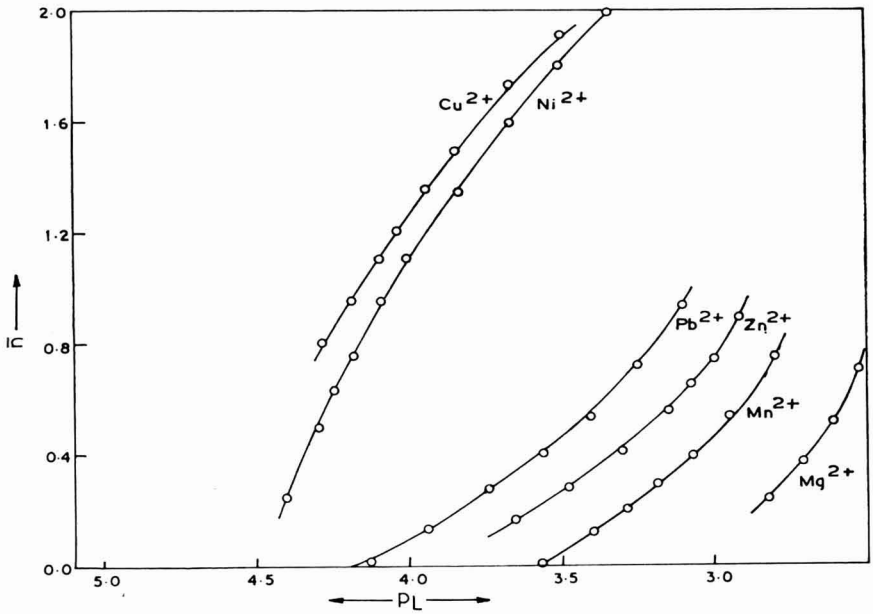

FIG. 2. Formation curves of bivalent metal complexes with thiovioluric acid.

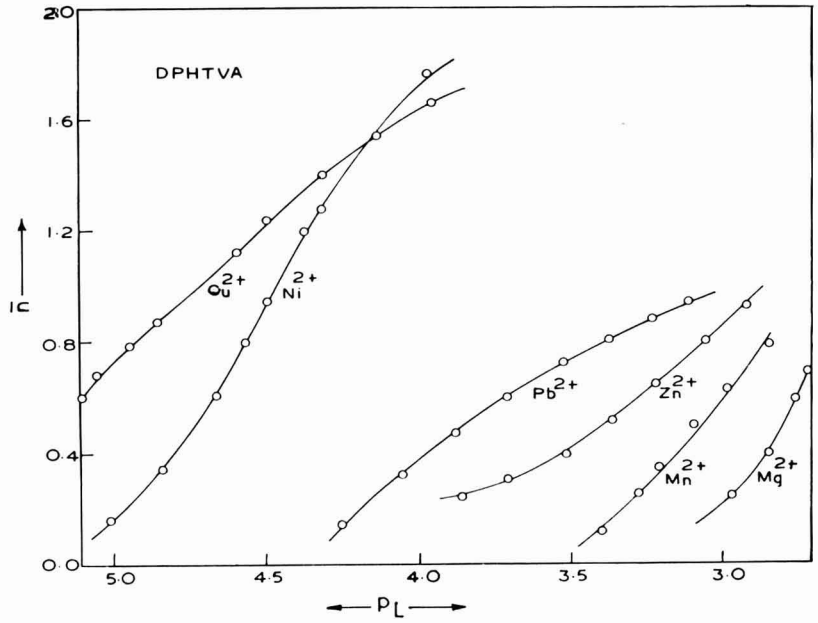

FIG. 3. Formation curves of bivalent metal complexes with diphenylthiovioluric acid.

dioxan medium while that of diphenylthiovioluric acid was in 75% dioxan medium. The pK_a value of diphenylthiovioluric acid in 50% dioxan has been reported in the literature. Even with that value (5.00), the order remains the same. The increase in pK_a value by substitution by phenyl groups may be explained in the following way:

In thiovioluric acid, carbonyl groups being electron attracting, are expected to pull electrons from the lone pair on the nitrogen atom. This would lead to decrease in pK_a value of the oxime group. However, in diphenylthiovioluric acid, two phenyl groups have been introduced. The carbonyl groups would then also exert a pull on the electrons of the two benzene rings and thus the pull on the lone pair of electrons on the nitrogen atom of the oxime group would be diminished, resulting in an increase in its pK_a value.

Since in DPHTVA, the two imino groups are blocked by phenyl groups, it is concluded that reacting groups for complexation is –(NOH)–CO– in both the ligands as it is the only bidentate functional grouping common to both and imino group does not take part in complexation. Similar observations have been made by Leermaker and Hoffmann (*5*) in the case of violuric acid and dimethylvioluric acid.

In the case of nickel complexes, it is observed that $\log K_2 > \log K_1$. This is is probably due to the formation of square planar 1:2 complex of nickel with these ligands which stabilizes the 1:2 complex as compared to 1:1 complex. The stabilities of complexes formed by diphenylthiovioluric acid are slightly higher than those of thiovioluric acid. This is attributed to the more basic nature of the ligand. However, this cannot be said with certainity since stability constants have been determined in different media (50 and 75% dioxan).

TABLE 1

STABILITY CONSTANTS OF BIVALENT METAL COMPLEXES WITH HTVA AND DPHTVA [a]

	HTVA (50% dioxan)			DPHTVA (75% dioxan)		
Metal ion	$\log K_1$	$\log K_2$	$\log \beta_2$	$\log K_1$	$\log K_2$	$\log \beta_2$
H	4.93	—	—	5.38	—	—
Cu(II)	4.44	3.91	8.35	5.13	4.30	9.43
Ni(II)	3.82	4.30	8.12	3.80	5.10	8.90
Pb(II)	3.44	—	—	3.84	—	—
Zn(II)	3.21	—	—	3.37	—	—
Mn(II)	2.93	—	—	3.09	—	—
Mg(II)	2.63	—	—	2.79	—	—

[a] HTVA = thiovioluric acid; DPHTVA = diphenylthiovioluric acid.

SUMMARY

Stability constants of complexes of some bivalent metal ions, viz, Cu(II), Ni(II), Zn(II), Pb(II) and Mg(II) with thiovioluric and diphenylthiovioluric acid have been determined potentiometrically in 50 and 75% dioxan media, respectively. The order of stability constants for the complexes investigated has been found to be: Mn < Ni < Cu > Zn. The stability of lead complexes has been found to be intermediate between Mn(II) and Ni(II) while magnesium forms the least stable complexes.

ACKNOWLEDGMENT

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VI. Direct Titrimetric Microdetermination of L-Serine and L-Lysine

VII. Simultaneous Stepwise Microdetermination of Combinations of Amino Acids without Separating

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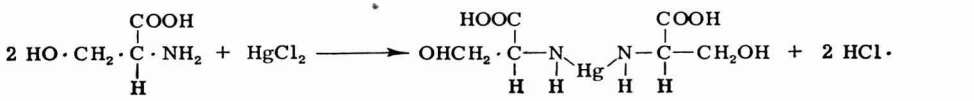
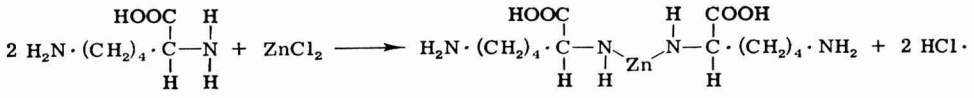
L-Serine and L-lysine have been determined separately by using various techniques but no technique could help in developing a quantitative method for the determination of amino acids in different combinations in one solution without separating. As such L-serine has been determined separately by: photometric method (*4, 12*); chromoconductometrically (*6*); using the dichromate heat of dilution method (9); the influence of cations on the Ninhydrin reaction (*10*) and chemically (*15*). L-Lysine has been determined chromatographically (*7, 8, 11, 13, 14*); and by iodometric titrations of their copper salts (*16*); and by direct titration against platinic chloride (*17*).

The present work was planned to estimate L-serine and L-lysine separately and in the presence of each other by direct titrations, without separating when present in the form of a mixture. L-Serine and L-lysine have been separately determined, quantitatively, by direct titration against mercuric chloride and zinc chloride solutions, respectively, using congo red solution as indicator. Potentiometric titrations show that in both cases a 1:2 complex is formed. In the form of mixture, L-serine and L-lysine were titrated in a particular order, using xylenol orange and congo red solutions as indicators. Probably the following reaction takes place:



L-Serine



L-Lysine

Besides this, titrations in combinations of L-serine–L-lysine, L-serine–L-histidine, L-lysine–L-histidine, L-lysine–L-arginine, L-histidine–L-serine-L-lysine, and L-histidine-L-lysine-L-arginine have also been done.

EXPERIMENTAL METHODS

Reagents used. L-Serine, L-lysine, L-histidine, xylenol orange, and congo red (B.D.H. grade); L-arginine and zinc chloride (E. Merck grade); lead nitrate, ferrous ammonium sulfate, mercuric chloride, and sulfuric acid (AnalaR, B.D.H. grade).

Apparatus used: Micropipettes and microburettes used had least count = 0.01 ml. Pyrex glass beakers with 100-ml capacity were used.

Standard L-serine, L-lysine, L-histidine, L-arginine, lead nitrate, mercuric chloride solutions were prepared by dissolving exactly weighed amount in distilled water.

Ferrous ammonium sulfate solution was prepared by dissolving an exactly weighed amount in 0.01 N H_2SO_4.

Zinc chloride solution was prepared by dissolving in distilled water and further standardized (*18*).

0.1 g of congo red and xylenol orange were dissolved in 100 ml of distilled water, and thus the two indicator solutions were prepared.

PROCEDURE

I. Direct Determination Of L-*Serine Separately.*

A known volume of standard L-serine solution was placed in a beaker to which 10 ml distilled water were added. Indicator congo red solution was added in drops (2–3) and the entire solution was shaken well. Standard mercuric chloride solution was run in to the beaker containing L-serine solution (through a microburette) until the red solution changed sharply to pink violet at the end point.

II. Direct Determination Of L-*Lysine Separately*

In a beaker was placed a known volume of standard L-lysine solution (through a micropipette) and diluted by adding 20 ml of distilled water. Few drops (2–4) of congo red solution were added to the L-lysine solution, which became red. From a microburette, standard zinc chloride solution was run into the beaker, containing L-lysine solution and the indicator, until the red changed sharply to violet at the end point.

III. Direct Determination Of L-*Serine and* L-*Lysine Together Without Separation*

Known volumes of standard L-serine and L-lysine solutions were placed in a beaker, through micropipettes, to which 20 ml of distilled

water were added, which gave a mixture of the two amino acids. Few drops (2–3) of xylenol orange solution were added to the solution mixture, which was colored pink. Standard mercuric chloride solution was run into through a microburette, the beaker until the pink color changed sharply to light rose at the end point. Thus, L-serine was first titrated in presence of L-lysine without separation.

In order to titrate L-lysine in the same solution mixture, after titrating L-serine, few drops (2–4) of congo red solution were added, which turned the light rose solution to red. Standard zinc chloride solution was added, in drops, until the red color changed sharply to violet.

It is important to note that in titrations of mixture, if the order is changed, then none of the amino acids would be titrable.

IV. Direct Titration of L-*Histidine and* L-*Serine in One Solution Without Separation*

L-Histidine and L-serine solutions were placed in a beaker with 20 ml of distilled water to form a mixture, to which few drops (1–3) of xylenol orange solution were added, and the solution became yellowish rose in color. At the first instant, L-histidine was titrated against standard lead nitrate solution until the appearance of pink color at the end point.

After titrating L-histidine, no further indicator solution was used, and in the same mixture, L-serine was titrated against standard mercuric chloride solution until the pink color changed sharply to light yellow at the end point.

V. Direct Determination of L-*Histidine and* L-*Lysine in One Solution Without Separation*

Known volumes of L-histidine and L-lysine solutions were placed in a beaker to which 20 ml of distilled water were added (forming the mixture) and few drops (2–4) of xylenol orange solution were added. L-Histidine was first titrated, as already mentioned, to the sharp appearance of pink color at the end point. In the same solution L-lysine was titrated next by adding a few drops of congo red solution; titration was then carried out, as previously mentioned, to a sharp change in color from red to violet.

VI. Direct Determination of L-*Arginine and* L-*Lysine Together in One Solution*

Different volumes of standard L-argnine and L-lysine solutions were placed in a beaker to which, 20–25 ml of distilled water were added. In

such mixture, L-arginine was first titrated against ferrous ammonium sulfate solution, by first adding few drops of congo red solution as indicator, to a sharp change in color from red to orange yellow at the end point. L-lysine was titrated in the second instance in the same mixture (using xylenol orange as indicator, as already mentioned) to a sharp change in color from rose red to light orange violet at the end point.

VII. Direct Determination of L-*histidine,* L-*serine, and,* L-*Lysine in One Solution Without Separation*

Known volumes of standard L-histidine, L-serine, and L-lysine solutions were placed in a beaker, 20–25 ml of distilled water were added, which formed the solution mixture; and a few drops of xylenol orange were added and the solution mixture became light yellow. In the first instance L-histidine was titrated against standard lead nitrate to rose or pink color at the end point (depending upon the concentration of L-histidine).

In the same solution, L-serine was titrated, without adding any further indicator, against standard mercuric chloride solution to a sharp yellow color at the end point.

After titrating L-histidine and L-serine, L-lysine was titrated last in the same mixture by adding a few drops of congo red solution, which colored the solution violet-red. Standard zinc chloride solution was run into the beaker until violet color appeared sharply at the end point.

VIII. Direct Determination of L-*Histidine,* L-*Arginine in One Solution Without Separation*

Known volumes of standard L-histidine, L-lysine, and L-arginine solutions were placed in a beaker (through micropipettes) to which 30 ml of distilled water were added (forming a mixture of the three amino acids). In this mixture, L-histidine was first titrated (by adding few drops (2–3) of congo red solution, which colored the solution red) against lead nitrate solution until orange yellow color appeared sharply at the end point.

In the same mixture, L-lysine was titrated against zinc chloride solution by adding few drops of xylenol orange solution. The rose colored solution turned pink rose or purple violet (depending on the concentration of L-lysine) at the end point.

After titrating L-histidine and L-lysine, L-arginine was titrated in the same solution mixture by adding few drops of congo red solution, when the solution mixture became pink red. Standard ferrous ammonium sulfate solution was run in until orange red color appeared sharply at the end point.

TABLE 1

MICRODETERMINATION OF L-SERINE

L-Serine, 0.0398 *M* (ml)	$HgCl_2$, 0.01 *M* (ml)	Amount of L-serine ($\times 10^2$ mg)		Error (%)
		Taken	Found	
0.1	0.2	41.82	42.03	
0.3	0.6	125.46	126.10	
0.4	0.8	167.28	168.14	0.5
0.5	1.0	209.10	210.18	

Thus, it was possible to titrate three amino acids in one solution without separation.

RESULTS AND DISCUSSION

Results are given in Tables 1 to 8 L-Serine and L-lysine were estimated in ranges of 42.03×10^{-2} to 210.18×10^{-2} mg; and 59.06×10^{-2} to 147.65×10^{-2} mg, respectively.

Tables 1 and 2 show that L-serine and L-lysine have been determined separately against mercuric and zinc chlorides, respectively, using congo red solution as indicator. Since the complexes are formed beetween mercuric chloride–L-serine and zinc chloride–L-lysine in the ratio of 1:2, in the calculations the observed experimental values were multiplied by 2. Maximum error in the cases of L-serine and L-lysine was 0.5 and 0.9% respectively.

In Table 3 L-serine and L-lysine have been titrated together in one solution without separation. L-Serine and L-lysine have been estimated in the descending and ascending order from the top. It is peculiar with these titrations that L-serine is titrated first using xylenol orange solution as indicator and then, in the same solution, L-lysine was titrated without separation using congo red solution as indicator. In case the

TABLE 2

MICRODETERMINATION OF L-LYSINE

L-Lysine, 0.02 *M* (ml)	$ZnCl_2$, 0.0101 *M* (ml)	Amount of L-lysine ($\times 10^2$ mg)		Error (%)
		Taken	Found	
0.2	0.2	58.48	59.06	
0.3	0.3	87.72	88.59	
0.4	0.4	116.96	118.12	0.9
0.5	0.5	146.20	147.65	

TABLE 3

MICRODETERMINATION OF L-SERINE AND L-LYSINE

I L-Serine, 0.0398 *M* (ml)	$HgCl_2$, 0.01 *M* (ml)	Amount of L-serine ($\times 10^2$ mg) Taken	Found	II L-Lysine, 0.02 *M* (ml)	$ZnCl_2$, 0.0101 *M* (ml)	Amount of L-lysine ($\times 10^2$ mg) Taken	Found
0.5	1.0	209.10	210.18	0.2	0.2	58.48	59.06
0.4	0.8	167.28	168.14	0.3	0.3	87.72	88.59
0.3	0.6	125.46	126.10	0.4	0.4	116.96	118.12
0.2	0.4	83.64	84.07	0.5	0.5	146.20	147.65

order of titration is changed, then neither of these two amino acids would be titrable.

Table 4 shows that L-serine and L-histidine have been titrated together in one solution without separation. In this combination, L-histidine is first titrated, using xylenol orange solution; and then, in the same solution without separation, L-serine is titrated against mercuric chloride solution without adding any further indicator. L-Histidine and L-serine were estimated in the ascending and descending order from the top, respectively.

Table 5 shows that L-histidine and L-lysine were estimated together in the ascending and descending order from the top, respectively. In this combination L-histidine was first titrated using xylenol orange solution and then in the same solution L-lysine was titrated, using congo red solution, against zinc chloride solution.

Table 6 shows a new combination in which L-arginine and L-lysine were titrated together in the ascending and descending order, respectively, from the top. L-Arginine was first titrated, and then, in the same

TABLE 4

MICRODETERMINATION OF L-HISTIDINE AND L-SERINE

I L-Histidine, 0.0024 *M* (ml)	$Pb(NO_3)_2$ 0.01 *M* (ml)	Amount of L-histidine Taken, Found ($\times 10^2$ mg)	II L-Serine, 0.0398 *M* (ml)	$HgCl_2$, 0.01 *M* (ml)	Amount of L-serine ($\times 10^2$ mg) Taken	Found
0.5	0.06	27.37	0.5	1.0	209.10	210.18
1.0	0.12	54.74	0.4	0.8	167.28	168.14
1.5	0.18	82.11	0.3	0.6	125.46	126.10
2.0	0.24	109.48	0.2	0.4	83.64	84.07

TABLE 5

MICRODETERMINATION OF L-HISTIDINE AND L-LYSINE

I			II		
L-Histidine, 0.0024 *M* (ml)	$Pb(NO_3)_2$ 0.01 *M* (ml)	Amount of L-histidine found ($\times 10^2$ mg)	L-Lysine, 0.02 *M* (ml)	$ZnCl_2$, 0.0101 *M* (ml)	Amount of L-lysine found ($\times 10^2$ mg)
0.5	0.06	27.37	0.5	0.5	147.65
1.0	0.12	54.74	0.4	0.4	118.12
1.5	0.18	82.11	0.3	0.3	88.59
2.6	0.24	109.48	0.2	0.2	59.06

solution, L-lysine was titrated by using only the congo red solution as indicator.

Table 7 shows that in another combination L-histidine, L-serine, and L-lysine were titrated together in one solution without separation. L-Histidine and L-lysine were taken in the ascending order but L-serine was taken in the descending order from the top. In this combination, the order of titration is from L-histidine, L-serine, and L-lysine, respectively. First two amino acids were titrated by using xylenol orange solution as indicator; but in the same solution, after titrating the first two amino acids, congo red solution was added as indicator and then L-lysine was titrated as mentioned.

Results of the last combination, comprising L-histidine–L-lysine–L-arginine, are presented in Table 8. In this combination, the order in which titrations are accomplished starts from L-histidine, L-lysine, and L-arginine, respectively. L-Histidine and L-arginine were taken in the descending order from the top; but L-lysine was taken in the ascending order from the top. L-Histidine was titrated first and then, in the same

TABLE 6

MICRODETERMINATION OF L-ARGININE AND L-LYSINE

I			II		
L-Arginine, 0.02 *M* (ml)	$FeSO_4 \cdot (NH_4)_2 SO_4$, 0.0098 *N* (ml)	Amount of L-arginine found ($\times 10^2$ mg)	L-Lysine, 0.02 *M* (ml)	$ZnCl_2$, 0.0101 *M* (ml)	Amount of L-lysine found ($\times 10^2$ mg)
0.1	0.1	34.14	0.5	0.5	147.65
0.2	0.2	68.29	0.4	0.4	118.12
0.3	0.3	105.04	0.3	0.3	88.12
0.4	0.4	139.98	0.2	0.2	39.06

TABLE 7

MICRODETERMINATION OF L-HISTIDINE, L-LYSINE AND L-SERINE IN ONE SOLUTION

L-Histidine, 0.0024 *M* (ml) I	$Pb(NO_3)_2$, 0.01 *M* (ml)	L-Histidine found ($\times 10^2$ mg)	L-Serine, 0.0398 *M* (ml) II	$HgCl_2$, 0.01 M (ml)	L-Serine found ($\times 10^2$ mg)	L-Lysine, 0.02 *M* (ml) III	$ZnCl_2$, 0.0101 *M* (ml)	L-Lysine fouhd ($\times 10^2$ mg)
0.5	0.06	27.37	0.5	1.0	210.80	0.2	0.2	59.06
1.0	0.12	54.74	0.4	0.8	168.14	0.3	0.3	88.59
1.5	0.18	82.11	0.3	0.6	126.10	0.4	0.4	118.12
2.0	0.24	109.48	0.2	0.4	84.07	0.5	0.5	147.65

TABLE 8

MICRODETERMINATION OF L-HISTIDINE, L-LYSINE AND L-ARGININE IN ONE SOLUTION

L-Histidine, 0.0024 *M* (ml) I	$Pb(NO_3)_2$, 0.01 *M* (ml)	L-Histidine found ($\times 10^2$ mg)	L-Lysine, 0.02 *M* (ml) II	$ZnCl_2$, 0.0101 *M* (ml)	L-Lysine found ($\times 10^2$ mg)	L-Arginine, 0.02 *M* (ml) III	$FeSO_4 \cdot (NH_4)_2SO_4$, 0.0098 *N* (ml)	L-Arginine found ($\times 10^2$ mg)
2.0	0.24	109.48	0.2	0.2	59.06	0.4	0.4	139.98
1.5	0.18	82.11	0.3	0.3	88.59	0.3	0.3	105.84
1.0	0.12	54.74	0.4	0.4	118.12	0.2	0.2	68.29
0.5	0.06	27.37	0.5	0.5	147.65	0.1	0.1	34.14

solution, xylenol orange indicator solution was added for titration of L-lysine. In a third instance, L-arginine was titrated, after titrating the first two amino acids, in the same solution mixture after adding more drops of congo red solution.

It is important with these titrations that the eye should be well trained to perceive sharp color changes. Besides this, one of the most important precautions is that after each set of titrations the beakers must be well cleansed and dried carefully; failing which, desired results may not be obtained. Individual titrations of, and in combinations of, amino acids gave quick, accurate, and reproducible results. In combinations of amino acids, these titrations may be used as stepwise titrations, because there is a particular order for each set of combinations.

SUMMARY

L-Serine and L-lysine have been quantitatively determined in microamounts separately and in presence of each by direct titrations against mercuric and zinc chlorides, respectively. The two amino acids form complexes with mercuric and zinc ions in the ratio of 1:2. Maximum error for L-serine and L-lysine is 0.5 and 0.9%, respectively. These two amino acids in double and triple combinations with L-histidine and L-arginine have been titrated successfully in one solution without separation. In separate titrations, and in other combinations, congo red and xylenol orange solutions have been used only as indicators. Titrations are accurate, quick, and reproducible.

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Spectrophotometric Microdetermination of Cu(II) and Fe(III) Using 2,4-Dinitrosoresorcinol

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INTRODUCTION

The reaction of 2,4-dinitrosoresorcinol (DNR) with transition metal ions leads to the formation of colored chelate compounds. The method was applied for the microdetermination of ferrous iron *(3)* and palladium *(4)*. The reaction of DNR with Cu(II) and Fe(III) has been studied by some physicochemical methods *(2, 5)*, the results revealed the formation of some different chelates of varying stability and stoichiometry. Also it was found that Fe(III) is reduced through the organic ligand to Fe(II).

The present investigation deals with the application of DNR as a colouring reagent for the microdetermination of Cu(II) and Fe(III) either alone in their binary mixture.

EXPERIMENTAL METHOD

Water used in the preparation of solutions was twice distilled from an all glass apparatus. The materials were either of AR or CP grade (B.D.H.).

SOLUTIONS

Dinitrosoresorcinol (10^{-2} *M*) (DNR) was prepared by dissolving the accurately weighed amount of the reagent in a small volume of water containing an equivalent amount of NaOH so as to give the disodium salt. The resulting solution was then diluted to the appropriate volume.

Cu(II) (10^{-2} *M*) solution was prepared by dissolving copper chloride in water acidified with HCl (2 ml of the concentrated stock per liter). The exact Cu(II) content was determined electrolytically *(6)*.

Fe(III) (10^{-2} *M*) solution was obtained by dissolving ferric nitrate in water acidified with HNO_3 (3 ml of the stock per liter). The solution thus obtained was then analysed for its Fe(III) content *(6)*. More dilute solutions were obtained by accurate dilution.

Other salt solutions used to test their interference were prepared by dissolving the required weight of the salt in the appropriate volume of water. Buffer solutions (acetate buffer pH 3–5 and borate buffers pH 6–10) were prepared as given by Britton *(1)*.

Procedures

Twenty ml of the supporting electrolyte or water placed in a 25-ml certified measuring flask followed by the necessary quantity of 10^{-2} *M* DNR (0.5–1.0 ml). The required volume of 10^{-3} *M* Cu(II) or Fe(III) solution was then added, and the reaction mixture thereafter was completed to the mark with the supporting electrolyte. The absorbance of the resulting solutions was measured on a Unicam S.P. 500 or Hilger and Watts spectrophotometers using 1-cm matched silica cells, the blank contained the same concentration of DNR and the supporting electrolyte as in the test solution.

RESULTS AND DISCUSSION

Determination of Cu(II)

The reaction of Cu(II) with DNR, studied in different media, yields a yellowish to yellowish-brown colored complex. The media favoring maximum color development alternatively maximum complex formation are water, 10^{-4} *M* HCl, 10^{-4} *M* HNO_3, and acetate buffer of pH $\simeq$ 5. The absorption curves in such solutions exhibit one maximum within the wavelength range 360–370 mμ (Fig. 1 A). When the DNR concentration is kept constant at 2×10^{-4} *M* while that of Cu(II) is varied, the absorbance increases steadily. A plot of absorbance vs Cu(II) concentration yields a linear relation within the range $1–5 \times 10^{-5}$ *M* as shown in Fig. 2. At lower or higher concentrations, negative deviations are observed due to hydrolysis of the complex in more dilute solution and the formation of complexes with low stoichiometry in the more concentrated ones. When the medium contains a higher concentration of DNR ($3–4 \times 10^{-4}$ *M*), the concentration limit can be extended to $7–8 \times 10^{-5}$ *M*. Thus to obtain satisfactory results, the DNR concentration should be at least four times as much as that of Cu(II).

The addition of indifferent electrolytes such as NaCl, KCl, Na_2SO_4, and $NaClO_4$ to the Cu(II)–DNR mixture in water increases the absorbance due to suppressed dissociation of the Cu(II)–DNR complex. The increase in absorbance increases with the concentration of the indifferent electrolytes but attains a more or less constant value when the medium contains 0.005–0.01 *M* of the electrolyte. Under such conditions, the increase in absorbance amounts to $\sim$ 10% from that in absence of the electrolyte. The effect of interfering ions was tested with

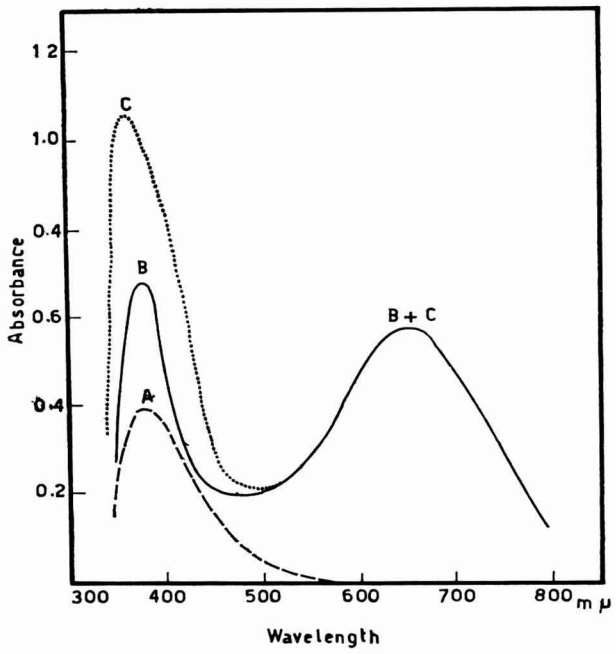

FIG. 1. Absorption spectra of complexes in solution, (*A*) Cu(II), (*B*) Fe(III), (*C*) Cu(II), and Fe(III), in binary mixture.

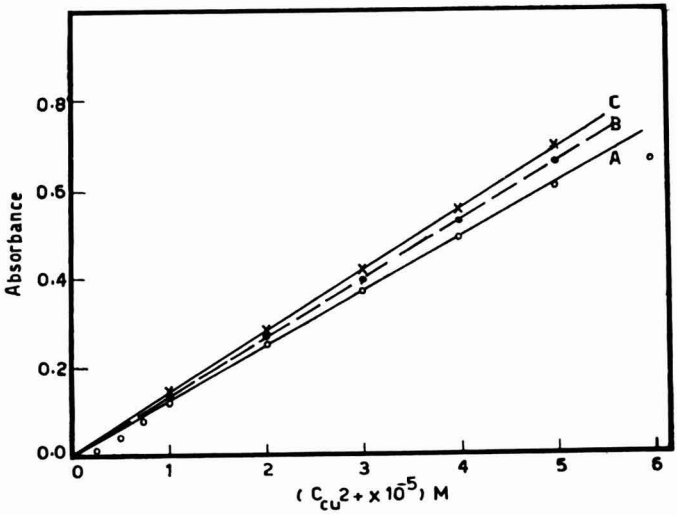

FIG. 2. Absorbance–concentration curves of Cu(II)–DNR complex. $Concn_{DNR} = 2.0 \times 10^{-4}$ *M*. (*A*) Water, (*B*) 10^{-4} *M* HNO_3, (*C*) acetate buffer solution, pH = 5.

a solution containing 1.0 ppm Cu(II) and 2×10^{-4} *M* DNR in 0.01 *M* $NaClO_4$, representative results are depicted in Table 1. From these data, it is apparent that metal cations, that are capable of forming complexes with DNR, causes positive deviations. On the contrary, ions which form complexes with Cu(II) leads to negative deviations. Accordingly, to obtain promising results the Cu(II) solution should be deprived of such interfering species.

Determination of Fe(III)

The reaction of Fe(III) with DNR yields a complex, the color of which is strongly dependent on the pH of the medium. In solutions of ph < 3, the complex formed exhibits a bluish color which changes to bluish green at pH 4–5 and green at pH 7–8. At pH 9–10 the solution is yellowish. The spectra of solutions of pH 3–8 show two maxima within the wavelength ranges 360–390 and 640–680, respectively (Fig.

TABLE 1

EFFECT OF INTERFERING IONS ON THE ABSORBANCE OF THE Cu(II)–DNR AND Fe(III)–DNR COMPLEXES

	Effect of anions				Effect of cations		
No. [a]	Ion	Concentration (ppm)	Deviation (%)	No.	Ion	Concentration (ppm)	Deviation (%)
1	NH_4^+	200	− 0.7	17	NO_2^-	100	− 2.0
2	Zn^{2+}	20	+ 1.0	18	HPO_4^{2-}	100	− 1.0
3	Cd^{2+}	3.0	+ 0.5	19	Cl^-	100	− 0.5
4	Pb^{2+}	5.0	+ 0.5	20	Br^-	100	− 0.5
5	Pd^{2+}	1.0	+ 5.0	21	I^-	20	− 8.5
6	Fe^{3+}	0.2	+ 8.5	22	SO_4^{2-}	100	− 0.5
7	Mn^{2+}	20	+ 1.0	23	AcO^-	100	− 0.5
8	Ca^{2+}	50	+ 0.8	24	Tartrate	50	− 1.0
9	Ba^{2+}	50	+ 1.3	25	Citrate	50	− 1.0
10	K^+	500	+ 4.0	26	Cl^-	500	+ 4.0
11	Na^+	500	+ 3.5	27	SO_4^{2-}	500	+ 2.0
12	Mg^{2+}	1.0	+ 2.0	28	NO_3^-	500	− 1.5
13	Zn^{2+}	1.0	+ 2.0	29	NO_2^-	500	−41.5
14	Cd^{2+}	1.0	−14.0	30	AcO^-	350	− 2.0
15	Cu^{2+}	1.0	− 6.0	31	Tartrate	350	− 3.5
16	Ba^{2+}	1.0	−15.0	32	Citrate	200	−32.0

[a] Numbers 1–9 and 17–25 using 1.0 ppm Cu^{2+}, 1.0×10^{-4} *M* DNR and 0.01 *M* $NaClO_4$[2] as supporting electrolyte, $\lambda = 370$ mμ. Numbers 10–16 and 26–32 using 1.0 ppm Fe^{3+}, 1.0×10^{-4} *M* DNR in medium deprived of supporting electrolyte. Solution aged for $\sim$ 48 hours, $\lambda = 670$ mμ.

1 B). With increasing pH, λ_{max}, shifts to higher values. On the other hand the more alkaline solutions exhibit the short wavelength band only which has a low absorbance. The highest absorbance values are obtained in solutions of pH 5–7 and as well in 10^{-4} *M* HNO_3. In solutions of pH $>$ 8, the absorbance of the short wavelength band reaches a constant value after $\sim$ 30–40 minutes while that of the long wavelength band does not attain a constant value except after 48–72 hours. The time needed to reach the limiting value increases with decreasing pH. Since ions with d^5 systems does not exhibit a measurable absorbance in the visible side, it seems that Fe(III) is reduced in such media to Fe(II). This opinion is substantiated by the fact that the absorption spectra at saturation are comparable with those of Fe(II) in such media. Also color development is enhanced by the addition of reducing agents such as sodium sulphite or hydroxylamine. Warming the solution also enhances the development of the greenish color. Rapid complete color development can be attained by addition of 0.1 g sodium sulphite or 1 ml of 10% hydroxylamine solution. When the DNR concentration is maintained constant at 2–4 $\times$ 10^{-4} *M* while that of Fe(III) is varied the absorbance of the solution is a linear function of Fe(III) content. The maximum limit for the validity of Beer's law depends on the medium and DNR concentration. Representative curves are given in Fig. 2.

The effect of interfering ions on the absorbance of the reduced Fe(III) solution is studied in medium deprived of supporting electrolytes. Metal ions generally cause a strong positive deviation with the short wavelength band while the one at longer wavelength is not apparently influenced since no overlap between this absorption band and the absorption bands of other metal-DNR complexes occurs. However, if the DNR concentration is less than four times as much as the additive concentration of Fe(III) and the other metal ion, negative deviations are observerd. This can be explained by the competitive effect of the interfering metal ions, hence the DNR present in solution would be insufficient to yield the limiting absorbance with both complex forming ions. Ions which form complexes with Fe(III) cause negative deviations, citrate ions inhibit the reaction of Fe(III) with DNR to a very large extent. Some results are collected in Table 1.

Analysis of Cu(II) and Fe(III) in a Binary Mixture

The spectrum of a solution containing both iron and copper in acetate buffer of pH 5 or 10^{-4} *M* HNO_3 exhibits two bands. The band at longer wavelength is due to the absorption of the iron complex while that at shorter wavelength is due to both iron and copper complexes.

Thus, in a binary mixture of copper and iron, it is only possible to determine the iron concentration from direct measurements. The copper content can be determined if the absorption due to iron under the short wavelength band is known. (a) If we consider that

A_1 = absorption of long wavelength band (λ = 670 mμ);
A_2 = absorption of short wavelength band (λ = 370 mμ);
ϵ_1 = molar extinction of Fe(II)–DNR complex at λ = 670 mμ;
ϵ_2 = molar extinction of Fe(II)–DNR complex at λ = 370 mμ;
ϵ_3 = molar extinction of Cu(II)–DNR complex at λ = 370 mμ;

the concentration of Fe(II) and Cu(II) can be determined from Eq. (1) and (2), respectively.

$$\text{Concn}_{\text{Fe(II)}} = A_1/\epsilon_1, \tag{1}$$

$$\text{Concn}_{\text{Cu(II)}} = \left(A_2 - \frac{A_1\,\epsilon_2}{\epsilon_1}\right) \cdot \frac{1}{\epsilon_3}. \tag{2}$$

However, the reduction of Fe(III)–DNR complex to that of Fe(II) without affecting the Cu(II)–DNR complex is only achieved by allowing the reaction mixture to stand for ~ 48 hours. This lengthy time renders the method inapplicable for the analysis of such a mixture. (b) A rapid satisfactory analysis can be achieved by considering the absorption of the short wavelength band only. The Cu(II)–Fe(III)–

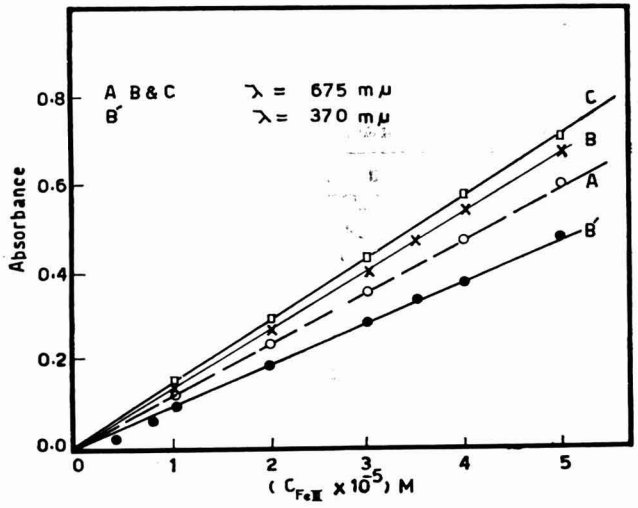

FIG. 3. Absorbance–concentration curves of Fe(III)–DNR complex. $\text{Concn}_{\text{DNR}}$ = 4.0 × 10^{-4} *M*. (*A*) Water, (*B*) 10^{-4} *M* HNO_3, (*C*) acetate buffer solution, pH = 5.

DNR mixture is allowed to stand for $\sim$ 40 minutes then recording the absorbance at $\lambda = 370$ mμ (A_2). A similar mixture is treated with 2.0 ml hydroxylamine solution and warmed at 60°C for $\sim$ 5–10 minutes, after cooling the mixture is centrifuged to allow the formed Cu_2O to settle down. The absorbance of the supernatant liquid is then recorded (A'_2). From these results Cu(II) and Fe(III) concentrations can be determined from Relations (3) and (4).

$$\text{Concn}_{\text{Fe(III)}} = A'_2/\epsilon_2, \tag{3}$$

$$\text{Concn}_{\text{Cu(II)}} = \frac{A_2 - A'_2}{\epsilon_3}. \tag{4}$$

The molar extinction coefficients can be determined from absorbances of solutions containing known quantities of Cu(II) or Fe(III) in the same medium applied for the analysis of the mixture. Representative results are given in Table 2.

SUMMARY

The colored complexes formed by the reaction of dinitrosoresorcinol with Cu(II) and Fe(III) is utilized for the microdetermination of both metal ions either alone or in a binary mixtures. Satisfactory results are obtained when the proper media are utilized in the presence of an excess of the organic ligand. The interference of some ions is also investigated.

TABLE 2

ANALYSIS OF Cu(II) AND Fe(III) IN A BINARY MIXTURE

	$\text{Concn}_{Fe^{3+}}$ ($\times 10^{-5}M$)		$\text{Concn}_{Cu^{2+}}$ ($\times 10^{-5}M$)	
No. [a]	Added	Found	Added	Found
1	1.0	1.02	1.0	0.96
2	2.0	1.95	1.0	1.03
3	2.0	1.98	2.0	2.05
4	2.0	2.0	3.0	3.06
5	3.0	3.05	1.0	0.98
6	5.0	4.95	1.0	0.98
7	2.0	2.06	1.0	0.96
8	2.0	1.98	1.0	1.0
9	2.0	2.05	1.0	0.98
10	3.0	2.95	2.0	2.02
11	4.0	4.06	2.0	1.96
12	5.0	4.92	2.0	1.96

[a] (1–6) Results obtained by method (a) in acetate buffer pH = 5.0, solutions aged for $\sim$ 48 hours. (7–12) Results obtained by method (b) in medium deprived of supporting electrolyte.

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Electroanalysis with Tungsten Electrodes

I. The Tungsten/Platinum Bimetallic Pair in Potentiometry at Zero Current

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INTRODUCTION

Use of bimetallic electrode pairs in potentiometric titration permits the elimination of the calomel electrode with its salt bridge so that a more sturdy assembly results. Several combinations have been experimentally tested in neutralization (*1,2*), oxidation/reduction (*3,4*), precipitation (*5*), and complexometric (*6*) titrations. Neutralization reactions have also been reported in nonaqueous media (*7*). The suitability of a particular pair for a certain titration is judged by the occurrence of a sharp change in the bimetallic pd at, or very near to, the equivalence point. Although the principles underlying the choice of pairs are still unclear, yet the essential feature is that each electrode in a pair should vary its potential independent of the other electrode with change in solution composition during titration. It appears that a combination comprising a valve metal and a noble metal will give the highest difference in the extent of surface oxidation and in the electron conductivity of both metals.

The W/Pt pair offers wide prospects since W not only behaves as a valve metal (*8*), but also possesses a high resistance to corrosion and is easy to obtain. Therefore, the present investigation was undertaken to study the use of this pair in various classical potentiometric titrations. To afford an explanation for the success or failure of the pair in a titration, it is felt necessary to establish the shape of the titration curves for the separate electrode potentials of W and Pt. This also helps to determine the relative response of each electrode during titration. Since the occurrence of oxide films on W surface may interfere with the results, it is important to investigate the effect of these oxides on the titration curves.

EXPERIMENTAL METHODS

Standard techniques of potentiometric titration were employed. The W was in the form of a spec-pure rod sealed to glass. Its surface was abraded with fine metallurgical paper before each experiment. Electrode potentials were measured against sat calomel, once for W and another time for Pt in the same experiment. The solution was stirred by a magnetic stirrer during titration, and each potential reading was followed with time until a constant value was established. While the time factor was negligible in most results on Pt, the potential of W usually took 1–2 minutes to become constant, and a larger time interval was needed when W was immersed in alkaline solutions. Two anodic oxide films were formed on W surface by anodization in 1.0 *N* H_2SO_4:

(i) a thin film formed at 100 $\mu A/cm^2$ for 50 minutes (0.3 C/cm^2), and (ii) a thicker film formed at $2mA/cm^2$ for 42 minutes (5 C/cm^2). Solutions were prepared from AR grade reagents and twice-distilled water. These were standardized by conventional analytical procedures. All titrations were repeated several times to ensure reproducibility, and the mean was computed. The mean deviation was less than $\pm$ 10mV. Measurements were made at room temperature (28–30°C).

RESULTS AND DISCUSSION

Neutralization

Strong and weak acids were titrated with standard NaOH. The volumes and concentrations of solutions were so chosen as to allow a clear graphical representation of the results. Forward titrations in which the alkali was added to the acid, as well as backward titrations in which the reverse was made, were performed in each case. Figure 1a shows the results of forward (A) and backward (B) titrations of 5-ml samples of 1.0 *N* H_2SO_4 and 0.81 *N* NaOH. The shape of the curves is the same for metallic W, anodized W, and Pt. Sharp inflexions are observed at the equivalence point. These are on the whole smaller for Pt than for W electrodes. Thus while the inflexion pd on Pt amounts to 110 mV (A) and 160 mV (B), it increases on W reaching 280–290 mV for both forward and backward directions. It is worth noting that the occurrence of anodic oxide films on the W surface increases the inflexion pd in the forward titration to 380 mV in the case of the thin film and to 430 mV for the thicker film. However, both oxide films have but a little effect on the inflexion pd in the backward titration. It is possible to attribute this behavior to the solubility of W oxides in NaOH with the result that oxide-coated W behaves like metallic W from the beginning of backward titration until all alkali is consumed. In presence

of excess acid, spontaneous oxidation of W may commence; and this may be responsible for the slight deviations observed in this range.

Figure 1b shows the results obtained for 0.28 *N* acetic acid/0.3 *N* NaOH. Titrations were made with 10 ml of acetic acid in the forward, and 5 ml of NaOH in the backward direction. Similar to the experiments with strong acids, the inflexion occurs here at the equivalence point. This inflexion is more pronounced on W (170 mV for forward and backward) than on Pt (25–35 mV for both).

Measurements were also made with oxalic and phosphoric acids. Forward titration of 17 ml of 0.1 *N* oxalic acid by 0.27 *N* NaOH (Fig. 2a) shows two steps on both W and Pt. These represent half and complete neutralization to form the mono- and dibasic salts, respectively. The inflexion pd are of the same magnitude on the two metals, thus amounting

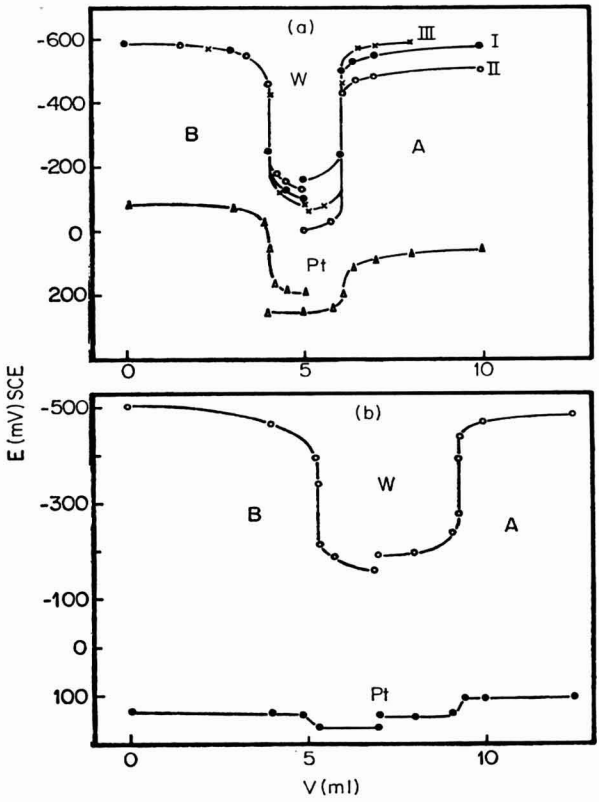

FIG. 1a. Titration of: (A) 5 ml of 1.0 *N* H_2SO_4 by 0.81 *N* NaOH; (B) 5 ml of 0.81 *N* NaOH by the same acid; (I) W metal; (II) thin oxide; (III) thick oxide. (b) Titration of: (A) 10 ml of 0.28 *N* acetic acid by 0.3 *N* NaOH; (B) 5 ml of 0.3 *N* NaOH by the same acid; (●) Pt; (○) W.

to 50 mV for the first step and 180 mV for the second. Backward titration of 5 ml of 0.27 *N* NaOH by 0.1 *N* acid gives one step only. This has a pd of 70–80 mV on W and Pt, and corresponds to 13.5 ml of acid thus indicating the complete neutralization to the dibasic salt. This titration was repeated in five separate experiments which gave the same result.

In the forward titration of 30 ml of 0.23 *N* H_3PO_4 by 0.4 *N* NaOH, two steps are only observed (Fig. 2b). The first requires 5.8 ml of alkali in agreement with the value calculated for the formation of NaH_2PO_4. The volume of alkali consumed from the beginning of titration up to the end of the second step amounts to 11.6 ml, indicating the neutralization to Na_2HPO_4. No step could be detected for the trisodium salt. The inflexion pd are (i) first step: 110 mV for W and

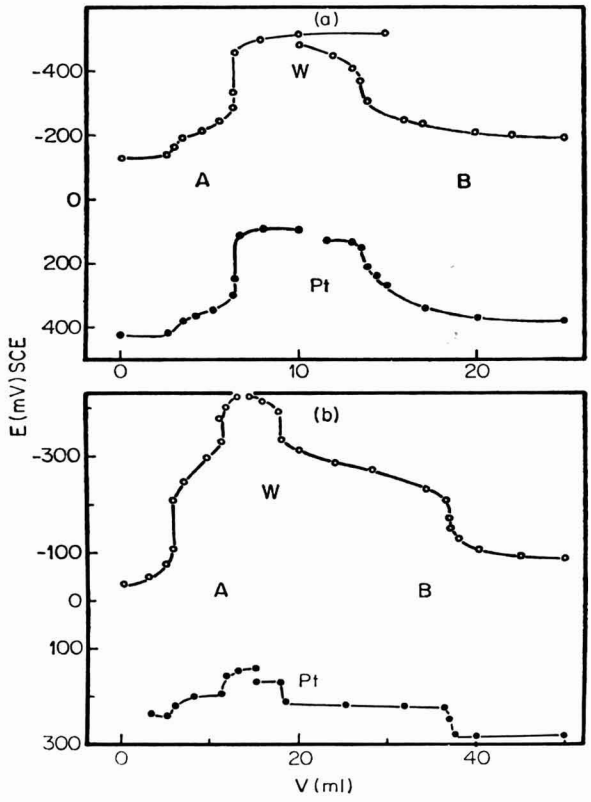

FIG. 2a. Titration of: (A) 17 ml of 0.1 *N* oxalic acid by 0.27 *N* NaOH; (B) 5 ml of 0.27 *N* NaOH by the same acid; (●) Pt; (○) W. (b) Titration of: (A) 30 ml of 0.23 *N* H_3PO_4 by 0.4 *N* NaOH; (B) 7 ml of 0.4 *N* NaOH by the same acid; (●) Pt; (○) W.

30 mV for Pt; and (ii) second step: 60 mV for W and 40 mV for Pt. Backward titration of 7 ml of 0.4 *N* NaOH by the same phosphoric acid solution also gives two steps requiring 18.1 and 36.5 ml acid, respectively. This titration proceeds as:

$$3\ NaOH + H_3PO_4 = Na_3PO_4 + 3H_2O, \quad (1a)$$

$$Na_3PO_4 + \tfrac{1}{2}\ H_3PO_4 = \tfrac{3}{2}\ Na_2HPO_4, \quad (1b)$$

$$\tfrac{3}{2}\ Na_2HPO_4 + \tfrac{3}{2}\ H_3PO_4 = 3\ Na\ H_2PO_4. \quad (1c)$$

Reaction (1a) theoretically requires 12.2 ml of the acid used. However, this does not appear as an independent step, but the neutralization proceeds to the completion of (1b). Up to this stage, the volume of acid consumed is 1.5 times the volume required for (1a) alone, giving a total of 18.3 ml compared to the experimental result of 18.1 ml. Reaction (1c) also requires 18.3 ml, i.e., a total of 36.6 ml from the beginning of titration in agreement with the experimental value of 36.5 ml. The inflexion pd are (i) first step: 70 mV for W and 50 mV for Pt; and (ii) second step: 60–70 mV for both metals.

Titrations of oxalic and phosphoric acid solutions by NaOH were repeated on anodized W surfaces (thick oxide formed at 5 C/cm^2). The results give the same end points as with W, but the inflexion pd are noticeably increased. Thus with oxalic acid the new pd are 100 and 325 mV for the two steps of the forward titration, respectively. The corresponding values in the case of H_3PO_4 are 175 and 125 mV. Preliminary measurements on thermally oxidized W surfaces show that oxide films prepared in this manner have the same effect as anodic oxides.

Variation of the bimetallic pd during titration is shown in Fig. 3 for the forward direction of the four neutralization reactions studied here. The magnitude of change of this pd with change in volume of titrant at the end point is given in Table 1. In the titration of oxalic acid, the change of electrode potential is nearly the same for both W and Pt, and hence the bitmetallic pd is so small as to be of any practical use. However, when oxide-coated W is used in combination with Pt, the two electrodes behave differently and the bimetallic pd starts to increase. In this case the first neutralization step, which is associated with an inflexion of 60 mV, is not sharp and extends over 1.5 ml of titrant giving the low value of 4 mV/0.1 ml. A similar behavior is observed with the glass electrode. The first neutralization step occurs over a range of 2 pH units and 2 ml of titrant. On the other hand, the second step is sharply defined (5 pH units/0.1 ml).

The present results indicate that the electrode potential of W is

generally more sensitive to variations in solution composition near the equivalence points of acid/base titrations than Pt. This may be explained in terms of the relative ability of each metal to form an oxide film in aqueous solutions. Although the following reactions have been suggested for Pt in acid and neutral solutions (*9*):

$$\begin{aligned} Pt + 2H_2O &= Pt(OH)_2 + 2H^+ + 2e, \\ Pt(OH)_2 &= PtO_2 + 2H^+ + 2e, \end{aligned} \qquad (2)$$

Thus showing the dependence of electrode potential on H^+ ion activity, yet it appears from more recent work (*10*) that the primary process on Pt is the adsorption of oxygen. The adsorbed film may be partially

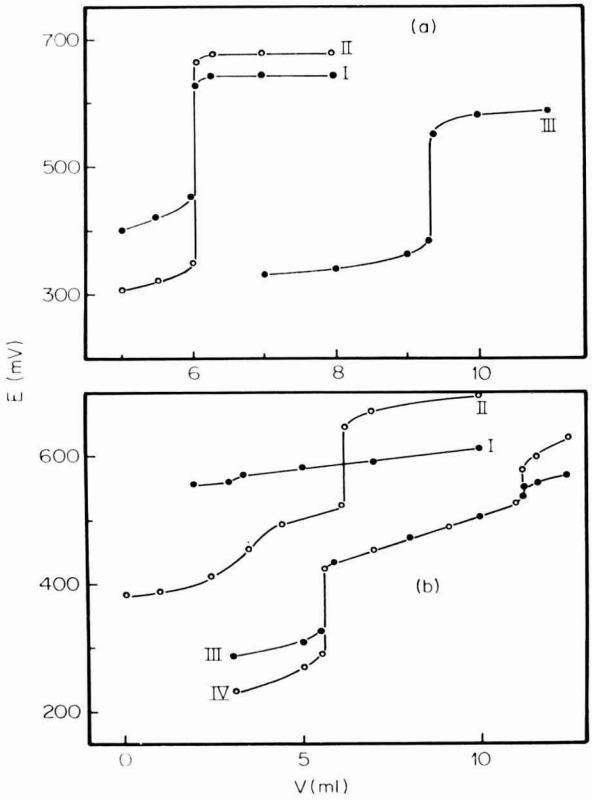

FIG. 3. Bimetallic pd in the forward titration of: (a) H_2SO_4 and acetic acids: (I) H_2SO_4 using W; (II) H_2SO_4 using W oxide; (III) acetic using W. (b) Oxalic and H_3PO_4 acids: (I) oxalic using W; (II) oxalic using W oxide; (III) H_3PO_4 using W; (IV) H_3PO_4 using W oxide.

TABLE 1

$\Delta E/\Delta V$ FOR THE BIMETALLIC POTENTIAL DIFFERENCE AT END POINT OF NEUTRALIZATION

Neutralization of/by	$(\Delta E/\Delta V)$ (mV/0.1 ml)	Pair [a] W or oxide/Pt
1.0 *N* H_2SO_4/0.81 *N* NaOH	180	W
	310	W oxide
0.28 *N* CH_3COOH/0.3 *N* NaOH	165	W
0.1 *N* $(COOH)_2$/0.27 *N* NaOH	—	W
	4 (1st) [b]	W oxide
	120 (2nd)	W oxide
0.23 *N* H_3PO_4/0.4 *N* NaOH	100 (1st)	W
	25 (2nd)	W
	130 (1st)	W oxide
	50 (2nd)	W oxide

[a] The oxide used here is the one formed at 5 C/cm².
[b] Neutralization steps.

transformed into an oxide film of a limited thickness. W on the other hand is known to form a number of oxides:

$$\begin{aligned} W + 2H_2O &= WO_2 + 4H^+ + 4e, \\ 2\,WO_2 + H_2O &= W_2O_5 + 2H^+ + 2e, \\ W_2O_5 + H_2O &= 2\,WO_3 + 2H^+ + 2e. \end{aligned} \tag{3}$$

Previous work *(11)* on the dependence of the W electrode potential on pH shows that the final oxide formed in acid and neutral solutions is likely to be a mixture of W_2O_5 and WO_3. Furthermore, since W behaves as a valve metal *(8)*, the most probable anodic reaction of the mixed electrode system attained on open circuit is the growth of an oxide film over the thin air-formed film already present. Therefore, the oxide thickness on W is larger than that on Pt. Hence, the behavior of W as pH-indicating electrode is more pronounced than that of Pt. In alkaline solutions, the pH-dependence of the W electrode potential arises from the dissolution of either W_2O_5 or WO_3 or both to form tungstates.

No simple explanation can be given for the similarity observed in the behavior of W and Pt electrode potentials during the titration of oxalic acid by NaOH. The same situation is encountered in the titration of other dicarboxylic acids, e.g., succinic acid by NaOH. However, to throw some light on these results, the open circuit potential was meas-

used for both metals in stirred solutions of sodium oxalate and ammonium succinate. The final steady state potentials are recorded in Table 2. The results indicate that: (i) the two electrode potentials are of opposite signs; (ii) increase of salt concentration or pH increases both potentials, to more positive values for Pt, and to less negative values for W; (iii) the increase of potential lies between 48 and 58 mV; and (iv) the difference between the two electrode potentials is constant (547–558 mV for both salts) independent of concentration. The direction of the pH effect on the electrode potential of either metal in both solutions cannot be explained on the basis of a metal/metal oxide equilibrium, since the latter requires a decrease of 60 mV for each unit increase of pH [cf. Eqs. (2) and (3)]. Since the anions of the dicarboxylic acids are capable of undergoing anodic oxidation to give the unsaturated hydrocarbons and carbon dioxide, the possibility exists that oxidation/reduction equilibria involving these species are established at electrodes left on open circuit. Hence, the potential is determined by thes rather than by metal/metal oxide equilibria, and the present results may therefore be qualitatively interpreted in terms of the general considerations to be given below for oxidation/reduction equilibria.

Oxidation/Reduction

The following oxidation/reduction titrations were studied: ferrous by permanganate and dichromate, thiosulfate by iodine, and ferrocyanide by ceric sulfate. In all these experiments both forward (oxidizing agent added) and backward (reducing agent added) titrations were performed. Figure 4a shows the results obtained for 0.04 *N* ferrous/0.05 *N* permanganate when 5 ml of either solution is titrated with the other after acidifying with H_2SO_4. In the forward direction, both W and Pt give inflexions at the equivalence point. These are associated with a pd

TABLE 2

OPEN CIRCUIT POTENTIAL FOR TUNGSTEN AND PLATINUM IN OXALATE AND SUCCINATE SOLUTIONS [a]

Conc	$(COONa)_2$			$(CH_2COONH_4)_2$		
(*M*)	pH	*E* (mV) Pt	$-E$(mV)W	pH	*E* (mV) Pt	$-E$(mV)W
0.15	7.1	215	337	—	—	—
0.10	—	—	—	6.3	295	258
0.05	6.8	195	352	6.1	278	278
0.025	6.4	175	370	6.0	250	305
0.005	6.2	165	385	5.9	243	315

[a] Potentials vs sat calomel.

of 520 mV for Pt and 80 mV for W. The presence of oxide films on W slightly increases this pd by about 20–30 mV, but the position of inflexion is simultaneously shifted to lower volumes of titrant. In the backward titration, however, sharp inflexions are observed at the equivalence point for Pt, W, and oxide-coated W. The inflexion pds are 360, 80, and 70 mV, respectively.

Figure 4b shows the results obtained for the titration of 0.095 *N* ferrous/0.1 *N* dichromate. In the forward titration, 20 ml of the ferrous solution were used, while the backward titration was made with 10 ml of dichromate solution. In both cases, Pt and W give inflexions at the equivalence point. Similar to the results with permanganate, the inflexion pd observed here on Pt (70 mV for forward and 140 mV for backward) are always higher than those on W (40–50 mV). During

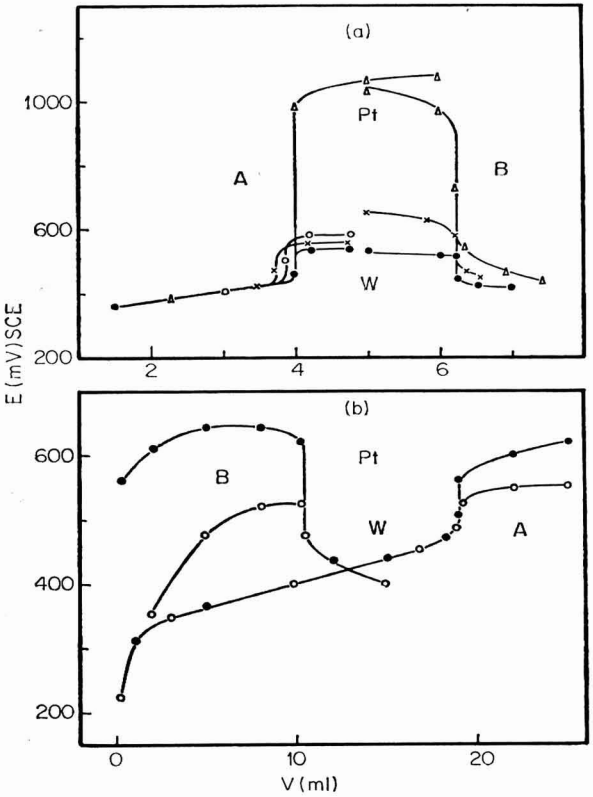

FIG. 4a. Titration of: (A) 5 ml of 0.04 *N* ferrous ammonium sulfate by 0.05 *N* $KMnO_4$; (B) 5 ml of 0.05 *N* $KMnO_4$ by the same ferrous solution; (△) Pt; (●) W; (○) thin W oxide; (×) thick oxide. (b) Titration of: (A) 20 ml of 0.095 *N* ferrous ammonium sulfate by 0.1 *N* $K_2Cr_2O_7$; (B) 10 ml of 0.1 *N* $K_2Cr_2O_7$ by the same ferrous solution; (●) Pt; (○) W.

the initial stage of either titration, the potential of both W and Pt shows a noticeable rise with the volume of titrant.

Iodimetric titrations were made with 0.14 *N* iodine and 0.1 *N* thiosulfate solutions using 20 ml of the latter for forward and 12.5 ml of the former solution for the backward. The results are shown in Fig. 5a which indicates that W gives inflexion pd which are higher than those associated with Pt. Thus while the pd on Pt amounts to 170 mV in the forward titration, it increases on W reaching about 200 mV. In the backward direction the pd are 100 and 140 mV, respectively. Apart from the disappearance of the metallic lustre of W, the electrode surface does not undergo any other apparent change during the above three oxidation/reduction titrations.

Results of the titration of 0.011 *N* ferrocyanide with 0.01 *N* ceric sulfate are shown in Fig. 5b. While the titration curves on Pt show the

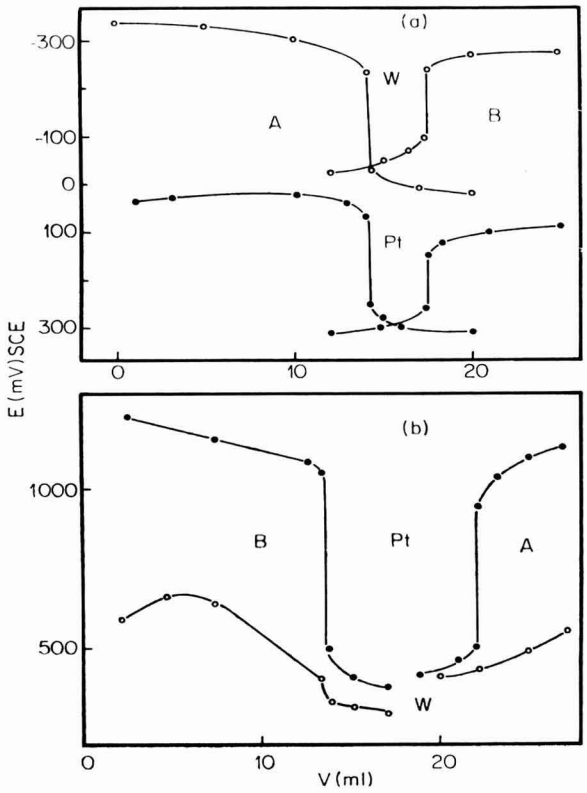

FIG. 5a. Titration of: (A) 20 ml of 0.1 *N* $Na_2S_2O_3$ by 0.14 *N* iodine; (B) 12.5 ml of 0.14 *N* iodine by the same $Na_2S_2O_3$ solution; (●) Pt; (○) W. (b) Titration of: (A) 20 ml of 0.011 *N* $K_4[Fe(CN)_6]$ by 0.01 *N* $Ce(SO_4)_2$; (B) 15 ml of 0.01 *N* $Ce(SO_4)_2$ by the same ferrocyanide; (●) Pt; (○) W.

normal behavior with well-defined inflexions (420 mV for forward and 550 mV for backward), those on W either show ill-defined inflexions or no inflexions at all. Moreover, the curves for W lie below those for Pt throughout the whole range. In this titration, the surface of W acquires a blue color which is not easily removed by rubbing with a filter paper. This is probably caused by the formation of a surface layer of tungsten blue.

The dependence of the bimetallic pd on the volume of titrant is shown in Fig. 6 for the forward direction of the present four oxidation/reduction titrations. With the exception of the iodine/thiosulfate reaction, sharp inflexions are obtained. The values of $\triangle E/\triangle V$ at the end point of the titration amount to 480 mV/0.1 ml for the oxidation of ferrous by permanganate, 460 mV/0.1 ml for the oxidation of ferrocyanide by ceric ions, and only 30–40 mV/0.1 ml for the oxidation of ferrous by dichromate. In the oxidation of thiosulfate by iodine the bimetallic pd decreases, at first reaching a slight minimum (about 20 mV) at the equivalence point, and then increases again.

The essential requirements in an indicator electrode for oxidation/reduction titrations are: (i) a good electrical conductivity which enables the electrode to respond rapidly to variations in the activity of reactants and products; and (ii) a high degree of chemical stability which prevents the dissolution of electrode material, as well as any interaction

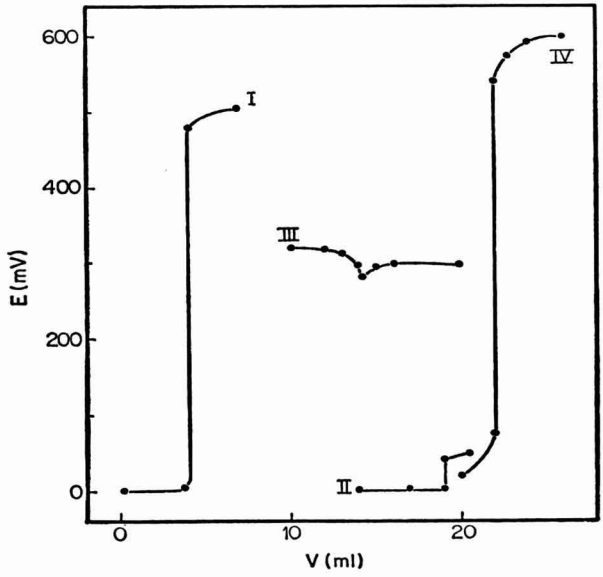

FIG. 6. Bimetallic pd in the forward titration of: (I) ferrous by $KMnO_4$; (II) ferrous by $K_2Cr_2O_7$; (III) $Na_2S_2O_3$ by I_2; (IV) K_4 [Fe(CN)$_6$] by Ce^{4+}.

between the surface and the components of solution. In this connexion, the presence of oxide films is undesirable since they not only decrease the electrical conductivity of the surface but may also act as oxidizing agents. This is the case with tungsten oxides which possess a very low electron conductivity and a considerably higher ionic conductivity, and whose participation in the oxidation of ferrous ions is indicated here (Fig. 4a). The fact that the electrode potential of metallic W is generally less sensitive to variations in solution composition than Pt particularly near the equivalence point, may be attributed to a thin oxide film formed on W in air or in solution. However, this cannot account for the opposite behavior observed in the case of iodine/thiosulfate reaction (Fig. 5a).

This apparent discrepancy may be clarified by close consideration of the role of metal in oxidation/reduction equilibria. A metallic electrode acts as a catalyst for charge transfer reactions, i.e., as electrocatalyst, and various factors have been found to affect its electrocatalytic power *(12)*. These include: (i) a chemical factor inherent in the rate constant for electrocatalysis; (ii) an electrochemical factor represented by the position of electrode potential relative to the potential of zero charge (pzc); and (iii) the presence of specifically adsorbed species capable of bonding with the surface. In absence of specific adsorption, the electrode potential is a function of: (i) the crystallographic properties of the surface, and the chemical nature of the species participating in the oxidation/reduction equilibrium; both factors being included in the rate constant; and (ii) the electronic work function of the metal which affects the pzc and consequently the ionic concentration in the double layer. Therefore, both the nature of metal and the chemical species present are important in determining the relation between electrode potential and solution composition. Hence, the titration curves for two different metals may be nearly similar in some solutions (cf. the results on W and Pt in oxalate and succinate solutions) and completely different in others (cf. Figs. 4 and 5). This explanation cannot be subjected to quantitative verification since the pzc, its variation with electrolyte composition, and the rate constants are not known with certainty even for Pt. Specific adsorption, e.g., of iodide ions, introduces a new factor, and the potential behavior of each electrode in the pair during titration depends also on the extent and strength of adsorption on each metal.

Precipitation

Three precipitation reactions involving $AgNO_3$ with chloride, iodide, and sulfocyanide ions were studied. The forward titration is considered

the one made by addition of $AgNo_3$. Figure 7a shows the results obtained for 0.01 *N* $AgNO_3$/0.0122 *N* NaCl. Forward and backward titrations were made with 10 ml of NaCl and $AgNO_3$, respectively. The inflexion pd at the equivalence point is higher for W (325 mV for forward and 280 mV for backward) than for Pt (100–110 mV for both directions). Results of the precipitation by iodide ions are shown in Fig. 7b. Forward titration (A) of 20 ml of 0.0081 *N* KI by 0.01 *N* $AgNO_3$ gives an inflexion pd of 320 mV on W and 55 mV on Pt. The corresponding values obtained in the backward titration (B) of 15 ml 0.01 *N* $AgNO_3$ by the same iodide solution are 280 mV and 45 mV, respectively. The titration involving sulfocyanide (Fig. 8a) gives an inflexion pd of 240 mV on W and 70 mV on Pt for both forward and backward directions. In all these precipitation reactions, the W electrode

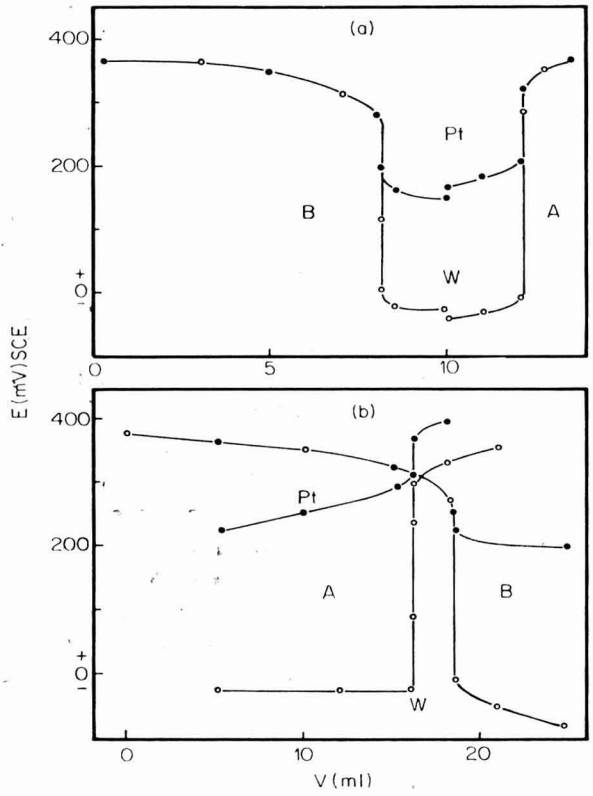

FIG. 7a. Titration of: (A) 10 ml of 0.0122 *N* NaCl by 0.01 *N* $AgNo_3$; (B) 10 ml of 0.01 *N* $AgNo_3$ by the same chloride solution; (●) Pt; (○) W. (b) Titration of: (A) 20 ml of 0.0081 *N* KI by 0.01 *N* $AgNo_3$; (B) 15 ml of 0.01 *N* $AgNo_3$ by the same iodide solution; (●) Pt; (○) W.

shows a higher change in potential at the equivalence point than observed with Pt.

The bimetallic pd is shown in Fig. 8b as a function of the volume of titrant for forward and backward precipitation titrations. It is clear that sharp inflexions are obtained at the equivalence points for all cases studied here. These are associated with the following values for $\Delta E/\Delta V$ (mV/0.1 ml):

chloride	210 (A)	175 (B)
iodide	260	230
sulfocyanide	175	175

Complexometric Titrations

These were made by 0.05 *M* EDTA. Solutions of $CuSo_4$ (15 ml, 0.049 *M*), $MgSO_4$ (10 ml, 0.053 *M*), and $CaCl_2$ (5 ml, 0.048 *M*) were

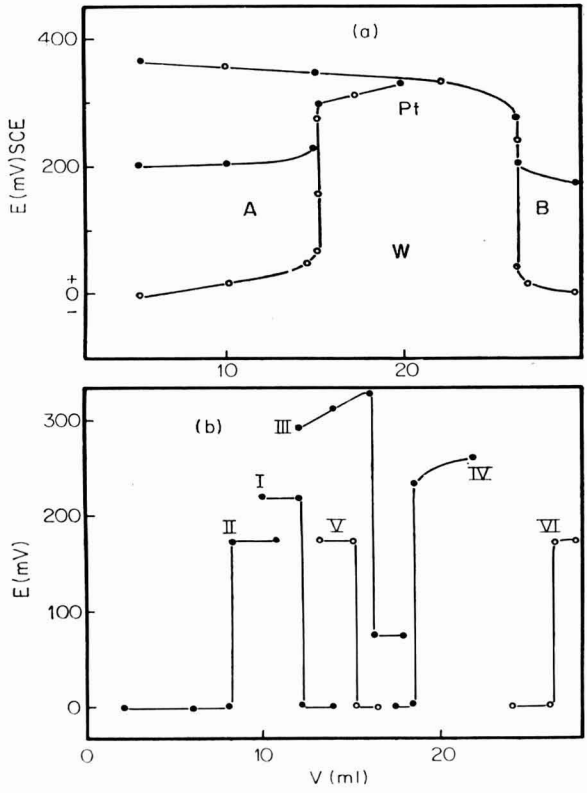

FIG. 8a. Titration of: (A) 20 ml of 0.0076 *N* KCNS by 0.01 *N* $AgNO_3$; (B) 20 ml of 0.01 *N* $AgNO_3$ by the same sulfocyanide solution; (●) Pt; (○) W. (b) Bimetallic pd in precipitation reactions with $AgNO_3$: chloride (I) forward, (II) backward; iodide (III) forward, (IV) backward; sulphocyanide (V) forward, (VI) backward.

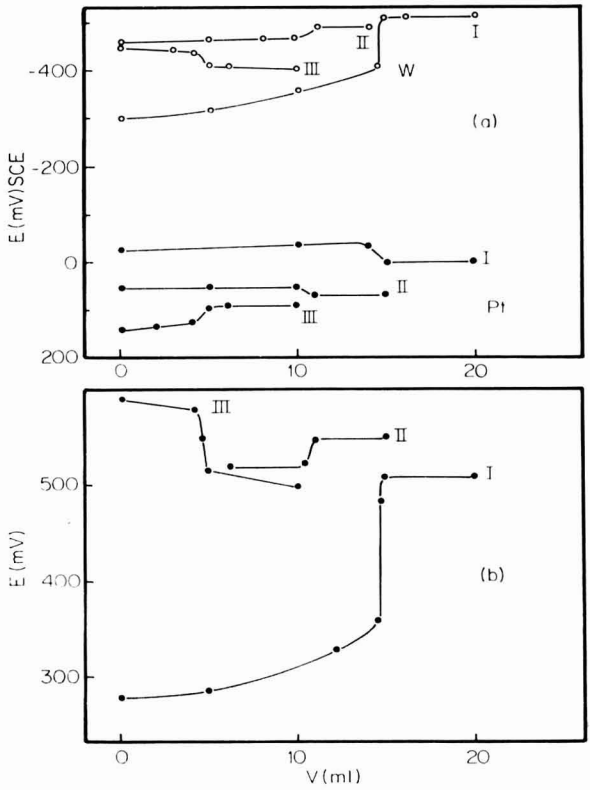

FIG. 9a. Titration with 0.05 *M* EDTA on (●) Pt; and (○) W: (I) 15 ml of 0.049 *M* $CuSO_4$; (II) 10 ml of 0.053 *M* $MgSO_4$; (III) 5 ml of 0.048 *M* $CaCl_2$. (b) Bimetallic pd in the titration with EDTA; (I) Cu; (II) Mg; (III) Ca.

used. The first was made alkaline to pH 11 by adding 4 ml of conc-NH_4OH before titration. To each of the other two solutions, 10 ml of NH_4Cl/NH_4OH buffer (pH 10) were added. The results of electrode potential measurements during titration are shown in Fig. 9a. Except for the titration of copper on W where a sharp inflexion occurs at the equivalence point, all other inflexions are not sharp and extend over a volume of 0.8–1.0 ml of titrant. Moreover, the complexation reaction with copper gives higher inflexion pd (100 mV on W and 40 mV on Pt) than observed with magnesium and calcium (20–30 mV on both metals). Results of bimetallic pd (Fig. 9b) give a sharp inflexion at the equivalence point for copper only (150 mV/0.1 ml).

SUMMARY

The W/Pt bimetallic pair has been applied in various potentiometric titrations. Except for the neutralization of dicarboxylic acids, e.g., oxalic, the pair is suitable

for neutralization titrations where $\Delta E/\Delta V$ values at the equivalence point are higher on W than on Pt. Tungsten oxides increase the inflexion pd. The pair is also suitable for oxidation/reduction titrations using permanganate, dichromate, and ceric sulfate, and for precipitation reactions with silver nitrate. However, it gives a small inflexion pd in the oxidation of thiosulfate by iodine. EDTA titrations of copper, magnesium, and calcium give a sharp inflexion in the case of copper only. This pair may be useful in routine analyses requiring a robust electrode assembly but not a high degree of accuracy.

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Spectrophotometric Determination of Rhodium Using Acenaphthenequinone Monoxime (AQM)

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Numerous organic reagents have been employed for the spectrohotometric determination of rhodium. A critical evaluation of the different methods has been made by Beamish *(1)*. Among the most important and the recently introduced reagents are stannous chloride *(1)*, 5-amino-2-mercapto-benzimidazole *(1)*, *N*, *N'*-bis(3-dimethylamino propyl) dithiooxamide *(1)*, *p*-nitrosodimethylaniline *(2)*, thiosalicylamide *(3)*, oximidobenzotetronic acid *(4)*, chrome azurol *(5)* and 1-(2-pyridylazo)-2-naphthol *(6)*.

Acenaphthenequinone monoxime forms colored complexes and precipitates with many metal ions *(7)*. It has been used for the spectrophotometric determination of osmium *(8)*. In the present study, a water-insoluble complex of rhodium with acenaphthenequinone monoxime has been investigated after extracting into chloroform.

EXPERIMENTAL METHODS

REAGENTS AND EQUIPMENT

AQM solution. The requisite amount of AQM was weighed and the solution (1×10^{-2} *M*) was prepared in ethanol. Working solutions were prepared from this stock solution by dilution.

Rhodium Solution. A standard rhodium solution was prepared by dissolving rhodium trichloride (Johnson Matthey) in 1 *M* hydrochloric acid. The rhodium content was determined gravimetrically by precipitating rhodium as the sulfide, followed by ignition to the oxide and then reduction to the metal in a current of hydrogen and cooling in carbon dioxide. One ml of this solution contained 2.02 mg of rhodium. Subsequent dilutions were made from this stock solution according to requirements.

Chloroform. Chloroform (B.D.H., AnalaR) was freshly distilled before use. In no case was chloroform, which had been stored for more than 1 week, used without distillation.

Reagent grade chemicals were used in the study of interferences.

Buffers. Buffers were prepared by mixing 0.2 *M* sodium acetate and 0.2 *M* acetic acid in requisite proportions.

Unicam, SP 600, spectrophotometer was used for taking absorbance readings. Measurements of pH were made on a Metrohm pH meter, type E-350. A Tempo (India) electric shaking machine was employed for shaking the solutions.

Procedure for Extration of the Complex

To a solution of rhodium(III) is added an excess of AQM in ethanol. pH is then suitably adjusted with buffer. The contents are heated on a boiling water bath under reflux and cooled. The complex, extracted into the chloroform layer, is separated, centrifuged, and its absorbance is read.

Absorption spectra. Absorption spectra of the reagent and a series of solutions, containing a fixed amount of rhodium and excess of the reagent (10 itmes) at different pH values are recorded in Fig. 1. The contents were extracted into 15 ml of chloroform in each case and absorbance was read against reagent blank. The curve shows an ab-

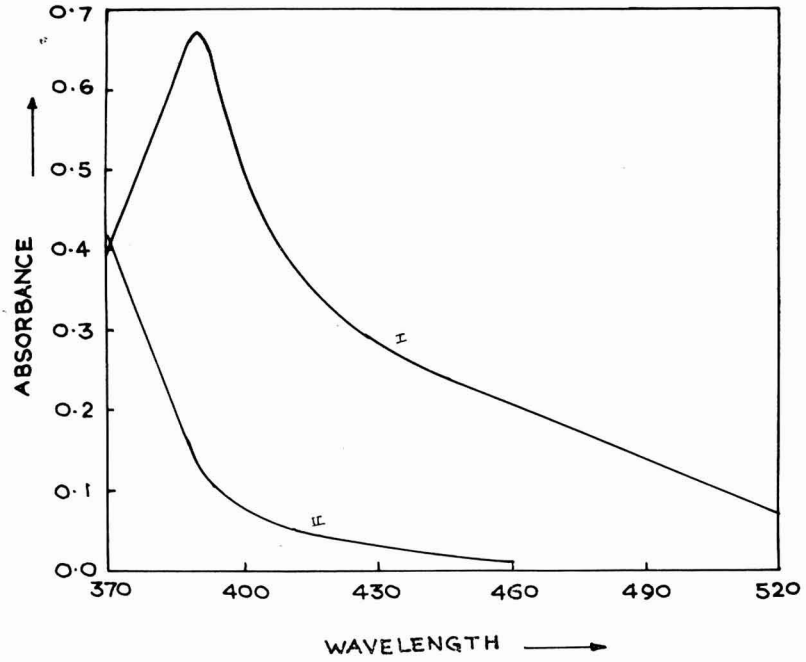

Fig. 1. Absorption spectra of reagent and its rhodium complex. (I) rhodium complex vs reagent; (II) reagent alone vs chloroform; rhodium, 2.5 × 10^{-5} *M*; reagent, 6.6 × 10^{-4} *M*.

sorption maximum at 390 nm. Wavelength of 390 nm was, therefore, employed in subsequent measurements. As the reagent absorbs at this wavelength, ligand blanks were used in all studies. Below pH 2.0 and abov pH 8.0, very little color formation seemed to take place. The nature of all absorption curves is similar, which shows the formation of only one complex under the conditions of study.

Sequence of additions of reagents. It has been found that the order of addition of reagents is very important and the following sequence must be strictly adhered to: rhodium solution, reagent, and buffer solution. Beamish *(9)* has stressed the importance of this point in such cases. If the order of addition is changed, low values are obtained arising probably from the appearance of colloidal hydrated oxide, which are usually insensitive to the color reagent.

Recommended Procedure

Suitable aliquot of the sample solution containing between 12 to 25 μg of rhodium is taken in a Pyrex stoppered bottle and 10 times excess of reagent in ethanol is added, followed by 10 ml of sodium acetate–acetic acid buffer solution to give the desired pH values, viz, between 4.4 to 6.0. The contents are made to 15 ml and heated on a water bath for nearly 2 hours. After cooling to room temperature, the contents are extracted in 15 ml of freshly distilled chloroform by shaking on a shaking machine for nearly 10 minutes. The phases are separated, centrifuged to get rid of any droplets of water present in the organic phase and the absorbance is measured against reagent blank. Knowing the absorbance of the chloroform extract, the rhodium content is deduced from the calibration curve drawn under identical conditions.

Effect of pH. The effect of pH on color development was studied by preparing a number of samples varying in pH from 2 to 8. Absorbance measurements were made at 390 nm. A constancy in absorbance was obtained over the pH range 4.40 to 6.0. At higher or lower pH values, absorbance of the complex decreases, Subsequent studies were made at pH 4.80.

Effect of reagent concentration. It has been found that 6 moles of reagent/mole of rhodium are sufficient for complete color development, beyond which a constancy in absorbance was observed. However, 10 times excess was used in subsequent studies for full color development.

Effect of time of heating and stability. The reaction of rhodium and AQM at room temperature is very slow and color development was found to be insignificant even on standing for several hours. Aliquot amounts of rhodium solution, reagent, and buffer were heated on a water bath for different intervals of time and it was found that com-

plete color formation takes place after 2 hours. The complex is stable in solution and no change in the absorbance of chloroform extracts was observed even after 2 days.

Adherence to Beer's law and physical constants. The system was found to adhere to Beer's law up to 3.087 ppm of rhodium. The optimum concentration range, for determination of rhodium, as evaluated by Ringbom's method, was found to be 1.20–2.572 ppm. The sensitivity of the reaction, as calculated from Beer's plot, is 0.0036 $\mu g/cm^2$ at 390 nm for log I_o/I 0.001. The molar extinction coefficient of the complex is 27,300, as evaluated from Beer's law plot.

Composition of the complex. The composition of the complex was determined by Job's method of continuous variations *(10)*. For this purpose, a series of solutions was prepared by mixing equimolar solutions of rhodium and acenaphthenequinone monoxime, in which the sum of the molar concentration of the reactants was constant, while their ratio varied. The curve obtained indicates the formation of a complex of rhodium in which the metal:ligand ratio is 1:3 (Fig. 2). The results obtained by the mole ratio method *(11)* gave the same metal:ligand ratio in the complex, viz, 1:3 (Fig. 3).

Stability constants of the complex. The stability constant calculated by using the conventional relationships *(12)* $(E_m - E_s)/E_m$, and K $[C(1 - \alpha)/\alpha C)(nC)^n]$, was found to be 1.17×10^{16} at room temperature and pH 4.80 (where C is the concentration of the complex

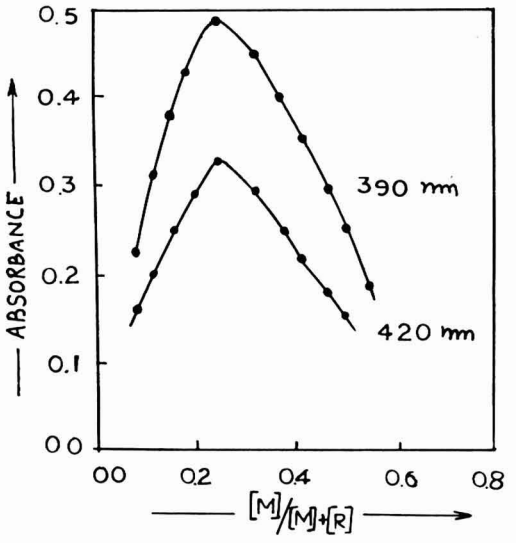

FIG. 2. Composition of complex by Job's method: total molarity, 5.33×10^{-5} *M*.

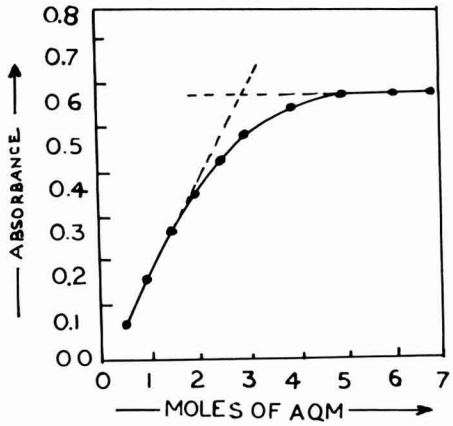

FIG. 3. Composition of rhodium–AQM complex by mole ratio method.

in moles/liter and n is 3). The values of E_m and E_s were obtained from the mole ratio plot (Fig. 3), where E_s is the absorbance at the mole ratio 1:3 and E_m is the absorbance that is obtained at the point in the absence of dissociation.

Absorbance Deviations. Measured under experimental conditions, the absorbance of solutions containing 2.572 ppm of rhodium gave an average absorbance deviation value ± 0.002 and maximum absorbance deviation value ± 0.006. The average relative percentage error was ± 0.34, and maximum relative percentage error was ± 0.89.

Effect of Diverse Ions. To study the effect of diverse ions in the determination of rhodium, solutions were prepared containing 1.72 ppm of rhodium, known concentration of ion to be tested, and excess of reagent. The solutions were buffered to pH 4.8, extracted in chloroform, and absorbance of chloroform extracts were measured. EDTA, NTA, thiocyanate, gold(III), iridium, platinum(IV), copper, iron, and cerium(IV) caused serious interference. Attempts to mask them were unsuccessful. The interference due to cobalt, palladium, and nickel were obviated by precipitating them in cold and extracting the complexes into chloroform before heating. After the removal of these metals, the aqueous phase was buffered and rhodium determined.

With 1.72 ppm of rhodium, the following ions present in amounts shown in parantheses did not cause any interference: tartrate (160), phosphate (160), oxalate (400), fluoride (1000), bromide (320), nitrite (80), pyrophosphate (100), nitrate (2480), sulfate (1000), borate (120), sulfite (100), bromate (200), antimony(III) (80), tellurium(VI) (60), ruthenium(III) (0.1), palladium (II) (4), cobalt(II) (10), nickel(II) (40), zinc(II) (80), manganese(II) (50),

TABLE 1

SENSITIVITIES OF DIFFERENT METHODS FOR THE DETERMINATION OF RHODIUM

Method	Sensitivity (μg of Rh/cm^2)	at (nm)
Tin(II) bromide	0.0035	427
Tin(II) chloride	0.026	479
5-Amino-2-mercapto-benzimidazole	0.0084	430
	0.004	390
N-*N*′-bis(3-dimethylaminopropyl)dithiooxamide	0.012	420
p-Nitrosodimethylaniline	0.0015	510
Thiosalicylamide	0.014	377
Oximidobenzotetronic acid	0.07	475
Thiosalicylic acid	0.0208	365
Chrome azurol-S	0.008	570
AQM (present reagent)	0.0036	390

silver (20), uranium(VI) (20), bismuth(III) (1), osmium(VIII) (2), mercury(II) (60),[1] tin(II),[2] and aluminium(III) (10).

DISCUSSION

Very few satisfactory methods are available for spectrophotometric determination of rhodium. One of the most sensitive reagents reported so far is tin(II) bromide. The sensitivity is 0.0035 μg/cm^2, but the composition of the complex is not known. The color is stable for 3 hours. The use of tin(II) chloride for the determination has also been reported. Several metals, especially those of the eighth group, interfere in this method.

Perhaps the most sensitive reagent for rhodium is *p*-nitrosodimethylaniline which has been introduced by Wilson and Jacobs(2). The color develops within 10 minutes of heating. The volume of buffer solution used was found to have a pronounced effect on the absorbance of the complex.

Recently, the use of thiosalicylamide and oximidobenzotetronic acid has been reported for the spectrophotometric determination of rhodium. Ruthenium and osmium interfere in the case of thiosalicylamide but selectivity is high in the case of oximidobenzotetronic acid. Both the reagents lack sensitivity. The chief disadvantage of thiosalicylamide is its susceptibility to oxidation in presence of strong oxidizing agents.

Acenaphthenequinone monoxime has successfully been used for

[1] Phosphate used as masking agent.

[2] Fluoride used as masking agent; silver removed by precipitating as chloride.

determination of micro quantities of rhodium. It is highly sensitive and in selectivity it compares well other reagents. Small amounts of eighth group metals do not interfere. Effect of buffer, pH, and heating time are not critical factors as they are in some other methods. Sensitivities of some well known and widely employed methods are compared with AQM in Table 1.

SUMMARY

Acenaphthenequinone monoxime forms a yellow coloured complex with rhodium which is extractable into chloroform. The complex exhibits maximum absorption at 390 nm. The composition of the complex, as determined by Job's and mole ratio methods comes out to be 1:3. The pH for maximum colour development is 4.4 to 6.0. The sensitivity of the colour reaction is 0.0036 μg Rh/cm^2 at 390 nm. The effect of various foreign ions has been investigated. The method is very sensitive and selective for determination of rhodium.

ACKNOWLEDGMENT

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Titration of Aspartic Acid with Mercuric Chloride

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Northrop (*1*) and Dimick (*2*) suggested titrimetric methods for the quantitative estimation of amino acids by formol titration and ninhydrin–carbon dioxide reaction, respectively. Arhimo (*3*) estimated aspartic acid by oxidation and bromination methods. According to Pucher (*4*), aspartic acid, free from tyrosine, is treated with bromine and potassium permanganate, and the resulting compound, dibromoacetaldehyde, is then estimated colorimetrically with dinitrophenyl hydrazine by Suomalainen (*5*). Later on Libicky (*6*) gave a direct titrimetric method for the determination of the amino acid with cupric sulphate solution.

A number of schemes for the quantitative estimation of aspartic acid have been proposed, but, still there is no procedure for the direct titration of aspartic acid in the presence of other natural amino acids. This paper describes a method for the complexometric titration of aspartic acid in microquantities separately, as well as in the presence of other natural amino acids, such as lysine, methionine, tryptophane, tyrosine, glutamic acid, glycine, alanine, asparagine, cystine, cysteins, arginine, norleucine, ornithine, histidine, threonine, leucine, and serine.

It has been observed that at pH 4.0, aspartic acid combined quantitatively with the mercuric ion. Further, it was found that at the above pH range, 1-(2-pyridyl-azo)-2-naphthol (PAN) reacted with the mercuric ion resulting in a pink-colored complex. To a mixture of buffered solution with aspartic acid, using PAN as indicator, which was yellow in color, when solution of $HgCl_2$ was added, no PAN + Hg complex was formed till all the aspartic acid present in the mixture completely reacted with mercuric ion, whereafter, the excess of Hg^{2+} resulted in the formation of a pink-colored complex. This change from yellow to pink was taken as the end point of the reaction. Titrations were carried out with 0.01 to 0.02 *M* aspartic acid employing a 0.01 to 0.0033 *M* metal-ion solution of mercuric chloride and gave almost quantitative results. The presence of other natural amino acids in the reaction mixture does not interfere with the titrations.

EXPERIMENTAL METHODS

Aspartic acid (E. Merck, at 0.01 and 0.02 *M*) was prepared in double distilled water.

Mercuric chloride (A. R., 0.01 and 0.0033 *M*) was dissolved in double distilled water.

Five milligrams of 1-(2 Pyridyl-azo)-2-naphthol was dissolved in absolute alcohol and made up to 100 ml.

Buffer solutions of 0.1 *M* citric acid and 0.1 *M* sodium citrate were made in double distilled water; a pH of 4.0 was used.

Procedure

The titration was carried out by employing semimicropipettes and burettes with minimum division up to 0.01 ml.

Two milliliter samples of citrate buffer of pH value 4.0 were placed in different beakers with different volumes of standard aspartic acid solution in each beaker, and 0.10 ml of dye (PAN) and 5 ml of doubly distilled water were added in each mixture. A standard solution of mercuric chloride was added in each beaker from a microburette till a sharp change in color (from yellow to pink) was observed at the end point. The titrations were also carried out at 0.01 and 0.02 *M* of aspartic acid with 0.01 and 0.0033 *M* of metal-ion solutions at the same pH value.

The stability of the reaction at pH 2–9 was investigated, however, the best result of the change in color at the equivalence point was obtained at pH 4.0.

Further, the titration was performed to estimate the amount of aspartic acid in a mixture containing other amino acids such as lysine, methionine, tryptophane, tyrosine, glutamic acid, glycine, alanine, asparagine, cystine, cysteine, arginine, norleucine, ornithine, histidine, threonine, leucine, and serine. It was observed that these amino acids, even when present together, do not interfere in the titration, and this process for the estimation of aspartic acid could be used even in the presence of other natural amino acids.

DISCUSSION

Tables 1, 2, and 3 indicate that aspartic acid can be estimated quantitatively by direct complexometric titration against a standard solution of $HgCl_2$ using 1-(2-pyridyl-azo)-2-naphthol as indicator in citrate buffer medium at pH 4 within a fairly wide range of aspartic acid. This is plotted in Fig. 1. Tables 4 and 5 showed that titration of aspartic acid can also be carried out in the presence of natural amino acids, such as lysine, methionine, tryptophane, tyrosine, glutamic acid, glycine,

TABLE 1

TITRATION OF 0.01 *M* ASPARTIC ACID AGAINST 0.01 *M* MERCURIC CHLORIDE USING PAN AS INDICATOR

Aspartic acid (ml)	Mercuric chloride (ml)	Amount of aspartic acid (mg)	
		Obsd	Calcd
1.00	0.32	1.33	1.33
2.00	0.64	2.66	2.66
3.0	0.96	3.99	3.99
4.0	1.28	5.32	5.32
5.00	1.61	6.65	6.69
6.00	1.92	7.98	7.98

TABLE 2

TITRATION OF 0.02 *M* ASPARTIC ACID SOLUTION WITH 0.01 *M* MERCURIC CHLORIDE USING PAN AS INDICATOR

Aspartic acid (ml)	Mercuric chloride (ml)	Amount of aspartic acid (mg)	
		Obsd	Calcd
0.5	0.32	1.33	1.33
1.0	0.64	2.66	2.66
1.5	0.98	3.99	3.99
2.0	1.28	5.32	5.32
2.5	1.60	6.65	6.65
3.0	1.92	7.98	7.98

TABLE 3

TITRATION OF 0.01 *M* ASPARTIC ACID WITH 0.0033 *M* MERCURIC CHLORIDE USING PAN AS INDICATOR

Aspartic acid (ml)	Mercuric chloride (ml)	Amount of aspartic acid (mg)	
		Obsd	Calcd
1.00	0.96	1.33	1.33
2.00	1.92	2.66	2.66
3.00	2.88	3.99	3.99
4.00	3.85	5.32	5.33
5.00	4.80	6.65	6.62

TABLE 4

TITRATION OF VARYING QUANTITIES OF ASPARTIC ACID IN THE PRESENCE OF OTHER AMINO ACIDS AGAINST MERCURIC CHLORIDE SOLUTION USING PAN AS INDICATOR

Mixture of amino acids (ml)	Aspartic acid 0.01 *M* (ml)	Mercuric chloride 0.01 *M* (ml)	Amount of aspartic acid (mg)	
			Obsd	Calcd
1.00	1.00	0.32	1.33	1.33
1.00	2.00	0.64	2.66	2.66
1.00	3.00	0.96	3.99	3.99
1.00	4.00	1.28	5.32	5.32
1.00	5.00	1.60	6.65	6.65

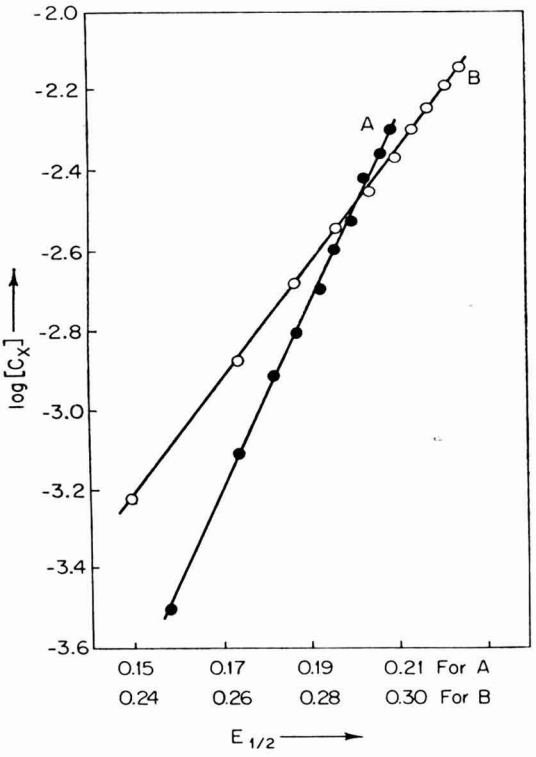

FIG. 1. Log plot of the concentration of complexing agent against $E_{1/2}$: (A) mercuric chloride concentration as in Tables 1 and 2, (B) mercuric chloride concentration as in Table 3.

TABLE 5

TITRATION OF ASPARTIC ACID IN THE PRESENCE OF VARYING QUANTITIES OF OTHER AMINO ACIDS AGAINST STANDARD MERCURIC CHLORIDE USING PAN AS INDICATOR

Mixed amino solution (ml)	Aspartic acid 0.01 *M* (ml)	Mercuric chloride 0.01 *M* (ml)	Amount of aspartic acid (mg)	
			Obsd	Calcd
0.50	1.00	0.32	1.33	1.33
1.00	1.00	0.32	1.33	1.33
1.50	1.00	0.32	1.33	1.33
2.00	1.00	0.32	1.33	1.33
2.50	1.00	0.32	1.33	1.33

alanine, asparagine, cystine, cysteine, arginine, norleucine, ornithine, histidine, threonine, leucine, and serine without their interference. Further it was found that a complex is formed with aspartic acid and mercuric chloride in the ratio of 1:3.

ACKNOWLEDGMENT

We are thankful to Dr. A. E. Smith of Sir George Williams University for supplying us with the indicator.

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MICROCHEMICAL JOURNAL 18, 699–702 (1973)

Titration of L-Cysteine Hydrochloride with Standard Nickel Sulphate Solution Employing 1-(Pyridyl-2′-azo)-naphthol-(2) as Indicator

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Received May 7, 1971

For about 40 years scientists suggested different methods for the estimation of cysteine. Baernstein (*1*) in 1930 furnished a method of gasometric estimation for cysteine and cystine. Hellerman *et al.* (*2*) in 1941 estimated cysteine and some other compounds in proteins. Colorimetric estimation of cysteine was performed by Nakamura *et al.* (*3*) in 1948. Thus efforts had been made from time to time. Cysteine and glutathione, in the presence of each other, were estimated by Wronski (*4*) in 1965. Santi *et al.* (*5*) in 1968 determined small amounts of thioamino acids potentiometrically.

But these methods do not represent any procedure of direct estimation of cysteine in micro amounts separately and also in the presence of other natural amino acids. This paper reports a procedure of such a direct titration of cysteine hydrochloride.

It was observed that cysteine hydrochloride combines with nickel sulphate quantitatively at pH 10.0. On the other hand, 1-(pyridyl-2′-azo)-naphthol-(2) (PAN) combines with nickel sulphate forming a complex of transient purple–violet color. If a solution of nickel sulphate is added to a mixture of cysteine hydrochloride, buffer of pH value 10.0 and PAN dye as indicator from a microburette, the reaction takes place in this way: PAN + Ni complex is not formed till all the cysteine hydrochloride has reacted with nickel. After this reaction, the excess of nickel reacts with PAN and forms a complex of transient purple-violet color. This sharp change from yellow to permanent purple-violet has been used to note the end point. The titration can be carried out very easily from 0.0001 to 0.01 *M* cysteine hydrochloride. The presence of other natural amino acids in the mixture does not interfere with the titration of cysteine by this procedure.

EXPERIMENTAL

Solutions of Cysteine hydrochloride, 0.0001, 0.001, and 0.01 *M* were prepared in glass-distilled water.

TABLE 1

TITRATION OF 0.0001 *M* CYSTEINE HYDROCHLORIDE AGAINST 0.0001 *M* NICKEL SULPHATE USING PAN AS INDICATOR

Cysteine hydrochloride (ml)	Nickel sulphate (ml)	Amount of cysteine hydrochloride (mg)	
		Obsd	Calcd
1.0	0.70	0.01	0.01
2.0	1.40	0.03	0.03
3.0	2.20	0.04	0.04
4.0	2.80	0.06	0.06
5.0	3.50	0.07	0.07

Nickel sulphate solutions (B.D.H.) (0.0001, 0.001, and 0.01 *M*) were prepared in glass-distilled water.

Five milligrams of 1-(pyridyl-2′-azo)-naphthol-(2) from E. Merck was dissolved in absolute alcohol and made to 100 ml.

Buffer solutions of 0.2 *M* anhydrous sodium carbonate and 0.2 *M* sodium bicarbonate were prepared in glass-distilled water; pH 10.0 was used.

PROCEDURE

Titrations were carried out by using micropipettes and microburettes with minimum division up to 0.01 ml.

In a beaker, 1.0 ml of cysteine hydrochloride (0.0001 *M*), 0.2 ml of PAN dye as indicator and 10.0 ml of buffer of pH 10.0 was taken. The color of the reaction mixture was yellow (smaller amounts of PAN dye produce a pinkish color). To this reaction mixture, nickel sulphate solution (0.0001 *M*) was added by a microburette. As nickel sulphate was added to it, the appearance of the pinkish color darkened on further addition of nickel sulphate solution to a permanent transient

TABLE 2

TITRATION OF 0.001 *M* CYSTEINE HYDROCHLORIDE AGAINST 0.001 *M* NICKEL SULPHATE USING PAN AS INDICATOR

Cysteine hydrochloride (ml)	Nickel sulphate (ml)	Amount of cysteine hydrochloride (mg)	
		Obsd	Calcd
1.0	0.70	0.15	0.15
2.0	1.40	0.31	0.31
3.0	2.10	0.47	0.47
4.0	2.80	0.63	0.63
5.0	3.50	0.78	0.78

TABLE 3

TITRATION OF 0.01 *M* CYSTEINE HYDROCHLORIDE AGAINST 0.01 *M* NICKEL SULPHATE USING PAN AS INDICATOR

Cysteine hydrochloride (ml)	Nickel sulphate (ml)	Amount of cysteine hydrochloride (mg)	
		Obsd	Calcd
1.0	0.70	1.57	1.57
2.0	1.40	3.15	3.15
3.0	2.10	4.72	4.72
4.0	2.80	6.30	6.30
5.0	3.50	7.88	7.88

purple-violet, and this change was taken as the endpoint. In the same way, experiments were performed by taking different amounts of cysteine hydrochloride. The results are summarized in Table 1.

Titrations with 0.001 and .01 *M* cysteine hydrochloride were carried out against 0.001 and 0.01 *M* nickel sulphate solutions, respectively. These results are given in Tables 2 and 3.

The pH values ranging from 2.0 to 10.0 had also been tried, but the best results were obtained at pH value 10.0.

Further experiments were performed to determine the microamount of cysteine hydrochloride together with the mixture of different natural amino acids as DL-alanine, L-arginine hydrochloride, L-asparagine monohydrate, DL-aspartic acid, L-cystine, glutamic acid, glycine, L-hydroxyproline, DL-isoleucine, DL-leucine, DL-methionine, DL-norleucine, DL-ornithine hydrochloride, DL-phenylalanine, L-proline, DL-serine, DL-threonine, L-tyrosine, DL-tryptophane, and DL-valine. The mixture of amino acids did not interfere. These results are given in Tables 4 and 5.

TABLE 4

TITRATION OF VARIFYING QUANTITIES OF CYSTEINE HYDROCHLORIDE IN PRESENCE OF OTHER AMINO ACIDS AGAINST STANDARD NICKEL SULPHATE USING PAN AS INDICATOR

Mixture of amino acids (ml)	Cysteine hydrochloride 0.01 *M* (ml)	Nickel sulphate 0.01 *M* (ml)	Amount of cysteine hydrochloride (mg)	
			Obsd	Calcd
1.0	1.0	0.70	1.57	1.57
1.0	2.0	1.40	3.15	3.15
1.0	3.0	2.10	4.72	4.72
1.0	4.0	2.80	6.30	6.30
1.0	5.0	3.60	7.88	7.88

TABLE 5
TITRATION OF CYSTEINE HYDROCHLORIDE IN PRESENCE OF VARIFYING QUANTITIES OF OTHER AMINO ACIDS AGAINST STANDARD NICKEL SULPHATE USING PAN AS INDICATOR

Mixture of amino acids (ml)	Cysteine hydrochloride 0.01 *M* (ml)	Nickel sulphate 0.01 *M* (ml)	Amount of cysteine hydrochloride (mg)	
			Obsd	Calcd
1.0	1.0	0.70	1.57	1.57
2.0	1.0	0.70	1.57	1.57
3.0	1.0	0.70	1.57	1.57
4.0	1.0	0.70	1.57	1.57
5.0	1.0	0.70	1.57	1.57

DISCUSSION

On the basis of Tables 1, 2, and 3, it is noticed that cysteine hydrochloride can be estimated quantitatively against a standard solution of nickel sulphate using PAN dye as indicator in a buffer medium of pH 10.0. Tables 4 and 5 show that there is no interference of other natural amino acids in this titration even when these amino acids were present in much greater concentrations than cysteine. A complex is formed with a ratio of nickel and cysteine hydrochloride of 2:3.

SUMMARY

The estimation of cysteine hydrochloride can be carried out quantitatively against standard nickel sulphate solution using 1-(pyridyl-2′-azo)-naphthol-(2) in carbonate–bicarbonate buffer medium of pH 10.0. Other natural amino acids have no interference with the titration.

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MICROCHEMICAL JOURNAL 18, 703–705 (1973)

Book Reviews

Advances in Inorganic Chemistry and Radiochemistry, Vol. 15. Edited by H. J. EMELÉUS AND A. G. SHARPE. Academic Press, New York, 1972. vii + 451 pp. $24.00.

This 15th volume, an excellent continuation of the well-known "Advances in Inorganic Chemistry and Radiochemistry" series, presents authoritative up-to-date reviews on topics of the most current interest in the advances of the structural inorganic chemistry. It was gratefully accomplished by the splendid cooperation of a number of experts in the fields concerned and under the overall supervision of Professor H. J. Emeléus. It consists of five special topics under the headings of secondary bonding to nonmetallic elements; Mössbauer spectra of inorganic compounds: bonding and structure; metal alkoxides and dialkylamides; fluoroalicyclic derivatives of metals and metalloids; the sulfur nitrides; and so forth. All of these articles are well written and the treatment of the subject matter is comprehensive, critical, thoughtful, and in great detail. In addition, a total of 1350 references to the literature has been cited. Author index, subject index, and contents of the previous volumes are also available for the reader to use the text and the series conveniently and effectively.

The first article (58 pp., 189 refs.) by Professor Alcock discusses the secondary bonding to nonmetallic elements. The secondary bonding is defined on the basis of an approximately linear interactions of the bonds: Y—A···X with a single dash "Y—A" for a normal bond and triple dashes "A···X" for short secondary interactions. Although the only conclusive method of establishing the presence of secondary interactions is by crystal structure determination, an intermolecular interaction can be recognized as being significant by being shorter than the expected intermolecular distance. Therefore, by a comparison of a secondary bond length with the standard single-bond length a useful estimate of the strength of the secondary bond has been logically made. These comparisons, together with the bond angles and their original references for 110 compounds, classifying into Group VIII, VII, VI, V, IV, and cyanides, etc., according to the location of the constituent elements in Periodic Tables, are summarized and tabulated in four tables. Under each group, several typical examples are well examined and clearly illustrated with the aids of pictorial models. Thus, by including secondary bonding, the whole range of chemical interaction can be brought into one conceptual group, that is, the nature of the bonding is controlled by the electronegativity of the participating atoms.

The second article (200 pp., 577 refs.) by Professor Bancroft and Professor Platt is very interesting and dealing with Mössbauer spectra of inorganic compounds. Mössbauer spectroscopy considers transitions between nuclear energy levels with the recoilless emission and absorption of γ rays, that is, the nuclear resonance fluorescence of γ radiation or Mössbauer effect. In this chapter, some theoretical fundamentals and mathematic derivatives of the use of two Mössbauer parameters, the isomer or center shift (C.S.) and quadrupole splitting (Q.S.) are well illustrated and clearly discussed. In principles, the C.S. measures the *s* electron density at the nucleus of the metal, while the Q.S. is related to the asymmetry of the electric field at the nucleus, which is influenced by the electron configuration of the metal atom (Mössbauer atom) and its environment (ligands). On the basis of the measurements of these two Mössbauer parameters, a great number of experimental data concerning the fingerprint uses and the elucidation of the structure and bonding for about 1486 compounds have been extensively surveyed and tabulated in 59 tables. The majority of the information thus listed includes a large number of halide and coordination compounds of the Mössbauer

atoms such as Sn^{II} and Sn^{IV}; Fe^{II} high- and low-spin: Fe^{III} high- and low-spin: Fe^{0}, Fe^{-1} and Fe^{-II}; Ru^{III}; Ir^{III}; ^{127}I and ^{129}I; Au^{I} and Au^{III}; Sb^{III} and Sb^{V}; ^{125}Te; and ^{129}Xe; etc. The theoretical interpretation and practical demonstration of the bonding and structure for compounds of each category are so comprehensive, complete, and critical that this chapter really provides the most valuable information to the theoretical and structural inorganic chemists, coordination chemists, and synthetic chemists.

The third article (64 pp., 235 refs.) by Professor Bradley reviews metal alkoxides and dialkylamides. The metal alkoxides $M(OR)_x$ have potential industrial applications as components of soluble Ziegler–Natta catalysts for olefin polymerization and also as sources for the production of pure metal oxides. The materials reviewed covers the alkali metal alkoxides; alkoxides of Be, Mg, Zn, and the alkaline earths; Al and Ga alkoxides; transition metal alkoxides; alkoxides of lanthanides and actinides; double alkoxides (derivatives containing two different metals, i.e., $KZn(OMe)_3$); and metal trialkylsilyloxides $M(OSiR_3)_x$. The qualitative discussion is emphasized on the physicochemical and structural properties of alkoxides, the ligand field aspects of the alkoxo group (i.e., electronic spectra, magnetism, etc.) and on X-ray crystallographic and nmr structural determination. While the metal dialkylamides $M(NR_2)_x$ are of special interest in that they contain covalent metal–nitrogen bonds and occupy a position between metal alkoxides and metal alkyls. These compounds possess a considerable versatility in using as synthetic reagents and remain to be further investigated. In this chapter, the preparative methods; the chemical and physical properties; ir, Raman, NMR, and ESR spectra; structural determination by X-ray or electron diffraction, etc., are concisely described and critically reviewed.

The fourth article (52 pp., 220 refs.) by Professor Cullen is concerned with the preparation and properties of alicyclic fluorocarbon (fluoroalicyclic) derivatives of metal and metalloids. The many preparative methods, including carbene and carbenoid additions, cycloaddition reactions, hydride additions, metal–fluoride additions, metal–alkyl and metal–aryl additions, reactions with compounds containing metal–metal bonds, oxidative addition reactions, direct reaction with a metal, exchange reactions, and preparation by modification of existing fluoroalicyclic derivatives, and so forth, are comparatively demonstrated with structural formula and chemical equations. Some chemical properties and reactivities, thermal stability, and methods of characterization by ir and nmr for compounds of the category are also briefly discussed. In addition, many coordination complexes derived from fluoroalicyclic-bridged ditertiary phosphines and arsines with a variety of chelate capacity of monoligate, biligate monometallic, biligate bimetallic, and triligate bimetallic are extensively reviewed and discussed from the structural aspects. Consequently, this chapter should be found of particular interest to the synthetic, organometallic, and coordination chemists.

The last article (38 pp., 129 refs.) by Professor Heal considers the chemistry of the sulfur nitrides. Sulfur nitride, SN, or thiazyl monomer, is a radical with one unpaired electron. It has only a transient existence in the gaseous phase and polymerizes so rapidly that it cannot be isolated as monomeric solid or liquid. However, in this chapter, the preparation methods, physicochemical properties, molecular and electronic structure, spectra characteristics, thermochemical behaviors, oxidation-reduction reactions, reactions with electrophiles and nucleophiles, the Diels–Alder type addition reactions, and so forth, for the best-known tetrasulfur tetranitride, S_4N_4, are completely demonstrated. Other compounds such as S_2N_2; $(SN)_x$; S_4N_2; the coupled-ring nitrides, $S_7N—S_x—NS_7$; the fused-ring nitrides, $S_{11}N_2$; and polymeric saturated sulfur nitrides are also briefly reviewed. Furthermore, it is concluded that one promising area of research is the use of S—N compounds of metals for synthesis of new sulfur nitrides. The recently investigated anions of the sulfur imides are also attractive starting materials for new synthesis. The preparative-scale molecular exclusion chromatography offers a new experimental technique for successful synthesis of compounds of this category.

In summary, in view of the well-established traditional disciplines of the series, it is not necessary for the reviewer to recommend this volume to the reader again at this time. The authors and the editors are to be sincerely congratulated on having done such excellent work in surveying and compiling this volume. Therefore, this volume should be considered as an invaluable reference book not only for inorganic and radiochemists but also for theoretical and structural chemists, organometallic and synthetic chemists, and coordination chemists.

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Metallurgical Microscopy. By H. MODIN AND S. MODIN. Wiley, New York, 1973. 473 pp. $42.50.

This volume translated from the original Swedish by G. G. Kinnane could not possibly have lost anything in the translation for it is truly an excellent book. Even the novice could instantly follow the very complete, lucid and systematic approach laid down by the Modins for the microscopical examination of metallurgical samples and, if he went by the book, produce some very presentable results. Would that I had read it when I first encountered metallography! Reading it was both educational and delightful: Educational both in its practical and its theoretical treatment of the subject and delightful for its concise, succinct style which leaves no room for ambiguity and, throughout, points out the possible pitfalls. It was as if the authors had leaned over my shoulder when I was learning the hard way, had noted everything I had done wrong and were now showing me the right way to do it.

The complete range of light and electron microscopic examination is covered from sample preparation through to the finished micrograph, with the applications and limitations of each alley-way—phase contrast, polarization, interference contrast, microhardness, etc.—adequately discussed.

The quality of the many micrographs and line drawings is excellent and no effort has been spared on the captions, which are self-explanatory.

Altogether, a very worthwhile book which will be used by beginner and expert alike. Not a book for your library, it should live on your workbench.

IAN M. STEWART, *Walter C. McCrone Associates, Chicago, Illinois 60616*

Phytochemistry. Vol. 1. Edited by LAWRENCE MILLER. Van Nostrand Reinhold, New York, 1973. 375 pp. + index. $22.50.

This is the first of three volumes on the general subject of phytochemistry. The first volume is devoted to the photosynthetic apparatus and to the important carbohydrates of plants. Subsequent volumes will deal with other organic metabolites, and inorganic elements and special groups of compounds. The editor has brought together a distinguished group of scientists, each of whom has clearly communicated the information in his field of expertise. Most of the chapters, such as the one on glycosides, are presented in depth and contain numerous literature citations that should be valuable to investigators. The chapter on photosynthesis is a well-organized introduction to photosynthetic carbon metabolism, but it is not quite up to date with respect to two-carbon metabolism. The time required to produce a volume of this type makes problems such as this unavoidable in a field where intensive research is being conducted. Although this first volume will be useful as a reference, the complete three-volume series will undoubtedly have its greatest value as a textbook in phytochemistry.

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Announcements

4th Polish Conference on Analytical Chemistry
Warsaw, Poland
August 26–31, 1974

Contact:

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7th Materials Research Symposium on Accuracy in Trace Analysis: Sampling, Sample Handling, and Analysis
National Bureau of Standards
Gaithersburg, Maryland 20760
October 7–11, 1974

Contact:

Dr. Philip D. LaFleur
B108 Reactor Building
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Roselle Coviello, Vice President

Author Index

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INTRODUCTION TO AIR CHEMISTRY

By SAMUEL S. BUTCHER
Bowdoin College
Brunswick, Maine

ROBERT J. CHARLSON
University of Washington
Seattle, Washington

Here is a book on atmospheric chemistry specifically designed for those with no experience in the field — one that will introduce the reader to *successful* approaches to this important field. It covers air pollution, chemical cycles, and the chemical and physical behavior of trace atmospheric constituents. It uses the principles of both analytical chemistry and meteorology to cope with the most crucial problems of our atmospheric environment.

The first two chapters introduce the reader to air chemistry and outline the basic chemical and meteorological principles used throughout the book. The authors then discuss general methods of obtaining and evaluating air chemical data, emphasizing some of the analytical methods now available. Next, they deal with three main classes of chemical compounds (those containing sulfur, nitrogen, and carbon) which must be considered in the study of atmospheric trace constituents — covering significant atmospheric reactions, global budgets, and selected methods of analyzing these compounds. The final chapter describes the physical characteristics of aerosols.

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2. Pease, D. C., "Histological Techniques for Electron Microscopy," 274 pp. Academic Press, New York, 1960.
3. Stern, K. C., Electrophoresis and ionophoresis. *In* "Physical Techniques in Biological Research" (G. Oster, ed.), Vol. 2, pp. 61–121. Academic Press, New York, 1956.

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Quality Control in the Pharmaceutical Industry

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