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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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Briefs

Redox Reactions in Nonaqueous Media Determination of Amines via Dithiocarbamate Formation and Iodine Monobromide Titrations. BALBIR CHAND VERMA AND SWATANTAR KUMAR, *Department of Chemistry, Himachal Pradesh University, Simla 171001, India.*

Primary and secondary amines are determined by conversion with carbon disulfide in acetonitrile to the alkylammonium alkyldithiocarbamates, which are titrated with iodine monobromide. Both visual and potentiometric end-points are used.

Microchem. J. **21**, 237–241 (1976).

Microdetermination of Histamine and Serotonin Drugs using Bromine Monochloride in Water-Acetic Acid Medium. V. K. S. SHUKLA AND S. SHUKLA, *Department of Pharmacology, M. L. N. Medical College, Allahabad, India.*

The sample is reacted with a known excess of bromine monochloride at ice-bath temperature and the excess is back titrated iodometrically.

Microchem. J. **21**, 242–245 (1976).

Characteristics of the Oxidation Process in the Methyl Oleate–Propionic Acid System by Means of Dielectric Constant Measurements. JÓZEF ŚLIWIOK, HALINA WIRA, AND TERESA KOWALSKA, *Institute of Chemistry, Silesian University, Katowice, Poland.*

The influence of association in the methyl oleate–propionic acid system on the course of autoxidation of this system is discussed. The course of autoxidation of the a/m system is characterized with the help of peroxide number and dielectric constant measurements.

Microchem. J. **21**, 246–249 (1976).

Mechanism of the Hydroperoxide Destruction in the Oxidized Methyl Oleate, Oleyl Alcohol, and Oleic Acid. JÓZEF ŚLIWIOK, JÓZEF RZEPA, AND TERESA KOWALSKA, *Institute of Chemistry, Silesian University, 40-006 Katowice, Poland.*

The differentiated mechanism of destruction of hydroperoxides derived from methyl oleate, oleyl alcohol, and oleic acid was explained with the influence of the hydrogen-bond interactions. The evidence for the proposed models was given through the quantitative yields of the five classes of the secondary products of autoxidation.

Microchem. J. **21**, 250–260 (1976).

Spectrophotometric Determination of Iron(III) with EDTA Tetrahydrazide. F. BERMEJO-MARTINEZ, J. M. GRAÑA-MOLARES, AND J. A. RODRIGUEZ-VAZQUEZ, *Departamento de Quimica Analitica y del Consejo Superior de Investigaciones Cientificas, Facultad de Ciencias, Universidad de Santiago de Compostela, Santiago de Compostela, Spain.*

The tetrahydrazide was obtained by reacting the ester with hydrazine hydrate. The reaction between the metal and the ligand was investigated.

Microchem. J. **21**, 261–266 (1976).

A Modified Assay for Cholinesterase, D. B. HOOVER, J. KOSA, B. K. COLASANTI, AND C. R. CRAIG, *Department of Pharmacology, West Virginia University Medical Center, Morgantown, West Virginia.*

A radioisotopic assay for cholinesterase activity is described, which was developed by combining parts of existing systems. Easily used microliter volumes are employed. Labeled acetylcholine and acetate are separated by liquid cation-exchange chromatography. The method is sensitive, rapid, and easy to perform.

Microchem. J. **21**, 267–271 (1976).

Micromethod for the Simultaneous Analysis of Phenobarbital, Diphenylhydantoin, Carbamazepine, and Primidone in Blood. C. V. ABRAHAM, *Lynchburg Training School and Hospital, Post Office Box 1098, Lynchburg, Virginia 24505.*

The method described uses gas–liquid chromatography with temperature programming. The methylated derivatives of the anticonvulsants were well resolved. 5-(*p*-Methylphenyl)-5-phenylhydantoin was used as the internal standard. The procedure required only 100 μl of blood, which can be collected by finger stick. The lower limit of detection for each of the drugs was 0.5 mg/l. Analytical recoveries of the drugs was excellent and standard curves were linear to twice the toxic concentration for serum.

Microchem. J. **21**, 272–278 (1976).

Heterometric Microdetermination of Cobalt Using Nitron as Titrant. SALAH SHAHINE, M. FATHY ELSHAHAT, AND SOAD KHAMIS, *Chemical Research Laboratory, Faculty of Engineering, Ain Shams University, Abbassia, Cairo, Egypt.*

Cobalt is determined in the presence of potassium thiocyanate by titration with nitron acetate solution. A large number of diverse ions do not interfere.

Microchem. J. **21**, 279–285 (1976).

Spectrophotometric Determination of Halogens in Water Using Phenolphthalin as Reagent. Determination of Residual Chlorine in Tap-Water. SALAH A. SHAHINE AND RAFAA M. MAHMOUD, *Faculty of Engineering, Ain Shams University, Abbassia, Cairo, Egypt.*

Excess ferrocyanide is added to the sample solution and the produced ferricyanide is estimated spectrophotometrically with phenolphthalin. The method was applied to the determination of residual chlorine in tap-water and compared with the o-tolidine method.

Microchem. J. **21**, 286–290 (1976)

Lower Limits of the Potentiometric Microtitration of Chloride with Silver Ions. WALTER SELIG, *Lawrence Livermore Laboratory, University of California, Livermore, California 94550.*

The lower levels of the potentiometric titration of chloride with silver ions were investigated. The titrant was 0.001 *N* acetonic silver perchlorate. The titration media were acetone and acetic anhydride:acetone (4:1). A silver sulfide ion-selective indicator electrode and a double-junction reference electrode were used to monitor emf's. This titration is limited only by the trace amounts of chloride in the reagents used. Satisfactory results and well-defined curves were obtained down to 7 μg of chloride per 50 ml of solution (0.2 μmol; 4×10^{-6} *N*). A small polarization current can be used to enhance the potentiometric breaks of this titration.

Microchem. J. **21**, 291-301 (1976).

A Spectrophotometric Method for the Determination of Nitrite. B. J. MEEHAN, S. A. TARIQ, AND R. J. MAGEE, *Department of Inorganic & Analytical Chemistry, La Trobe University, Bundoora, Melbourne, Victoria, Australia 3083.*

A method is described which is based on the reduction of chromium (VI) to chromium (III) by nitrite, the latter being determined by measurement of chromium (III) spectrophotometrically at 580 nm.

Microchem. J. **21**, 302–305 (1976).

Determination of Free Sulfur in Chemical Reagents by Means of Thin-Layer Chromatography. STANISŁAW BANASZKIEWICZ, *Technical University, Radom, Poland.*

The applicability of the TLC technique was established to determine free sulfur traces in chemical reagents which have sulfur in their molecules.

Microchem. J. **21**, 306–308 (1976).

Micromethod for Phosphorus Determination in Research Laboratories. BARBARA D. BELL, WILLIAM D. BELL, AND R. MORRISON HURLEY, *Department of Pediatrics and Academic Computing Services, McMaster University, Hamilton, Ontario L8N 1Y4, Canada.*

A simple micromethod for serum inorganic phosphorus determination is described. The absorbance of the phosphomolybdate complex is read on standard spectrophotometers in 1 ml cuvettes at 690 nm.

Microchem. J. **21**, 309–314 (1976).

A Rapid Method for the Microdetermination of Nitrogen in Organic Compounds Using a Flushed-Oxygen Combustion Tube. YOSHIKO BABA, *Tokyo College of Pharmacy, 1-10-19, Uenosakuragi, Taito-ku, Tokyo 110, Japan.*

A study has been made of a method to determine nitrogen in organic compounds using samples of 1–1.5 mg with an empty combustion tube flushed with oxygen and heated to 850°C. Excess oxygen and oxides of nitrogen are absorbed and reduced with reduced copper at 500–600°C.

Microchem. J. **21**, 315–324 (1976).

Ultramicrodetermination of Nitrogen in Organic Compounds. Centimilligram Determination of Nitrogen with Sealed Tube Method. KEIKICHI MIYAHARA AND TOMO TAKAOKA, *Shionogi Research Laboratory, Shionogi & Co., Ltd., Fukushima-ku, Osaka, 553 Japan.*

A new improved nitrometer was developed for centimilligram determination of nitrogen by the sealed-tube method. A combustion tube having an internal volume of 1 ml was used and the sample was weighed in a boat made of copper foil, which was prepared by a new device. The accurate volume of a gas granule of the blank was evaluated by observation with a micrometer microscope. A standard deviation of 0.24% was obtained with compounds in the range of 6–66% nitrogen.

Microchem. J. **21**, 325–336 (1976).

MICROCHEMICAL JOURNAL 21, 237–241 (1976)

Redox Reactions in Nonaqueous Media Determination of Amines via Dithiocarbamate Formation and Iodine Monobromide Titrations

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INTRODUCTION

Critchfield and Johnson *(1)* described a method for the determination of primary and secondary amines that were reacted with carbon disulphide in isopropyl alcohol or isopropyl alcohol/pyridine media and the resulting alkyldithiocrbamic acids titrated with standard aqueous sodium hydroxide.

$$RNH_2 + CS_2 \longrightarrow RHNCSSH$$
$$R_2NH + CS_2 \longrightarrow R_2NCSSH.$$

The endpoints were reported not to be sufficiently stable due to the expected formation of xanthates through the reaction of carbon disulphide with solvent alcohol. Przybylowicz and Rogers *(3)*, however, reported that the resulting alkyldithiocarbamic acids could be titrated coulometrically with electrolytically generated mercury.

In the course of our investigations on the oxidimetric determination of dithiocarbamates in nonaqueous media, we have found iodine monobromide in acetonitrile *(5)* to be a promising oxidimetric reagent for the visual and potentiometric determination of these compounds in acetonitrile medium. The dithiocarbamates are smoothly, rapidly, and quantitatively oxidized to the corresponding thiurum disulphides at room temperature without a catalyst.

$$2RR'NCSSM + I^+ \rightarrow RR'NCSSSSCNR'R + 2M^+ + I^-$$

R = alkyl group; R′ = hydrogen, or alkyl group; M = sodium, potassium, ammonium, alkylammonium, or dialkylammonium cation).

No indicator is required in visual titrations; the endpoint is signaled by the appearance of yellow tint imparted to the solution by the first drop of oxidant solution added in excess. The titrations also may be performed potentiometrically using a bright platinum wire indicator electrode and an antimony or modified-calomel reference electrode. A sharp jump in potential is observed at the equivalence point in each titration.

[1] To whom all correspondence should be directed.

The simplicity and reliability of the above method prompted us to investigate this nonaqueous method to see if it could be extended so as to determine amines after their quantitative conversion with an excess of carbon disulphide to the corresponding dithiocarbamates in nonaqueous medium.

Primary and secondary amines react with carbon disulphide in organic solvents in the ration of 2 to 1, yielding monoalkylammonium monoalkyldithiocarbamates and dialkylammonium dialkyldithiocarbamates *(4)* respectively. Tertiary amines, on the other hand, fail to do so..

$$2\,RNH_2 + CS_2 \longrightarrow \underset{\text{monoalkylammonium monoalkyldithiocarbamate}}{RHNCSS\cdot NH_3R}$$

$$2\,R_2NH + CS_2 \longrightarrow \underset{\text{dialkylammonium dialkyldithiocarbamates}}{R_2NCSS\cdot NH_2R_2}$$

These reactions have been extensively employed for the preparation of a large number of such dithiocarbamates *(4)*. Solvents such as isopropyl alcohol and ether have been employed to bring about these reactions, which have been reported to be nearly complete. We have found that in acetonitrile medium, primary and secondary amines are quantitatively converted with an excess of carbon disulphide to the corresponding monoalkylammonium monoalkyldithiocarbamates and dialkylammonium dialkyldithiocarbamates, respectively, which can be titrated in the same (acetonitrile) medium with iodine monobromide solution also prepared in acetonitrile. The excess of carbon disulphide does not cause any interference in these titrations. The method is simple, accurate, rapid, and has wide applications.

MATERIALS AND METHOD

Reagents. Acetonitrile: Distilled twice from phosphorus pentoxide (5g/1).

Iodine Monobromide 0.05 in acetonitrile: The solid compound was prepared by the method of Popov and Skelly *(2)*. The standard solution was prepared by dissolving a little more than the calculated amount in acetonitrile. The solution was standardized iodometrically in aqueous medium and preserved in the dark.

Amines: Commercial grade, were distilled before use.

Carbon Disulphide: Riedel (German), was used as such.

All other chemicals used in this investigation were of guaranteed quality.

Procedures. Aliquots of solution of each amine in acetonitrile were taken in a glass stoppered titration flask containing about 50 ml of acetonitrile and 2 to 5ml of carbon disulphide were added to it. The flask was stoppered and swirled to mix the reactants. The solution was cooled to room temperature in each case, and titrated visually and potentiometri-

TABLE 1
NONAQUEOUS OXIDIMETRIC DETERMINATION OF PRIMARY AND SECONDARY AMINES WITH STANDARD (0.05 *N*) IODINE MONOBROMIDE

Compound	Amount found[a] (mg) mean,[c] standard deviation (±)		Amount found[b] (mg) mean,[c] standard deviation (±)	
	Visual method	Potentiometric method	Visual method	Potentiometric method
$CH_3CH_2CH_2NH_2$	10.04, 0.05	10.03, 0.04	40.19, 0.08	40.17, 0.08
$(CH_3)_2CHCH_2NH_2$	9.97, 0.05	9.97, 0.05	39.84, 0.09	39.83, 0.08
$CH_3CH_2CH_2CH_2NH_2$	9.98, 0.05	9.99, 0.05	40.18, 0.08	40.15, 0.07
$HOCH_2CH_2NH_2$	10.03, 0.05	10.04, 0.04	40.12, 0.08	40.10, 0.07
$(CH_3CH_2)_2NH$	10.05, 0.04	10.02, 0.04	39.87, 0.09	39.90 0.08
$(OHCH_2CH_2)_2NH$	9.94, 0.05	9.98, 0.05	40.08, 0.09	40.07, 0.08
$(CH_3CH_2CH_2CH_2)_2NH$	10.02, 0.05	10.00, 0.05	40.15, 0.09	40.11, 0.08
$CH_2CH_2CH_2CH_2CH_2NH$	9.98, 0.05	9.98, 0.04	39.84, 0.08	39.82, 0.07

[a] Amount of each compound taken, 10 mg.
[b] Amount of each compound taken, 40 mg.
[c] Mean of 10 determinations.

cally with standard (0.05 *N*) iodine monobromide solution (in acetonitrile) run from a microburette provided with a guard tube for protection from atmospheric moisture. In visual titrations, the endpoint was detected by the yellow tint imparted to the solution by the first drop of oxidant solution added in excess. The potentiometric titrations were performed by using antimony or modified calomel (methanol saturated with potassium chloride used instead of aqueous saturated potassium chloride) reference electrode and a bright platinum wire indicator electrode. The solutions were magnetically stirred during potentiometric titrations. A sharp jump in potential was observed at the equivalence point in each titration.

From the volume of standard (0.05*N*) iodine monobromide solution used corresponding to the endpoint in visual and potentiometric titrations, the amount of each dithiocarbamate, and subsequently, the amount of each amine was calculated. The results are given in Table 1.

RESULTS AND DISCUSSION

The reactions of primary and secondary amines with carbon disulphide to form, respectively, the corresponding monoalkylammonium monoalkyldithiocarbamates and dialkylammonium dialkyldithiocarbamates in organic solvents may in fact be considered to involve two steps: The formation of mono- or dialkyldithiocarbamic acids, and their neutralization to give mono- or dialkylammonium, mono- or dialkyldithiocarbamates *(4)*. The reaction between primary amines and carbon disulphide thus may be represented as:

$$RNH_2 + CS_2 \rightarrow RHNCSSH$$
$$RHNCSSH + RNH_2 \rightarrow RHNCSSH \cdot H_2NR.$$

The preparation of many such dithiocarbamates *(4)* through analogous reactions in dry ether has been described in the literature. The formation of such dithiocarbamates on mixing amines with carbon disulphide in acetonitrile medium has separately been confirmed by isolating a few such compounds and characterizing them through their melting points. Moreover, the stoichiometry of the reaction of these dithiocarbamates with iodine monobromide, described in the communication, also establishes the stoichiometry of the reaction between primary (or secondary) amines and carbon disulphide in acetonitrile medium.

The results recorded in Table 1 show that *n*-propylamine, isobutylamine, *n*-butylamine, monoethanolamine, diethylamine, diethanolamine, di-*n*-butylamine, and piperidine, can be determined visually and potentiometrically after conversion to the corresponding mono- or dialkylammonium, mono- or dialkyldithiocarbamates with carbon disulphide in acetonitrile medium. The overall standard deviation from the pooled data of all the visual and potentiometric titrations performed with 10 mg of each amine has been found to be 0.05 and 0.05 respectively. The same for 40 mg of each compound are 0.09 and 0.08 respectively.

In potentiometric titrations, the potentials attained stable values immediately on addition of each instalment of the oxidant solution. A sharp jump in potential of the order of 140 to 225 mV/0.05 ml of 0.05 *N* oxidant solution was observed at the equivalence point using platinum–antimony electrode assembly. With platinum-modified calomel electrode assembly, however, the jump in potential was of the order of 75 to 115 mV/0.05 ml of 0.05 *N* oxidant solution.

The replicate samples of each amine under investigation were not weighed individually, but rather, single large volume was weighed, dissolved in known volume of acetonitrile, and the aliquots taken for analysis in the titration flask. Now that the replicate aliquots of amine solution delivered the same amount of amine each time, they were checked by independent acidimetric titrations of that amine in a few such aliquots. This check was applied to all the amines.

SUMMARY

A simple and accurate nonaqueous oxidimetric method of wide applicability has been developed for the determination of primary and secondary amines after their quantitative conversion with carbon disulphide in acetonitrile to the alkylammonium alkyldithiocarbamates, which are titrated with iodine monobromide solution also in acetonitrile at room temperature. The endpoint is detected visually by the yellow tint imparted to the solution by the first drop of oxidant solution in excess; and potentiometrically by using a bright platinum wire indicator electrode, and antimony or modified calomel reference electrode. The method is simple, rapid, accurate, and widely applicable.

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MICROCHEMICAL JOURNAL 21, 242–245 (1976)

Microdetermination of Histamine and Serotonin Drugs using Bromine Monochloride in Water–Acetic Acid Medium

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Bromine monochloride has been used as a good brominating reagent for the determination of olefinic unsaturation in a number of compounds *(1, 3, 8)*. It has also been used for the substitutive halogenation of aromatic compounds *(6)* in aqueous solutions, selective bromination of phenols *(2, 5)* and volumetric determination of alcohols *(4)*. Schulek and Burger *(7)* employed standard 0.1 *N* bromine monochloride as titrant for phenylhydrazines and isonicotinic hydrazides, on the semimicro scale. In the present work the reaction of bromine monochloride with histamine and serotonin drugs in water–acetic acid medium has been utilized to develop a rapid and convenient method for their microdetermination.

VALIDITY OF THE REACTION FOR THE QUANTITATIVE DETERMINATION

Before applying the reaction for the determination of histamine and serotonin drugs, the stoichiometry of the reaction was established as follows: 2–10 mg of the sample dissolved in glacial acetic acid was reacted with a known amount of bromine monochloride solution. The reaction was allowed to proceed for about 15 min in an ice-bath, after which the excess of the reagent was back titrated iodometrically. Results obtained are presented in Table 1.

REAGENTS

Bromine monochloride (0.1 M). Potassium bromate [1.3917 g (A.R., B.D.H.)] and potassium bromide [1.9835 g (A.R., B.D.H.)] were dissolved in 125 ml of water in a 500 ml volumetric flask. The solution was cooled in ice. One-hundred milliliters of concentrated hydrochloric acid (M.A.R. Grade) were added and the solution diluted to the mark.

Glacial acetic acid. (A.R., B.D.H.).

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TABLE 1
DETERMINATION OF THE STOICHIOMETRY OF THE REACTION

Compound	Weight of the sample (mg)	Moles of BrCl consumed per mole of compound
Histamine	2.03	2.006
	5.08	2.002
	7.11	2.004
	10.16	2.002
Serotonin	2.13	3.004
	5.36	3.002
	7.47	3.004
	10.86	3.000

Sodium thiosulphate. Sodium thiosulphate (0.02 *N*, 4.964 g) (A.R., B.D.H.) was dissolved in 1 liter of distilled water and standardized against standard solution of copper sulphate.

Potassium iodide. 15% (w/v) solution of potassium iodide (Baker analyzed reagent) was used.

Starch solution. One percent (w/v).

Sample solution. A stock solution of histamine and serotonin drugs was prepared by exactly weighing (240–250 mg) and dissolving it in glacial acetic acid in a 250-ml volumetric flask. Different aliquots (2–10 ml) of the solution were used to give a concentration ranging from 2 to 10 mg.

PROCEDURE

An aliquot containing 2–10 mg of the sample was placed in a 100-ml iodine flask. Five milliliters of glacial acetic acid followed by 5 ml of bromine monochloride solution were introduced and the flask was stoppered and shaken well. The flask was placed in an ice-bath and the reaction mixture allowed to cool well for about 15 min. After the reaction was over the stopper was washed with 5 ml of distilled water and 5 ml of potassium iodide solution and the liberated iodine was titrated with sodium thiosulphate solution using starch as indicator.

A blank experiment was also run under the identical conditions except for the use of the sample.

CALCULATION

$$\text{mg Histamine or Serotonin} = \frac{\text{mol wt}}{n} \; N\text{Na}_2\text{S}_2\text{O}_3 \; (V_{\text{Blank}} - V_{\text{Sample}})$$

where mol wt = molecular weight of the sample; $N\text{Na}_2\text{S}_2\text{O}_3$ = normality of sodium thiosulphate; V_{Blank} = volume of sodium thiosulphate required to titrate blank (ml); V_{Sample} = volume of sodium thiosulphate required to

TABLE 2
MICRODETERMINATION OF HISTAMINE AND SEROTONIN DRUGS

Compound	Sample weight (mg)		Deviation (%)
	Taken	Recovered	
Histamine	2.03	2.00	−1.4
	5.08	5.06	−0.4
	7.11	7.16	+0.7
	10.16	10.12	−0.4
Serotonin	2.13	2.12	−0.4
	5.36	5.34	−0.3
	7.47	7.50	+0.4
	10.86	10.87	+0.09

titrate sample (ml); n = number of moles of bromine monochloride required per mole of the sample for a complete reaction.

RESULTS AND DISCUSSION

The proposed method has been applied for the determination of histamine and serotonin drugs and the results of the determinations are presented in Table 2. The results are precise, the maximum deviation is about 1.4%. Excess of bromine monochloride should be controlled as it leads to higher results. Acetic acid is a good solvent and the reaction medium for a large variety of organic compounds. Cooling of the reaction mixture is necessary to obtain good results.

SUMMARY

A micromethod for the determination of histamine, serotonin drugs has been developed. A 2–10 mg sample dissolved in glacial acetic acid is reacted with a known excess of bromine monochloride at ice-bath temperature and the excess reagent is back titrated iodometrically. The maximum deviation in the results is ± 1.41%.

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Characteristics of the Oxidation Process in the Methyl Oleate–Propionic Acid System by Means of Dielectric Constant Measurements

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INTRODUCTION

In our previous papers *(2,3)* the influence of association of oleic acid, oleyl alcohol and methyl oleate on the course of their autoxidation was discussed and the higher association of the compounds was correlated with the lower yields of the cumulated hydroperoxides and vice versa. The conclusion was made that the different energies of the hydrogen-bond associates formed in the examined substances influence their course of autoxidation in a different way.

The additional investigations of a correlation between association and the course of autoxidation for a selected system composed of methyl oleate and propionic acid were performed to confirm our previous statements and to find some additional interpretation of the experimental data.

EXPERIMENTAL METHODS

The 30-g samples of methyl oleate with an addition of 0.004, 0.008 and 0.02 *M* propionic acid/1 *M* methyl oleate (propionic acid produced by the B.D.H. Lab., England; methyl oleate synthesized and purified in our laboratory) were autoxidized at 60°C for 14 days with the oxygen from air. The comparative sample of pure methyl oleate was oxidized in the same conditions. Samples were placed in the Petri's discs (diameter of a disc 8.5 cm; thickness of a sample layer 0.8 cm) and the peroxide number values were determined every day *(1)*. For the unoxidized series of samples and after the 14 days of running this process the dielectric constant measurements were performed at 60°C with the help of DK-60 GK decameter (East Germany), used with the thermostatic condenser. The error of these measurements was ±0.2%. The obtained results are shown in Fig. 1.

DISCUSSION

The binary system composed of methyl oleate and propionic acid can be considered as a system composed of a solvent, characterized with a low dielectric constant and of an acid having a general formula HA. The

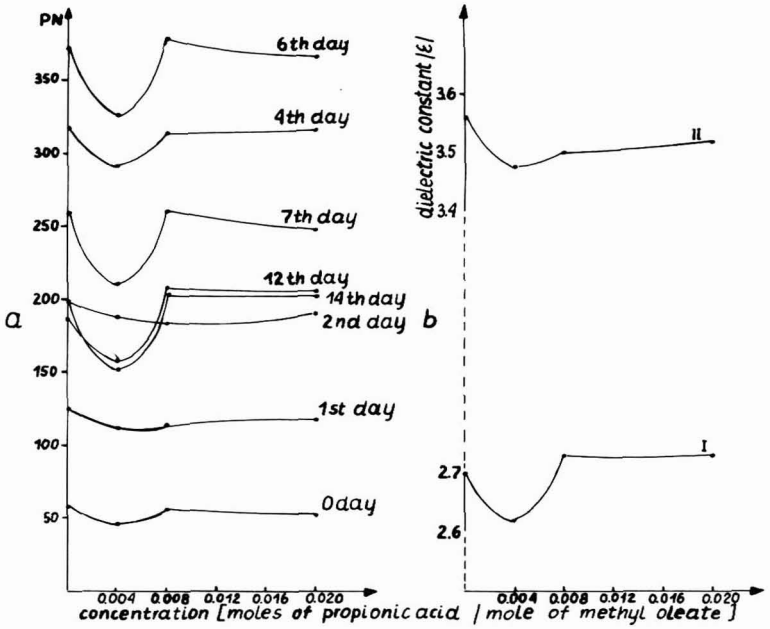


FIG. 1. (a) The peroxide number values measured in the course of oxidation at 60°C versus the mole amounts of propionic acid in methyl oleate for a series of the examined samples. (b) The dielectric constant values measured at 60°C versus the mole amounts of propionic acid in methyl oleate for the unoxidized samples (I) and these after 14 days of oxidation (II).

acidic properties of the compound HA can be revealed only in the presence of a protophilic substance B:

$$HA + B \rightarrow HB + A. \qquad (a)$$

This equation can be divided into two other equations, showing the ability of substances HA and HB to split off a proton:

$$HA \rightarrow A + H^{\oplus}; \qquad HB \rightarrow B + H^{\oplus} \qquad (b)$$

The corresponding equilibrium constants K_{HA} and K_{HB} cannot be determined in the direct way, because there is no possibility for the proton to independently exist in a solution. Therefore there is also no possibility to determine the absolute acid strength in the neutral solvents. The only measure of the relative acid strength can be formulated as the equilibrium constant in the reaction (a). Generally it is accepted, that with the very low values of the HA dissociation constants, the small addition of a component $H^{\oplus}$ or A turns back the dissociation.

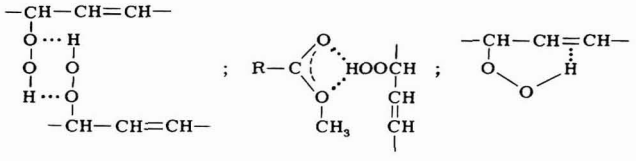
In our case the protophilic agent is methyl oleate, and more precisely its protophilic center consisting of the oxygen atoms belonging to the ester functional group. This situation creates the conditions for propionic acid to dissociate according to the Eg. (a). The complete equation of this reaction is as follows:

where R− = $CH_3-/CH_2/_7CH=CH-/CH_2/_7-$. These introductory considerations will be useful in the further part of our discussion.

Pure methyl oleate does not associate because of the lack of the sufficiently "acidic" hydrogen atoms. Addition of propionic acid in the amount of 0.004 *M*/1 *M* methyl oleate causes the decrease of dielectric constant by the value of 0.08. This fact gives an assumption to the conclusion, that the associated system is obtained with highly compensated electric charges. Addition of 0.008 to 0.02 *M* propionic acid/1 *M* methyl oleate can possibly turn back the dissociation of propionic acid to some extent, as mentioned above. It may be a reason of the less energetic association interactions between methyl oleate and propionic acid in these samples. This supposition finds a confirmation in the dielectric constant values for the unoxidized methyl oleate and methyl oleate−propionic acid samples (Fig. 1b, curve I).

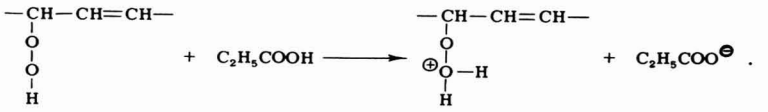
Considering the mechanism of autoxidation, the best conditions to propagate the longest chain of this radical reaction exist in the case of methyl oleate, because the unoxidized compound gives no association through the hydrogen bonds. In the case of methyl oleate with the addition of 0.004 *M* propionic acid/1 *M* methyl oleate one observes the strongest influence of association, which is possibly also connected with the highest number of coordinated molecules. Presumably this situation is unfavorable for propagation of the long chains of autoxidation, due to two different causes: (a) formation of macroaggregates slows down the mobility of the molecules in a liquid and statistically limits the number of the possible active collisons; and (b) it creates the steric hindrance for infiltration of oxygen into this liquid.

Oxidation of pure methyl oleate runs towards formation of the primary products of this process, i.e., hydroperoxides, which can associate as shown below:

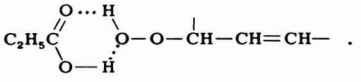


The proposed possibilities of the associated systems are arranged according to the decreasing energy of the hydrogen bonds.

Hydroperoxides formed in the course of autoxidation of methyl oleate with the addition of 0.004 *M* propionic acid/1 *M* methyl oleate can associate as shown in the above presented schemes, but there are also some additional possibilities. One of them is the particular case of Eq. (a):



The other one is as follows:



The energies of association in the last two cases are undoubtedly higher, than with hydroperoxides, formed in the pure methyl oleate. As it proceeds from our previous work *(2,3)*, the higher the hydrogen bond energy of the associates, involving hydroperoxides as a participant, the more suitable are the conditions for the decay of these hydroperoxides. Trying to sum up our considerations, we have to stress the fact, that the system composed of 0.004 *M* propionic acid/1 *M* methyl oleate shows the strongest effect of association, which is reflected in the comparatively lowest peroxide number values through the whole period of oxidation. Therefore, there is a basis for a generalization, that in some cases the estimation of the binary systems with the help of dielectric constant measurements can be applied to anticipate the course of autoxidation in these systems.

SUMMARY

In our paper the influence of association in the methyl oleate—propionic acid system on the course of autoxidation of this system was discussed. The course of autoxidation of the a/m system was characterized with the help of peroxide number and dielectric constant measurements.

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Mechanism of the Hydroperoxide Destruction in the Oxidized Methyl Oleate, Oleyl Alcohol, and Oleic Acid

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INTRODUCTION

In our previous paper *(5)* the models of association complexes between the functional groups of oleyl alcohol, oleic acid or methyl oleate and the corresponding hydroperoxides were proposed. On the basis of experimental material it was established, that the higher the hydrogen-bond energy of the discussed associates, the lower the durability of hydroperoxides and the higher the tendency towards their decay. In the case of the discussed compounds the highest tendency towards decay is observed with hydroperoxides derived from oleic acid, and the lowest one with those derived from methyl oleate. This fact induces the differentiated course of formation of the secondary oxidation products, i.e., of alcohols, glycols, aldehydes, and so on.

In this paper we attempted to propose the mechanism of hydroperoxide destruction, influenced by the presence of hydrogen bonds. The identified secondary products of autoxidation of methyl oleate, oleyl alcohol and oleic acid give the experimental evidence for our presumptions. In this way we tried to explain the differentiated mechanism of formation of the secondary oxidation products with ester, alcohol and acid.

EXPERIMENTAL AND DISCUSSION

Oleyl alcohol (K & K Laboratories, USA) used in our experiment was additionally purified, and oleic acid and methyl oleate were obtained in our laboratory. The purity of each reagent was higher, than 99.3% and they were not contaminated neither with their "*trans*" isomers, nor with the more unsaturated compounds.

Before the beginning of our experiment we attempted to estimate the hydrogen-bond energy values for the pure, unoxidized oleyl alcohol and oleic acid. Pure methyl oleate lacks similarly "acidic" hydrogen atom to give hydrogen bonds. The average hydrogen-bond energy values for oleyl alcohol and oleic acid were determined with the help of ir absorption spectroscopy *(8)*. According with this method the hydrogen-bond energy is calculated on the basis of the van't Hoff equation:

$$\frac{\Delta H}{RT^2} = \left(\frac{\partial \ln K}{\partial T}\right)_p , \tag{a}$$

where K = the equilibrium constant between the free and associated molecules.

The authors of the discussed method transformed Eq. (a) to adjust it to the situation, in which the high prevalence of the associated form over the free form of the examined compounds is observed. Then one has to perform the measurements of given, associated band at three, different temperatures, which fulfil the dependence:

$$T_2 = \frac{2\,T_1T_3}{T_1 + T_3} , \tag{b}$$

where T_1, T_2 and T_3 = three measuring temperatures.

Then, by the suitable transformation of the van't Hoff equation one obtains the following dependence:

$$\Delta H = \frac{RT_1T_2}{T_2 - T_2} \ln \frac{D_1/D_2 - D_3/}{D_3/D_1 - D_2/} , \tag{c}$$

where D_1 and D_3 = optical densities of the associated band at the temperatures T_1 and T_3; D_2 = optical density of the same band at the temperature T_3; the temperatures T_1, T_2 and T_3 should fulfil the dependence, given by Eq. (b).

In our investigations the calculations were performed for the valency vibration band of the associated hydroxyl groups ν_{O-H} of oleyl alcohol and oleic acid. Spectra were run with the help of the UR-20 model ir spectrophotometer produced by Carl Zeiss, East Germany, using the NaCl cells (thickness of a cell 0.025 mm) and the LiF prism. These measurements were performed at three temperatures: 40, 50 and 60°C, which approximately fulfil Eq. (b). The obtained results are shown in Table 1. The association energy for methyl oleate is assumed to equal $\Delta H = 0$.

TABLE 1

THE AVERAGE HYDROGEN-BOND ASSOCIATION ENERGY VALUES FOR OLEYL ALCOHOL AND OLEIC ACID

Substance	ΔH (kcal/mole)
Oleyl alcohol	0.10
Oleic acid	0.45

After presenting these introductory considerations we would like to discuss the autoxidation process of methyl oleate, oleyl alcohol and oleic acid. This process was conducted at 60°C in the following way: The investigated samples were placed in a flask of a gasometric equipment, which provided the constant oxygen pressure of 760 ± 0.4 mm Hg. The flat-bottomed reaction flask was equipped with the magnetic stirrer (200 turns/min and placed in darkness inside a thermostatic chamber at 60 ± 0.1°C. The examined substances were oxidized for 100 hr and in the 5–10 hr intervals the samples for analysis were collected with a syringe through the silicon septum closing one of the flask necks. The conditions of the performed analyses were as follows. The Varian Moduline Model 2868 gas chromatograph was used with the dual flame ionization detector, connected with the Varian 485 integrator. The results were registered with the help of Varian A-25 1 mV recorder. The *trans* isomers were determined on the capillary stainless steel column (length of a column, 50 m; inner ϕ, 0.1 mm, packed with the nonpolar silicon phase GE-SE-30; argonium as a carrier gas, its flow rate, 1.5 ml/min. Temperature of thermostat, 200°C; temperature of evaporizer, 260°C; temperature of the detector, 250°C. FID: H_2 flow rate, 20 ml/min; air flow rate, 250 ml/min.

The remaining products of oxidation were determined on the glass column (length of a column, 182 cm; inner ϕ, 2 mm, packed with the 3% OV-225 stationary phase on Gas-Chrom Q support 80/100 mesh; argonium as a carrier gas, its flow rate, 30 ml/min. Temperature of thermostat, 180°C; temperature of evaporizer: 280°C, temperature of detector, 250°C. FID: H_2 flow rate, 30 ml/min; air flow rate, 300 ml/min.

In each case the −COOH groups were esterified with methanol with a 1% addition of H_2SO_4; the −OH groups were silylated with the Tri-Sil mixture (Pierce Chemicals Co.). All the products of oxidation were identified with the help of chromatographic standards, using the tandem GC-MS model 9000 unit produced by the LKB. The quantitative determination was performed on the basis of chromatographic standards, and with the hydroxyl derivatives the difference was calculated between the peroxide number determinations and the hydroxyl derivatives obtained from the reduction of the reaction mixtures with the alc. solution of $SnCl_2$ (a sum of R−OOH and R−OH compounds).

In the course of autoxidation the partial isomerization of ester, alcohol and acid to the *trans* configuration is observed. As a result of this isomerization the elaidic alcohol is obtained from oleyl alcohol, elaidic acid from oleic acid and methyl elaidate from methyl oleate. The quantitative results are shown in Fig. 1.

As it arises from the data given in Fig. 1, the efficiency of the *trans* isomerization can be arranged in the following order:

$$\text{ester} > \text{alcohol} > \text{acid}.$$

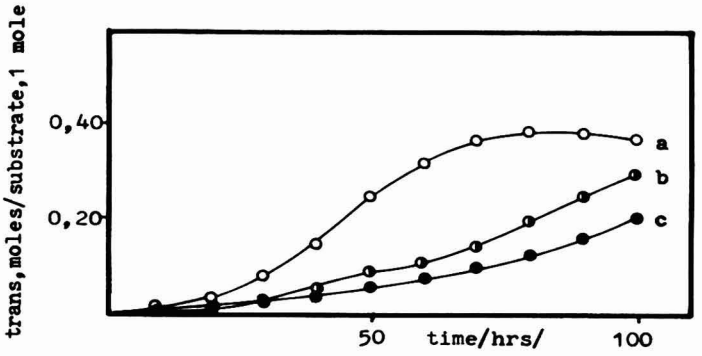


FIG. 1. The "*trans*" isomerizations yields of: (a) Methyl oleate; (b) oleyl alcohol; (c) oleic acid; autoxidized with oxygen for 100 hr at 60°C.

This observation can be explained with the influence of hydrogen bonds. With pure methyl oleate the hydrogen-bond association does not exist. Therefore there is practically no obstacle for the R· radical to give its mesomeric form:

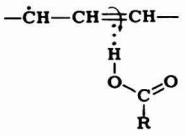
$$-CH-CH=CH- \leftrightarrow -CH\cdots CH\cdots CH-$$

In the case of the second mesomeric form a chance exists of a free rotation of the $-C-C-C-$ bonds, then followed by recombination:

$$R\cdot + R'H \rightarrow RH + R'\cdot;$$

in these circumstances the conditions exist for the *trans* isomerization of molecules.

With oleic acid the conditions occur for the strongest hydrogen-bond interactions with participation of its functional groups. Therefore, in the case of the R· radical its π-electrons can be stabilized through association:



Stabilization of the double bond through hydrogen bond in oleic acid prevents its molecules from the eventual *trans* isomerization. This presumption can be confirmed with the lowest yields of the *trans* product with oleic acid.

With oleyl alcohol its ability to associate through hydrogen bonds is medial between that of acid and ester. Unlike ester it gives the hydrogen bonds, but they are energetically weaker than those formed by acid. The

ability to *trans* isomerization for alcohol is also medial between acid and ester.

Perhaps besides the hypothesis with the associative stabilization of double bonds the other factor can be named, which raises difficulties with the *trans* isomerization. One can suspect that in the case of more efficient association the bigger aggregates are formed, which are less susceptible to develop the long chains of radical reactions. This difficulty is connected with the lower mobility of macromolecules, and simultaneously with the lower possibility of active collisions.

Now we shall discuss the determined products of autoxidation of methyl oleate, oleyl alcohol and oleic acid, and quantitative ratios among them.

The yields of peroxide products were determined according with Polish Standards *(4)*. The obtained results are shown in Fig. 2. These results confirm the observations presented in our previous paper *(5)*. Again the peroxidic products yield is highest with methyl oleate and lowest with oleic acid. We tried to explain this situation with the influence of association *(5)*. In this paper we discuss the yields of secondary products of autoxidation, i.e., of the substances coming from destruction of hydroperoxides and look for explanation of differences in these yields with ester, alcohol and acid with the differentiated influence of the hydrogen-bond association.

The following groups of the secondary products of autoxidation, de-

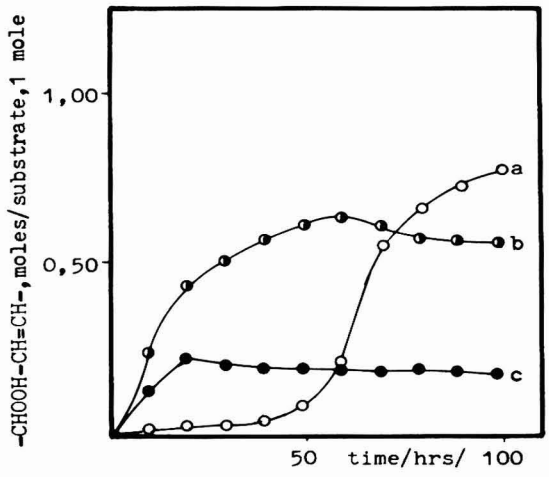


FIG. 2. The molar yields of peroxide products per mole of the oxidized substrate vs time of autoxidation at 60°C for: (a) methyl oleate; (b) oleyl alcohol; (c) oleic acid.

TABLE 2
THE MOLE PERCENTAGE YIELDS OF MONOHYDROXY-, DIHYDROXY-, KETO- AND KETOHYDROXYCOMPOUNDS AND OF MONOFUNCTIONAL PRODUCTS OF DEGRADATION OF THE OXIDIZED MOLECULES FOR METHYL OLEATE, OLEYL ALCOHOL AND OLEIC ACID AFTER 100 hr OF AUTOXIDATION AT 60°C

	Yield of product (mole%)				
	With preservation of the carbon skeleton				Monofunctional
Substance	Hydroxy-	Dihydroxy-	Keto-	Ketohydroxy-	products of degradation
Methyl oleate	2.1	1.8	3.8	4.1	0.8
Oleyl alcohol	3.5	4.1	3.5	0.6	0.9
Oleic acid	5.3	8.4	2.4	0.2	6.4

rived from ester, alcohol and acid were determined with the help of GLC: mono- and dihydroxycompounds, ketones, ketohydroxyderivatives and monofunctional products of degradation of the oxidized molecules (mainly aldehydes and acids). The obtained results are given in Table 2 and Fig. 3A–E.

As it proceeds from the quantitative data given in Table 2 and Fig. 3A–E, the products of autoxidation of ester, alcohol and acid can be divided into two groups. The first group should consist of the mono- and dihydroxyderivatives and monofunctional products of degradation of the examined substances, because in this case the yield of these products related to the original compounds can be arranged as follows:

$$\text{acid} > \text{alcohol} > \text{ester}.$$

The second group should include these products of autoxidation, which, related to the original compounds, give yields in the following order:

$$\text{ester} > \text{alcohol} > \text{acid}.$$

These are keto- and ketohydroxyderivatives.

This division of autoxidation products into two groups remains in agreement with Refs. *(1–3,7)*, concerning the mechanism of obtaining the individual classes of compounds through autoxidation. Thus with mono- and dihydroxyderivatives and also with the oxidative degradation products the prevalence of the proposed mechanisms accept the initial destruction of the primary product of autoxidation, which is hydroperoxide, and then the further chemical reactions.

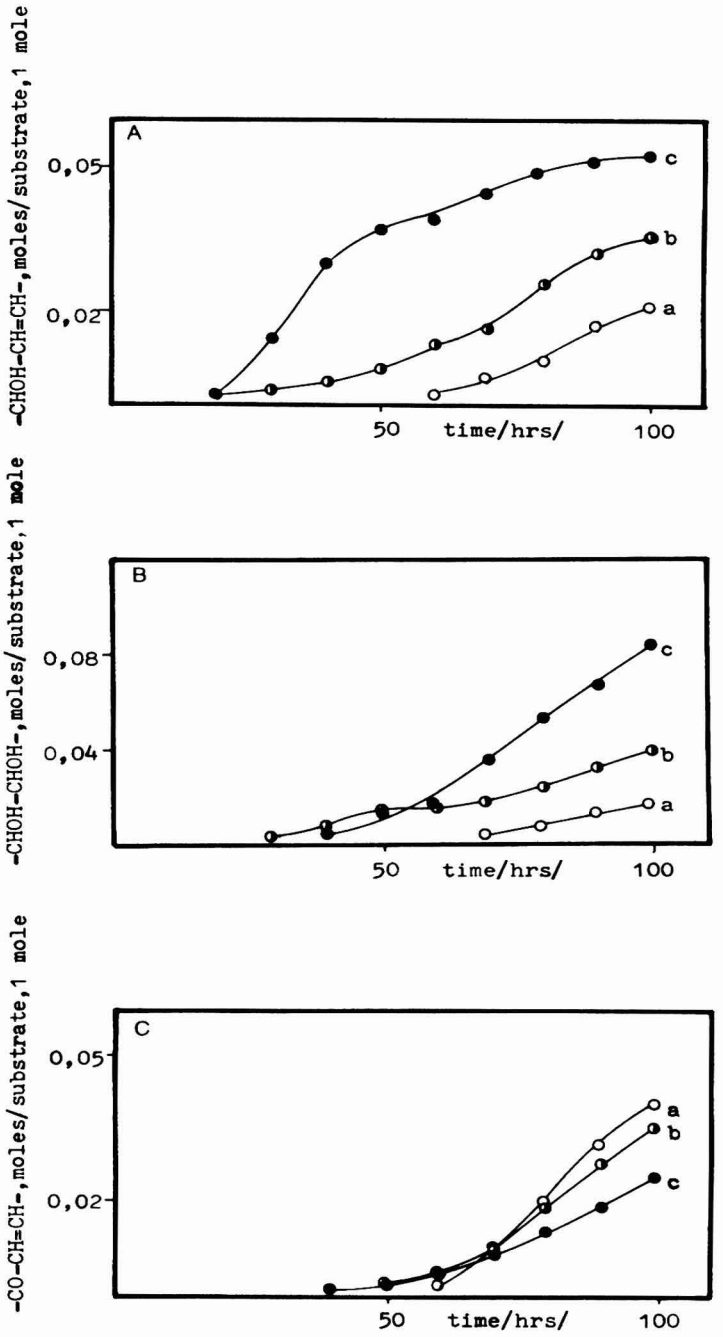


FIG. 3. (A) The molar yields of monohydroxylic products per mole of the oxidized substrate vs time of autoxidation at 60°C for: (a) methyl oleate; (b) oleyl alcohol; (c) oleic acid. (B) The molar yields of dihydroxylic products per mole of the oxidized substrate vs time of autoxidation at 60°C for: (a) methyl oleate; (b) oleyl alcohol; (c) oleic acid. (C) The molar yields of ketoproducts per mole of the oxidized substrate vs time of autoxidation at 60°C for: (a) methyl oleate; (b) oleyl alcohol; (c) oleic acid.

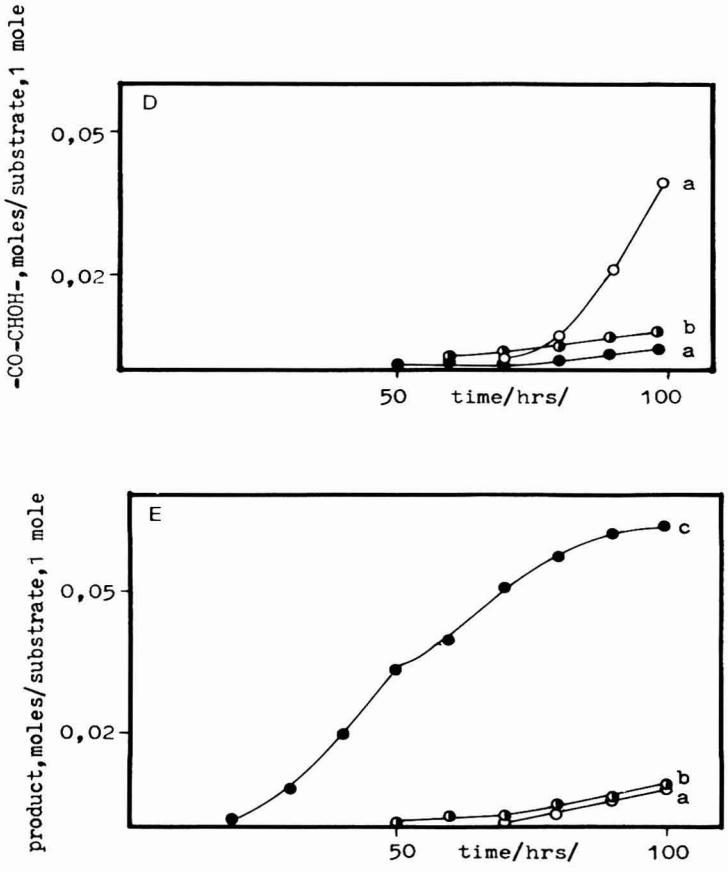
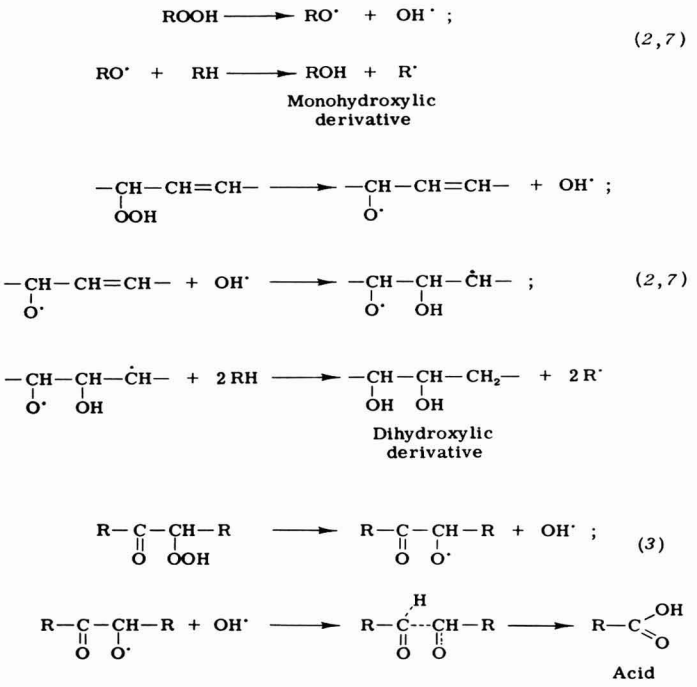
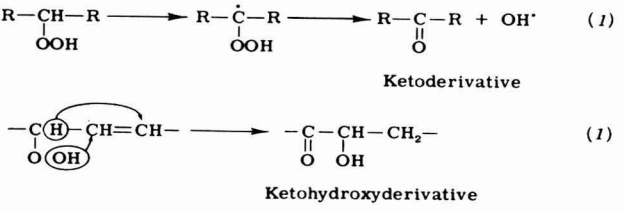


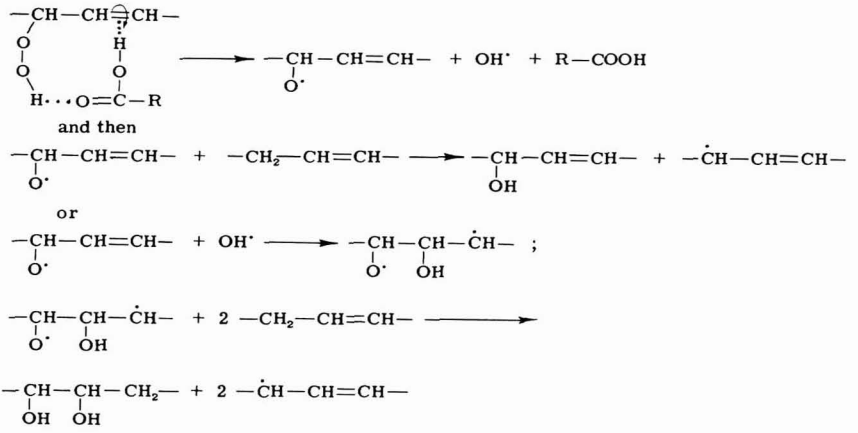
FIG. 3. (D) The molar yields of ketohydroxylic products per mole of the oxidized substrate vs time of autoxidation at 60°C for: (a) methyl oleate; (b) oleyl alcohol; (c) oleic acid. (E) The molar yields of monofunctional products of degradation of the oxidized molecules per mole of the substrate vs time of autoxidation at 60°C for: (a) methyl oleate; (b) oleyl alcohol; (c) oleic acid.



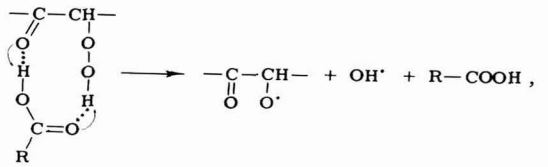
With the keto- and ketohydroxyderivatives the widely accepted mechanisms presume the intramolecular rearrangement of hydroperoxides, without degradation of the aliphatic chain:



With oleic acid the relatively highest yields of the products of destruction of hydroperoxides (i.e., of mono- and dihydroxycompounds and of monofunctional products of degradation of the molecules) are observed. With oleic acid the highest energy of hydrogen bonds is also noted (Table 1) and possibly both these facts can be correlated in the following way: Association through hydrogen bonds promotes destruction of hydroperoxides and is followed by recombination of radicals, which can give the a/m classes of products as a final effect. Taking into assumption the data concerning hydrogen-bond energy and given in Table 1, the scheme of the destruction of hydroperoxides derived from oleic acid can be presented in the following way:

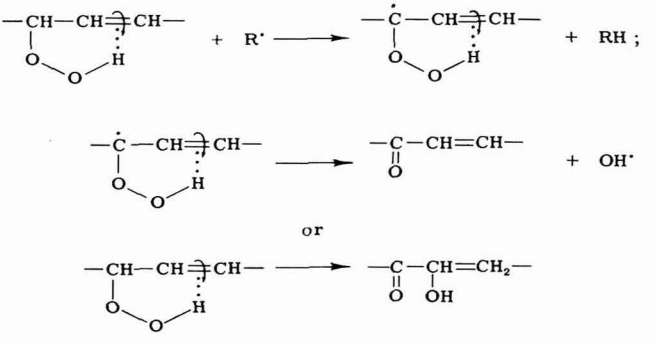


Similarly the split off of the carbon skeleton in oleic acid towards monofunctional products of degradation can be accelerated by the hydrogen-bond interactions with ketohydroperoxides:



which means further formation of monofunctional products of degradation of the oleic acid molecules, as it was shown before in the general scheme.

Methyl oleate represents the opposite situation. This substance reveals the relative weakest intermolecular influences. Simultaneously with this substance we observe the highest yields of these products, which are formed in the course of intramolecular rearrangement of hydroperoxides (keto- and ketohydroxyderivatives). Schematically it can be shown in the following way:



Consequently in the oxidized samples of methyl oleate the intramolecular reactions dominate, which finally bring keto- and ketohydroxyderivatives.

In respect of its ability to form hydrogen bonds oleyl alcohol is a medial link between ester and acid. Similarly its ability to give the secondary products of autoxidation stands in between this of ester and acid. In the case of the five examined groups of secondary products of autoxidation yields obtained from alcohol were lower than those from oleic acid and higher than those obtained from methyl oleate. It means, that the hydrogen-bond influence in alcohol is strong enough to give the reasonable yields of the hydroperoxide destruction products and sufficiently insignificant to bring such yields of hydroperoxide rearrangement products, which are also not negligible.

On the basis of our previous investigations *(5,6)* it was established, that the energy of hydrogen-bond association influences the level of hydroperoxides in the examined systems. Consequently the differentiated energy of hydrogen bonds (Table 1) influences and promotes the given mechanism of hydroperoxide destruction towards the corresponding secondary autoxidation products. Weak interactions through the hydrogen bonds, like in the case of our ester, promote the reaction mainly to the intramolecular rearrangement of hydroperoxides.

SUMMARY

The differentiated mechanism of destruction of hydroperoxides derived from methyl oleate, oleyl alcohol and oleic acid was explained with the influence of the hydrogen-bond interactions. The evidence for the proposed models was given through the quantitative yields of the five classes of the secondary products of autoxidation: monohydroxy-, dihydroxy-, keto-, ketohydroxyderivatives and monofunctional products of degradation of the oxidized molecules.

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Spectrophotometric Determination of Iron(III) with EDTA Tetrahydrazide[1]

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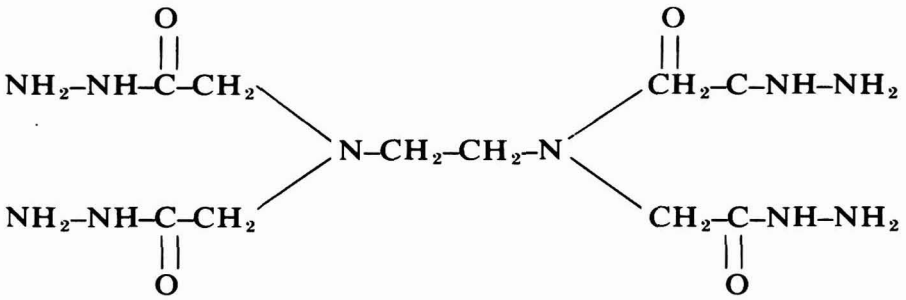
The EDTA tetrahydrazide, $(NH_2NH)_4$–EDTA, (I) was first synthesized by Badinand *et al.*[1] through the corresponding ester obtained after Fisher and used for the colorimetric determination of copper(II) in the presence of formaldehyde or acetaldehyde as major constituents; Beer's law is obeyed in the range 50–1000 μg Cu(II) ml^{-1} the only interfering species being cobalt(II).[2] They also studied the effect of the carbon chain of several aliphatic hydrazides, including the $(NH_2NH)_4$–EDTA, on the sensitivity of their reaction with copper(II).[3].



(I)

It was found that the ethylenediamine tetraacetic acid tetrahydrazide gives with iron(III) salts a very sensitive violet or yellow–red color depending on the pH value. In this paper the system was studied and a new procedure for the spectrophotometric determination of trivalent iron was developed.

EXPERIMENTAL

Synthesis of the $(NH_2NH)_4$–EDTA. The $NH_2NH)_4$–EDTA was obtained according to Badinand's method[1]

[1] Communication presented to EUROANALYSIS II, August 25–30, 1975, Budapest, Hungary. Part LXXXIII in the series "Analytical Applications of Chelons."

by preparing the corresponding ester which once extracted and purified was made to react for 1 hr on absolute ethanol with hydrazine hydrate, the formed precipitate being filtered and washed with cold ethanol. The resultant product recrystallized in 60% ethanol as white needles; melting point 93°C.

Elemental analysis. Cal: C 32.78; H 7.10; N 38.25. Found: C 32.27; H 7.05; N 37.41.

The resultant compound is water soluble, slightly soluble in methanol and ethanol, and insoluble in chloroform and ether; stable as a solid and sufficiently stable on aqueous solution; its infrared spectrum, run in potassium bromide, shows the characteristic bands of the hydrazide group.

Apparatus. A spectrophotometer Beckman model DU equipped with 10-mm glass cells was used for absorbance measurements. A Beckman model Electromate pH meter with glass and calomel electrodes (sensitivity ± 0.02 pH) was used for pH measurements.

Reagents. Standard iron(III) solution: prepared from iron trichloride hexahydrate, its concentration being determined by gravimetry, resulting 0.0978 *M*; from this other solutions were prepared by dilution.

Standard reagent solution: prepared by weighing the recrystallized reagent and dissolving it in distilled water, resulting 9.15×10^{-3} *M*.

0.3%(w/v) reagent solution: prepared by dissolving the appropriate amount of reagent in distilled water.

Buffer solution pH 4.5: prepared after Walpole[4] by adding to 60 ml of 0.2 *M* acetic acid solution, 40 ml of 0.2 *M* sodium acetate solution.

Buffer solution pH 11: prepared after Naegali and Tyabji[4] by adding to 100 ml 0.05 *M* borax solution, 100 ml of 0.1 *M* sodium hydroxide solution.

0.1 M hydrochloric acid and sodium hydroxide solutions: prepared from analytical grade reagents.

Recommended procedure. Transfer 5 ml of iron(III) solution to a 25-ml volumetric flask to give a final concentration in the range of 0.5–20.0 μg ml^{-1}. Add 2 ml of 0.3% reagent solution, drops of 0.1 *M* hydrochloric acid or sodium hydroxide aqueous solution to approximate the pH to 4.5 or 11.0, and 10 ml of buffer solution of the same pH, respectively; then dilute to the mark with distilled water. Measure the absorbance at 530 nm (pH 4.5) or at 450 nm(pH 11.0) against distilled water as blank.

RESULTS AND DISCUSSION

Absorption spectra. The spectral characteristics of the iron(III)–EDTA tetrahydrazide system in acidic and alkaline media was studied in the range 350–700 nm (Fig. 1). For pH values of 4.5 and 11.0 the complex under investigation shows two absorption maxima at 530 nm (pH 4.5) and

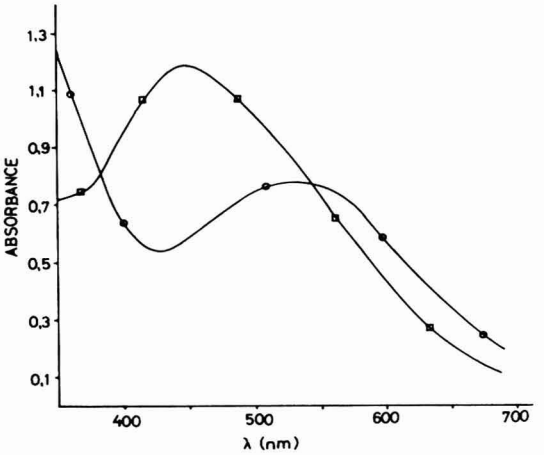


FIG. 1. Absorption spectra of iron(III)–$(NH_2NH)_4$–EDTA complex solutions at pH 4.5 (○) and 11.0 (□); concentration: 21.1 μg Fe(III) ml^{-1}.

450 nm (pH 11.0). As the pH value increases there is a shift towards smaller wavelength of the absorption maximum.

Effect of pH and amount of reagent. Samples containing a fixed amount of iron(III), 4.6 μg ml^{-1}, were developed and measured by the recommended procedure except that the pH was varied by addition of hydrochloric acid or sodium hydroxide aqueous solution in order to obtain pH values in the range 2–12 (see Figs. 2(a) and 2(b)). In these conditions maximal constant absorbance occurred with solutions of a pH value between 4.0–5.3 at 530 nm and 10.0–11.6 at 450 nm. Thus pH values of about 4.5 at 530 nm and 11.0 at 450 nm were chosen for further studies.

When the amount of reagent was varied it was found that 0.4 ml of 0.3% (w/v) aqueous solution of the reagent must be added for each 4.6 μg Fe(III). ml^{-1} and further additions of it did not appreciably affect the absorbance of the system. In order to ensure the complete chelate formation of somewhat larger amounts of iron, a 2 ml solution of reagent was used in the recommended procedure.

The reaction occurred instantaneously and was not affected by a variation in the order of mixing the reagents.

Effects of time and temperature. The effects of time and temperature on the stability of the system were also studied. At optimal conditions of other variables the absorbance of the complex measured as soon as the solution had cooled to room temperature, remained unchanged for up to 80°C. When measured at various intervals of time, absorbances were constant within experimental error over a period of up to 8 hrs.

Beer's law, optimal range, and molar absorptivity. The relationship between absorbed radiant energy and complex concentration was studied

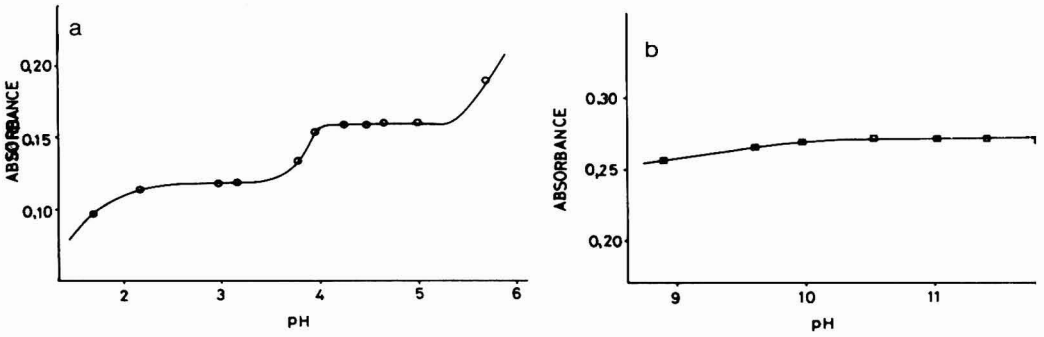


FIG. 2. Effect of pH on the absorbance of the iron(III) $(NH_2NH)_4$–EDTA complex solutions at 530 nm (Fig. 2(a)) and 450 nm (Fig. 2(b)); concentration: 4.6 μg Fe(III) ml^{-1}.

with the previously established conditions. Several standard solutions were prepared by the recommended procedure and their absorbances measured as indicated; the plot of absorbance versus concentration is a straight line, which passes through the origin, over the range investigated 1.0–20.0 μg Fe(III) ml^{-1} at 530 nm(pH 4.5), and 0.5–14.0 μg Fe(III) ml^{-1} at 450 nm(pH 11.0). The optimal Ringbom's[5] working range for measurement at 10.0 nm optical path is 3.0–18.0 μg Fe(III) ml^{-1} at 530 nm(pH 4.5), and 2.0–14.0 μg Fe(III) ml^{-1} at 450 nm(pH 11.0); the molar absorptivity is 1.95×10^3 1 mol^{-1} cm^{-1} at 530 nm and 3.35×10^3 1 mol^{-1} cm^{-1} at 450 nm, respectively (see Fig. 3).

Effect of diverse ions. The effect of other species on the formation of the iron(III)–$(NH_2NH)_4$–EDTA chelate was studied at pH 4.5 and 11.0. Varying amounts of the foregoing ions were taken with 4.6 μg Fe(III) ml^{-1} and the described general procedure was followed. Under these conditions it was found that at pH 11.0 wolframate, acetate, oxalate and nitrite and zinc(II), manganese(II), uranyl(II), nickel(II), vanadium(V), and copper(II) interfere when present in 100-fold and 10-fold amounts, respectively, with respect to iron concentration. At pH 4.5 nickel(II), vanadium(V), cobalt(II), and copper(II) interfere at a tenfold excess of the iron concentration. Those ions which interfere can be eliminated readily by using some of the methods already described[8].

Stoichiometry of the reaction. To establish the composition of the chelate the "mole ratio"[6] and "continuous variations"[7] methods were employed.

The mole ratio method was applied with a series of solutions containing iron at a fixed concentration of 9.81×10^{-4} M, the concentration of reagent being varied. A plot of absorbance versus moles of $(NH_2NH)_4$–EDTA per mole of iron showed a break at a 1:1 ratio of ligand to metal at pH 4.5 and at a 2:3 ratio at pH 11.0.

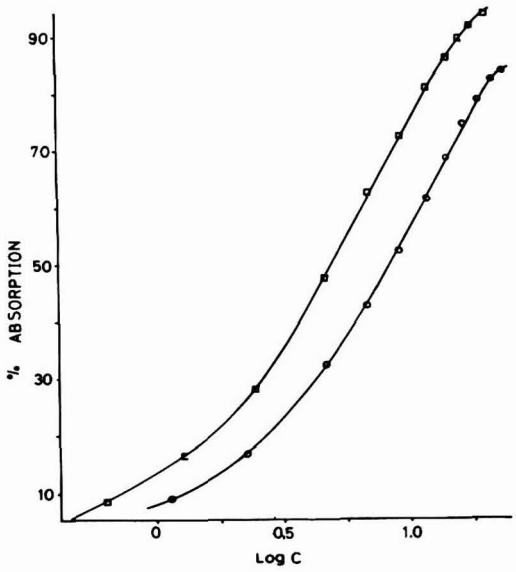


FIG. 3. Ringbom's plot for the iron(III) $(NH_2NH)_4$–EDTA complex solutions at 530 nm (○) and 450 nm (□), respectively.

The continuous variations method was applied with a series of solutions in which the total concentration of reactants—iron + reagent—was kept constant, but the mole fraction of components was varied. In a plot of absorbance versus mole fraction of iron, extrapolation of the initial and final portions of the curve gave an intersection at 0.5 mole fraction for iron at pH 4.5 and at 0.66 mole fraction for iron at pH 11.0, this confirming the stoichiometry metal:ligand reaction to be 1:1 at pH 4.5 and 2:3 at pH 11.0.

Application to the determination of iron in talcs. The method described above was applied to the determination of the iron content in various talc samples. The samples were treated in a platinum crucible with a sodium carbonate–potassium carbonate mixture in order to solubilize iron together with the accompanying elements. The filtrate obtained after the separation of silicon was made up to a volume of 250 ml from which an aliquot was transferred to a 25-ml volumetric flask and after oxydizing all the iron with bromine water, its excess being eliminated by heating, treated in the general procedure previously described.

The iron content was also evaluated by another method employed as a reference with DTPA as reagent[9]. Table 1 shows the results obtained for two samples when using both methods, the mean average values of iron content being indicated.

Although the method was applied to the determination of iron in talcs it can be used with a variety of materials.

TABLE 1
DETERMINATION OF IRON IN TALCS

Sample	Proposed method[a] (%)		DTPA method (%)	Average difference[a] (%)	
	(a)	(b)		(a)	(b)
1	5.41	5.32	5.56	0.15	0.24
2	5.45	5.25	5.56	0.10	0.31

[a] (a) Measurements made at 530 nm. (b) Measurements made at 450 nm.

SUMMARY

The tetrahydrazide of ethylenediamine tetraacetic acid $(NH_2NH)_4$–EDTA was synthesized from the EDTA ester and hydrazine hydrate in ethanolic solution, the resulting $(NH_2NH)_4$–EDTA being recrystallized in 60% ethanol. When the spectrophotometric study of the iron(III) $(NH_2NH)_4$–EDTA complex in aqueous solution was made two absorption maxima at 530 and 450 nm at pH 4.5 and 11.0, respectively, were found. Beer's law is obeyed in the range 1.0–20.0 μg Fe(III) ml^{-1} at 530 nm and pH 4.5 and 0.5–12.0 μg Fe(III) ml^{-1} at 450 nm and pH 11.0, the molar absorptivities being 1.95×10^3 1 mol^{-1} cm^{-1} at 530 nm and 3.35×10^3 1 mol^{-1} cm^{-1} at 450 nm, respectively. The Ringbom optimal interval falls between about 3 and 18 μg Fe(III) ml^{-1} at 530 nm and about 2–14 μg Fe(III) ml^{-1} at 450 nm. The reaction between the metal and the ligand was also investigated. The method has been successfully applied to the determination of iron in talcs.

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A Modified Assay for Cholinesterase

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INTRODUCTION

Many isotopic methods exist for the determination of cholinesterase activity. In these the incubation conditions vary somewhat, but the major differences are the methods used for separating the labeled reaction product from labeled acetylcholine. Among the techniques employed are the use of ion-exchange resins *(6, 7)*, thin-layer chromatography *(3)*, formation of the reinecke salt of acetylcholine *(4)*, liquid cation-exchange chromatography *(1, 8)* and the extraction of labelled acetic acid into organic solvents *(2, 5)*. The assay described here has been developed by combining some better parts of a number of existing assays to form a simple and rapid microassay.

MATERIALS

Acetyl-1-[14C] choline chloride was obtained from New England Nuclear Corp. Sodium tetraphenylboron was purchased from Sigma Chemical Co., St. Louis, Mo. Glass Lang–Levy micropipets used were from Bio–Rad Laboratories, Richmond, Ca. Incubations and extractions were done in 6 × 50 mm glass culture tubes (Fisher Scientific Co.). A device for mixing solutions in the microtubes was made by mounting an aluminum rod slightly off center on the end of the shaft of a small motor. Tubes were placed against the spinning rod to mix. Inserts for placing microtubes in centrifuge tubes were made from 1-inch diameter wooden rods. An Amana Model RR-4 microwave oven was used to obtain denatured enzyme. One dram sample vials were used for scintillation counting (Fisher Scientific Co.). Samples were counted in a Packard Liquid Scintillation Spectrophotometer, Model 2009.

METHODS

Frontal cerebral cortex from male Sprague Dawley rats killed by decapitation was homogenized in 25 volumes of cold distilled water, and an aliquot was diluted further to give a concentration of 3.5 μg tissue/μl enzyme stock. The incubation conditions from a previous report *(7)* were used, except that the total volume was only 30 μl. Ten-microliter aliquots of enzyme stock were placed in microtubes in an ice bath. Distilled water

was used as a blank. Homogenates were also prepared from cortex of rats killed by exposure to microwave irradiation for 10 sec. At timed intervals, 20 μl of a concentrated buffer and substrate solution containing approximately 23,000 cpm of acetyl-1-[14C] choline was added to each tube. Final concentrations of the pH 7.4 mixture were 0.033 *M* sodium phosphate, 0.1 *M* sodium chloride, and 1 m*M* acetylcholine. Tubes were mixed and incubated at 0 or 37°C. The reaction was stopped by adding 100 μl of a solution containing 75 mg tetraphenylboron/ml of 3-heptanone and mixing *(1, 8)*. The tetraphenylboron extracts acetylcholine, leaving acetate in the aqueous phase. The microtubes were then centrifuged at 1000 g for 10 min, and the upper organic phase was aspirated. A 15-μl aliquot of the aqueous phase was placed in a 1-dram sample vial, and 4 ml of a counting solution containing 2.4 g PPO and 75 mg POPOP in 300 ml of toluene and 150 ml of Triton X-100 was added. The sample vials were placed inside standard size glass scintillation vials for counting.

RESULTS

The activity of cholinesterase as a function of time of incubation is shown in Fig. 1. Under the conditions employed, this relationship was linear for a least 30 min. Acetylcholine was hydrolyzed at a rate of 460.4

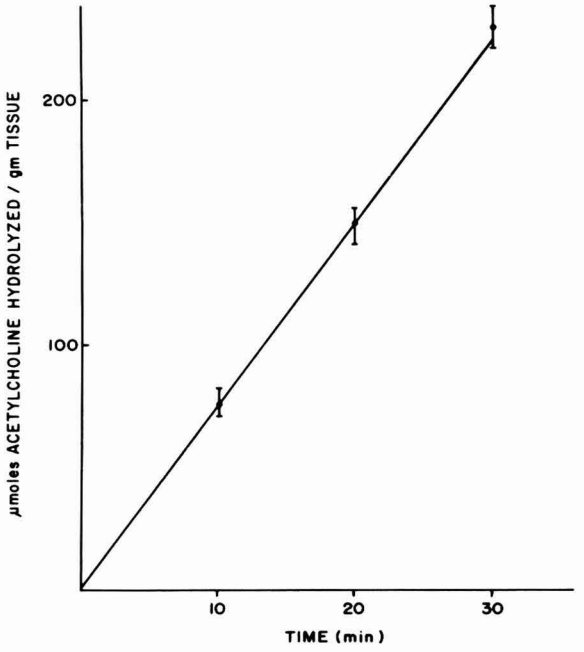

FIG. 1. Hydrolysis of acetylcholine by rat frontal cerebral cortex as a function of incubation time at 37°C. The values are the mean ± SEM for four rats.

μmol/g frontal cerebral cortex/hr. Distilled water was used as a blank here.

Figure 2 shows the counts obtained in the aqueous phase during a 30 min incubation at 37°C with distilled water or tissue subjected to microwave irradiation and intact enzyme at 0°C. The activity in tissue subjected to microwave irradiation is identical with that observed for water blanks. The presence of this amount of tissue does not alter the extraction. By comparison the activity of intact enzyme at 0°C is quite large. The results shown in Fig. 3 were obtained when samples from the same homogenates were incubated at 0 and 37°C. The activity at 0°C is even quite high when compared to that at 37°C.

Homogenizing and diluting the tissue with a 1% Triton X-100 solution did not increase the activity observed for the enzymes, but it did make pipeting more difficult.

DISCUSSION

We had been using an existing cholinesterase assay *(7)* but felt better methods were now available for separating acetylcholine and acetate. The liquid cation-exchange method was chosen for this purpose *(1, 8)*. At the same time, we decreased the total incubation volume to 30 μl. Skill in the

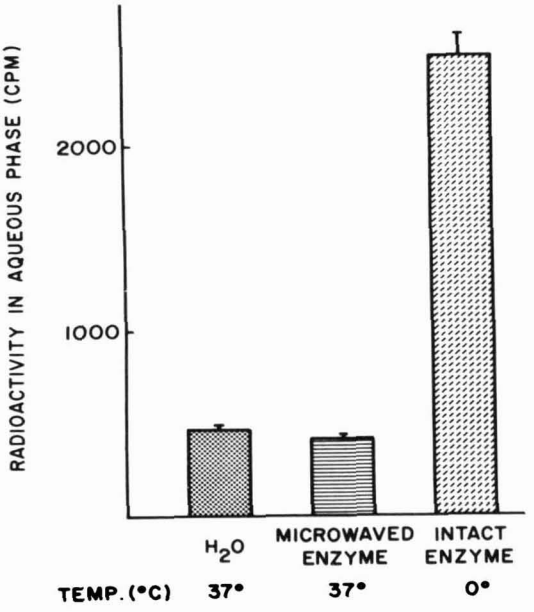

FIG. 2. Comparison of radioactivity found in the aqueous phase after 30 minute incubations with water or microwaved enzyme at 37°C and intact enzyme at 0°C. Total 14C per tube was 22,757 ± 56 cpm. Values represent the mean ± SEM for four rats or four determinations using water.

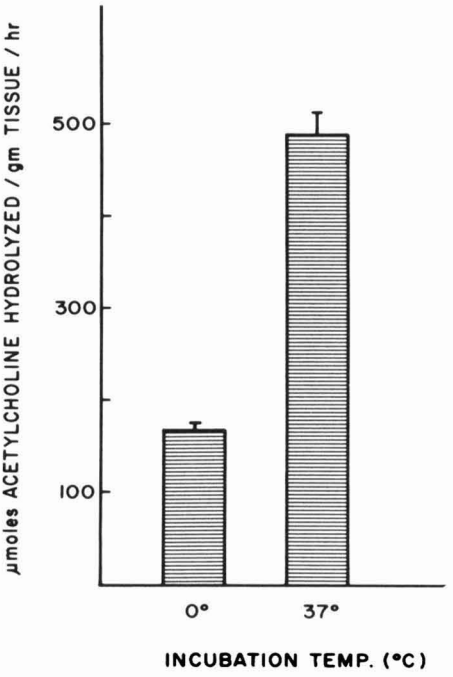

FIG. 3. Comparison of cholinesterase activity of rat frontal cerebral cortex at 0° and 37°C. Values represent the mean ± SEM for four rats.

use of Lang–Levy micropipets of the size employed is relatively easy to acquire. The use of only 100 μl of a solution containing 75 mg tetraphenylboron/ml 3-heptanone gave blanks of 2% of the total radioactivity added to the tubes. Counting half the aqueous phase simplified calculations when samples were run in duplicate. The use of sample vials for counting was included primarily as a conservation measure. Usage of such vials as incubation vessels would appear to place a limit on how small the incubation volumes could be *(2)*.

We feel that this assay will provide an easy and rapid method for the determination of cholinesterase activity over a wide range of tissue sizes. It has been used in our laboratory to examine rat brain and rabbit iris. The assay could be made more specific by the use of other substrates and selective enzyme inhibitors *(5, 7)*.

SUMMARY

A radioisotopic assay for cholinesterase activity is described, which was developed by combining parts of existing systems. Easily used microliter volumes are employed. Labeled acetylcholine and acetate are separated by liquid cation-exchange chromatography. The method is sensitive, rapid, and easy to perform.

ACKNOWLEDGMENTS

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Micromethod for the Simultaneous Analysis of Phenobarbital, Diphenylhydantoin, Carbamazepine, and Primidone in Blood

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INTRODUCTION

Gas–liquid chromatography is widely used for determining antiepileptic drugs (*1–3*). Its sensitivity and specificity allows simultaneous measurement of anticonvulsant drugs. Here, we describe a simple method for simultaneously determining phenobarbital, diphenylhydantoin, primidone, and carbamazepine in 100 μl of blood. The time required to complete the test is less than 30 min.

MATERIALS

1. Centrifuge tubes, 15 ml.

2. Syringe barrel filters.[1]

3. Standards. Drugs used were diphenylhydantoin (Dilantin; Parke, Davis & Co., Detroit, Mich. 48232), carbamazepine (Tegretol; CIBA Pharmaceutical Co., Summit, N.J. 07901), phenobarbital (Eli Lily and Co., Indianapolis, Ind. 46206), primidone (Ayerst Laboratory Inc., New York, N.Y. 10017) and 5-(*p*-Methylphenyl)-5-phenylhydantoin (Aldrich Chemical Co., Milwaukee, Wis. 53200).

4. Trimethylphenylammonium hydroxide, 0.2 mol/l. This was prepared from trimethylphenylammonium iodide (Fisher Scientific Co., Silver Spring, Md. 20901) that had been recrystallized three times from absolute ethanol: Into a 250-ml glass-stoppered Erlenmeyer flask with a Teflon stirrer, add 6.94 g of silver oxide, 10.52 g of trimethylphenylammonium iodide and 200 ml of absolute methanol. Stir (magnetic stirrer) for 2.5 hr and store at 4°C. The supernatant solution is stable for at least 1 month. Filter appropriate amounts of it through Whatman No. 1 filter paper immediately before use.

5. Internal standard. Dissolve 24 μg of 5-(*p*-Methylphenyl)-5-phenylhydantoin per milliliter of chloroform, and use 500 μl of this solution for the extraction.

[1] To the 2.5 ml syringe barrel (B–D Plastic Pak) four filter paper discs ¼-inch in diameter (A. H. Thomas & Co.) were inserted and pushed to the bottom of the syringe.

TABLE 1
CONCENTRATION OF WORKING STANDARDS (mg/l)

Standard	Phenobarbital	Diphenylhydantoin	Primidone	Carbamazepine
1	5	2.5	2.5	1
2	10	5	5	2.5
3	20	10	10	5
4	40	20	15	7.5
5	80	40	20	10

6. Drug standards. The stock drug standards were prepared in the following concentrations: Phenobarbital, 2 g/l; carbamazepine, 0.5 g/l; primidone, 1 g/l; and diphenylhydantoin, 2 g/l. The stock drug standards were added to a drug-free pool of serum to obtain five standards with the concentrations listed in Table 1. All four drugs were added to the same serum. These standards were kept frozen in screw-cap, Teflon-coated tubes until use.

METHODS

Standard curve. Standard curves were prepared for each drug. Each serum standard was extracted and chromatographed in duplicate as if it were a patient sample. The mean value of the relative peak height ratios from the duplicate analyses of each standard were plotted against the concentration of the respective standard (Fig. 1).

Instrumentation. We used a Model 2400 gas chromatograph (Fisher Scientific Co., Pittsburg, Pa. 15219) with dual hydrogen flame-ionization detectors and borosilicate glass columns (200 cm × 2 mm i.d.) packed with 3% SP 2250 on 100/120 mesh Supelcoport-type 50–50 Me–Ph (Supelco, Inc., Bellefonte, Pa. 16823). Before use, the columns were heat conditioned at 310°C for 24 hr with a carrier gas (nitrogen) flow rate of 50 ml/min.

The oven temperature was maintained at 190°C during periods of inactivity, with a carrier-gas flow rate of 50 ml/min. The injection ports were sealed with 10-mm septums (Supelco). Detectors and injection ports were heated to 310 and 250°C, respectively. The electrometer output was monitored with a Fisher Model 5000 series dual-pen recorder at a chart speed of 25 mm/min. The electrometer was operated at a range of 10^{-11} A/mV and the amplifier output was attenuated at 32.

Chromatographic conditions. Oven temperature was programmed from 190 to 300°C at a rate of 10°C/min, the program being started immediately after the sample extract was injected. Gas-flow rates were adjusted at: Nitrogen, 160 ml/min; hydrogen, 30 ml/min; and air, 300 ml/min.

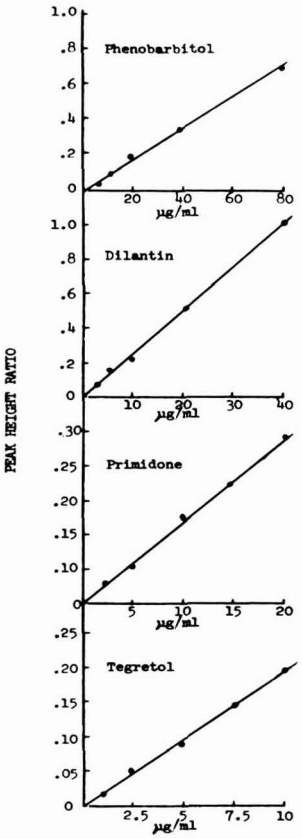

FIG. 1. Standard curve for phenobarbital, diphenylhydantoin, carbamazepine, and primidone.

Extraction procedure. One hundred microliters of blood were added to 50 μl of 0.25 M hydrochloric acid in a centrifuge tube (Teflon-lined screw cap). Five hundred microliters of chloroform containing 12 μg of internal standard (MPHH) were added to the tube and shaken for 30 sec on a Vortex mixer. The aqueous (upper) phase was aspirated and the organic phase filtered through the syringe barrel filter (filter paper was rinsed before use with few milliliters of chloroform) into a conical centrifuge tube and evaporated to dryness with the aid of a warm water bath (40°C) and a stream of nitrogen. The dried tubes were closed tightly with a cork until ready to inject to the gas chromatograph.

Chromatography. The dried residue in the centrifuge tube was reconstituted with 50 μl of TMPAH. One to two microliters of this extract was chromatographed using temperature programming with the SP 2250 column. Peaks were identified by comparing their relative retention times

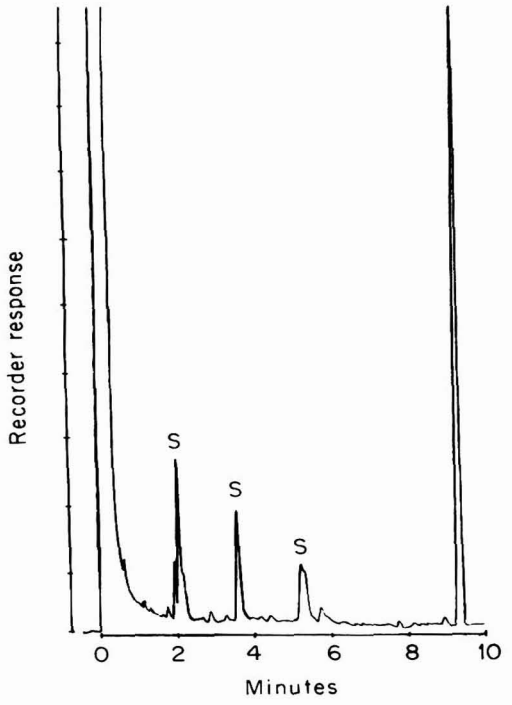

FIG. 2. Chromatogram obtained by methylation of blood extract from a person who is not on any medication. Temperature programmed from 190 to 300°C at 15°C/min. Column packing 3% SP 2250 on 100/120 mesh Supelcoport-type 50–50 Me–Ph. I.S., 5-(*p*-Methylphenyl)-5-phenylhydantoin; S, peak due to blood constituents.

(relative to the internal reference peak) to known standards. Drug concentrations were calculated from the standard curve.

The precision of the proposed method was checked by using aliquots of spiked blood pools which were kept frozen. The blood pool contained all four drugs. The standard deviation and percentage of recovery are given in Table 2.

TABLE 2
BETWEEN-DAY ANALYTICAL RECOVERIES

Drugs added to serum	Conc[a]	Mean conc[a] found (mg/l)	SD[a]	Recovery %
Phenobarbital	20	19.6	0.9	98
Diphenylhydantoin	10	9.7	0.2	97
Primidone	15	15.3	1.2	102
Carbamazepine	10	10.2	0.4	102

[a] $N = 31$.

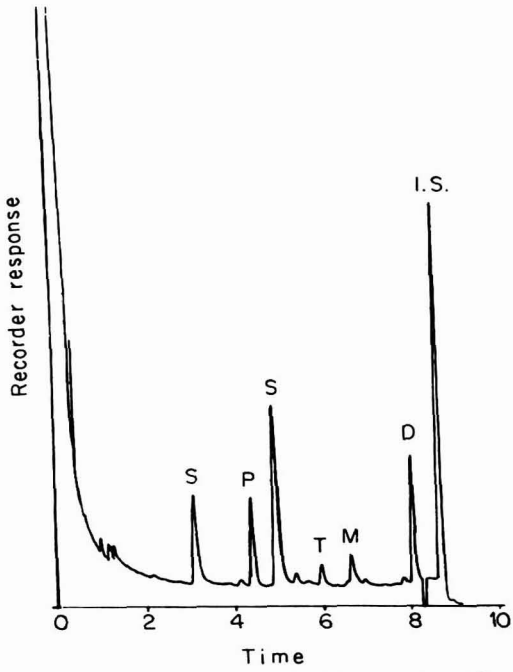

FIG. 3. Chromatogram obtained by methylation of a blood extract from a patient who is on all four antiepileptic drugs. Temperature programmed from 190 to 300°C at 15°C/min. Column packing 3% SP 2250 on 100/120 mesh Supelcoport-type 50–50 Me–Ph. P, phenobarbital; T, carbamazepine; M, primidone; and D, diphenylhydantoin; I.S., 5-(*p*-Methylphenyl)-5-phenylhydantoin; S, peak due to serum constituents.

RESULTS

Figure 1 is a standard curve of primidone, diphenylhydantoin, carbamazepine, and phenobarbital prepared by the peak height ratio technique utilizing MPHH as the internal standard. The standards were run in duplicate and the mean value used to plot the graph. All four standard curves were linear to approximately twice their toxic level. Recovery of the drugs from the blood was measured by adding known amounts of drugs (phenobarbital, diphenylhydantoin, carbamazepine, and primidone) to the same sample and analyzed for 31 consecutive days. The recovery was 97 to 102% (Table 2).

Chromatograms of serum specimens extracted and methylated according to our procedure are presented in Fig. 2, 3, and 4. The chromatogram in Fig. 2 with the internal standard (I.S.) is a typical pattern of the blood from a normal individual not receiving anticonvulsants. The three predominant peaks which are due to serum constituents are marked S. The chromatogram in Fig. 3 is from a patient who is on all four of the an-

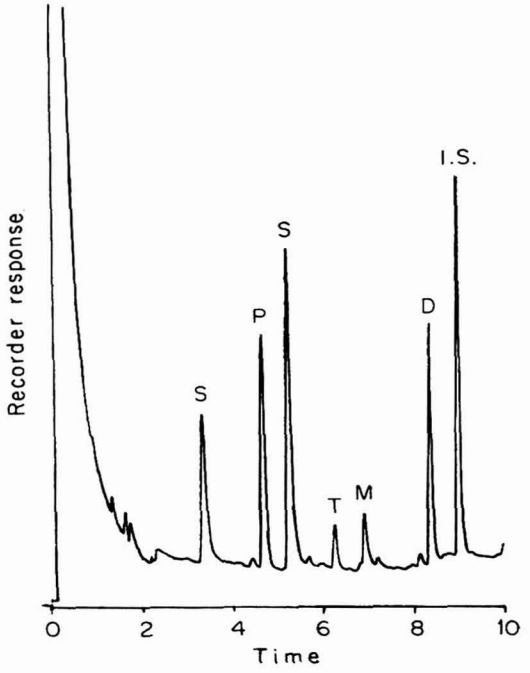

FIG. 4. Chromatogram obtained by methylation of a blood extract which was spiked with known amount of antiepileptic drugs. Temperature programmed from 190 to 300°C at 15°C/min. Column packing 3% SP 2250 on 100/120 mesh Supelcoport-type 50–50 Me–Ph. P, phenobarbital; T, carbamazepine; M, primidone; and D, diphenylhydantoin; I.S., 5-(*p*-Methylphenyl)-5-phenylhydantoin; S, peak due to serum constituents.

tiepileptic drugs. The concentrations calculated from this serum sample were primidone, 4 mg/l; phenobarbital, 28 mg/l; carbamazepine, 3 mg/l; and diphenylhydantoin, 11 mg/l. The chromatogram in Fig. 4 was prepared by adding known amounts of drugs to pooled blood. All four drug peaks were well separated and there were no interfering peaks.

Precision. The precision of the proposed method was evaluated by a between-day (Table 2) statistical analysis. The between-day standard deviation varied from 0.2 to 1.2 mg/l. Recoveries of the drugs were excellent and varied from 97 to 102%.

Drug concentrations as low as 0.5 mg/l were easily quantitated by adjusting the sensitivity of the analysis, e.g., injecting a more concentrated extract, increasing the volume of injection or increasing the electrometer output.

DISCUSSION

Rose, Smith, and Penry (*5*) emphasize the fact that the determinations of antiepileptic drugs are not readily available to the majority of physi-

cians who prescribe these medications. This is primarily because laboratories are reluctant to establish anticonvulsant assays due to the fact it requires considerable capital investment and commitment of experienced technical personnel. However, it is important to have rapid and accurate blood levels for better treatment of epilepsy.

The method we offered is simple and well suited for the clinical laboratory. A single extraction and Chromatogram System is used for the analysis of all four of the anticonvulsant drug levels. The use of a single internal standard and one temperature programming reduces the technical difficulties and standardization procedure. The temperature programming permits well-separated peaks for all of the four drugs. The on-column methylation, use of glass column and SP 2250 gives good peak characteristics and a linear standard curve.

The five standards which contain all the four drugs were prepared on the basis of the table given in (*4*). In this proposed method standard five is approximately twice the toxic level while the standard one is less than one-third the therapeutic level.

The most advantageous aspect of the procedure is the capability to determine the most commonly used antiepileptic drugs in a single procedure within thirty minutes using 100 μl of blood.

SUMMARY

We describe a simple, sensitive method for the determination of phenobarbital, diphenylhydantoin, carbamazepine, and primidone in whole blood by use of gas-liquid chromatography with temperature programming. The methylated derivatives of these anticonvulsants are well resolved. 5-(*p*-Methylphenyl)-5-phenylhydantoin was used as the internal standard. The proposed procedure requires only 100 μl of blood, which can be collected by finger stick. The lower limit of detection for each of the drugs is 0.5 mg/l. Analytical recoveries of drug from serum were excellent and standard curves were linear to twice the toxic concentration for serum.

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MICROCHEMICAL JOURNAL 21, 279–285 (1976)

Heterometric Microdetermination of Cobalt Using Nitron as Titrant

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Nitron is a very well-known reagent for the quantitative precipitation of nitrate, perchlorate, perrhenate, and tetrafluoroborate *(3)*, but its applications as a titrant have been less widely studied.

The reagent, which forms monovalent onium cations in acidic medium, gives sparingly soluble precipitates with some metal complex anions, especially the thiocyanato complexes. Thus, a standard nitron acetate solution was used for the heterometric titration of Au(III), Pd(II), and Pt(IV) in the presence of thiocyanate *(2)*. A similar procedure was reported *(1)* later for the estimation of ferric iron in concentrated thiocyanate solutions.

In the course of investigations in this laboratory, it was observed that nitron formed a sparingly soluble blue precipitate with cobaltous ion in presence of thiocyanate. It was further found that the reagent could be used for the heterometric determination of cobalt. In this titration 2 mol of nitron react with 1 mol of tetrathiocyanato cobalt ion to form the ion-association complex $(\text{Nitron} \cdot \text{H})_2[\text{Co(CNS)}_4]$.

EXPERIMENTAL

Apparatus and reagents. A Carl Zeiss Jena "Spekol" spectrocolorimeter supplemented with a T_i unit for photometric titration was used, titrations being carried out in rectangular cells of 30-ml capacity.

A 10-ml microburette was employed for titrations.

Gelatine, 0.25% solution, Gum Arabic, 0.2% solution, Agar–Agar, 0.25% solution and starch, 1% solution, were freshly prepared as required.

Standard cobalt solutions: A 0.05 *M* cobalt solution was prepared from Analar $\text{Co SO}_4 \cdot 7\text{H}_2\text{O}$ and standardized with EDTA. This was used for preparing more dilute standards.

Nitron acetate solution. A 0.01 *M* stock solution was prepared by dissolving 0.7802 g of nitron (mol wt. 312.16, Merck Reagent) and 7 ml of *N* acetic acid in water to give 250 ml of solution. More dilute solutions were freshly prepared by dilution with water. The solutions of nitron were kept

in dark brown bottles. The stock solution remained unchanged for a long period.

Potassium thiocyanate: A 4 *M* solution of AR grade reagent was used.

Sodium sulphate: A 10% aqueous solution of AR $Na_2SO_4 \cdot 10\,H_2O$ was used.

Procedure. Transfer the sample solution containing 0.01–0.25 mg of cobalt into a titration cell. Add 2 ml of 4 *M* potassium thiocyanate solution and 3 ml of 10% sodium sulphate solution, then make up to 15 ml with water. Adjust the galvanometer to zero optical density at 550 nm. Titrate with 0.002 *M* nitron acetate, adding the titrant rapidly and in small portions with vigorous stirring; and record the optical density after each addition until finally it becomes steady. The first maximum density point corresponds to the end point. The titration takes about 2–3 min.

RESULTS AND DISCUSSION

General. The reaction of cobaltous ion with nitron acetate was studied at different pH-values, in the presence and in the absence of thiocyanate. No noticable reaction took place in the absence of thiocyanate. In the presence of excess thiocyanate, however, a blue precipitate of nitron cobaltothiocyanate was formed. The reaction occurred instantaneously which proved to be of an ionic character in which nitron acts as cation. The precipitation of nitron cobaltothiocyanate was found to be quantitative in the pH range 0–6[1], since cobalt was not found in the filtrate. However, the method could not be used for the gravimetric determination of cobalt. A precipitate consisting of a mixture of nitron cobaltothiocyan-

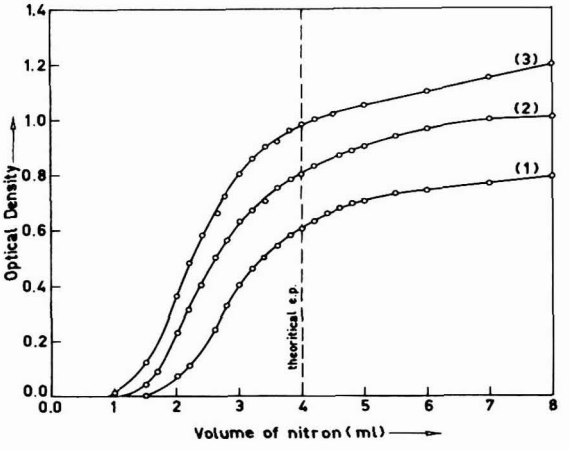

FIGURE 1

[1]Nitron cannot be used in basic solutions because it separates as the free organic base.

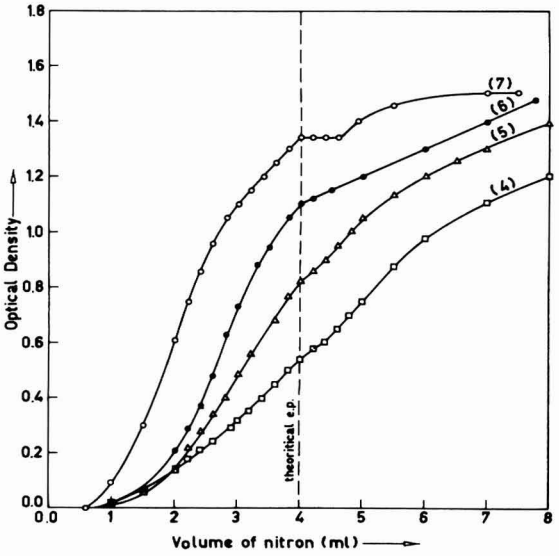

FIGURE 2

ate and nitron thiocyanate was always formed. Removal of the more soluble nitron thiocyanate by thorough washing was not practical, as the feebly stable cobaltothiocyanate complex decomposed to the aquated ion.

As minute amounts of cobalt could be precipitated quantitatively as nitron cobaltothiocyanate, and since the precipitate is stable only in thiocyanate solutions, it was possible to determine cobalt heterometrically with nitron as titrant. The molar ratio of nitron : Co^{2+} at the end point was found to be 2:1 indicating the formation of the ion-association complex (nitron · $H)_2[Co(CNS)_4]$. The effect of the medium on the end-point detection and the effect of foreign ions on the accuracy were studied. A selection of experiments, the composition of the solutions and the results obtained are compiled in Table 1. The course of some heterometric titrations is presented in Figs. 1, 2, and 3; the curves are given the same experiment numbers as in the table.

Effect of the medium on the end-point detection. Before establishing the procedure outlined above, it was difficult to determine the end point precisely. The optical density did not reach a steady value after the theoretical equivalence point, but it increased regularly. This was thought to be due either to the low stability of the complex or to the formation of nitron thiocyanate. Increasing the stability by increasing thiocyanate concentration, however, did not improve the curves (Experiments 1–3). Besides, when blank experiments were carried out by titrating concentrated thiocyanate solutions with nitron in the absence of cobalt, a slight white turbidity formed, but this never gave a high optical density (less than 0.1).

TABLE 1

GENERAL COMPOSITION : 2 ml m*M* $CoSO_4$ + *a* ml 4 *M* KCNS + *b* ml SUPPLEMENTS + *c* mg FOREIGN IONS[a] + H_2O TO 15 ml + *x* ml 0.002 *M* NITRON ACETATE. CONTENT: 0.118–0.236 mg COBALT

Expt.	$CoSO_4$ (*M*)	KCNS (ml)	Supplements	Foreign ions (mg)	% Co	Nitron acetate: Initial ppt at ml	Nitron acetate: End point at ml	Maximum o.d.	% error
1	0.0020	2				1.9			
2	0.0020	3				1.4			
3	0.0020	4				1.2			
4	0.0020	3	5 ml 0.2% Gum Arabic			1.3	4.0		0.0
5	0.0020	3	5 ml 0.25% gelatine			1.5	4.0		0.0
6	0.0020	3	5 ml 0.25% Agar–Agar			1.3	4.0		0.0
7	0.0020	3	5 ml 1% starch			0.8	4.0		0.0
8	0.0020	2	2 ml 10% Na_2SO_4			0.75	—	—	—
9	0.0020	2	3 ml 10% Na_2SO_4			0.50	4.0	1.40	0.0
10	0.0020	2	4 ml 10% Na_2SO_4			0.50	4.0	1.32	0.0
11	0.0020	3	2 ml 10% Na_2SO_4			0.50	—	—	—
12	0.0020	3	3 ml 10% Na_2SO_4			0.50	4.0	1.35	0.0
13	0.0020	3	4 ml 10% Na_2SO_4			0.40	4.0	1.28	0.0
14	0.0020	2	3 ml 10% Na_2SO_4			0.50	4.0	1.38	0.0
15	0.0020	2	3 ml 10% Na_2SO_4			0.50	4.02	1.36	+0.5
16	0.0015	2	3 ml 10% Na_2SO_4			0.50	3.00	0.92	0.0

TABLE 1—*Continued*

Expt.	$CoSO_4$ (*M*)	KCNS (ml)	Supplements	Foreign ions (mg)	% Co		Nitron acetate: Initial ppt at ml	Nitron acetate: End point at ml	Maximum o.d.	% error
17	0.0015	2	3 ml 10% Na_2SO_4				0.50	3.00	0.95	0.0
18	0.0010	2	3 ml 10% Na_2SO_4				0.60	2.00	0.66	0.0
19	0.0010	2	3 ml 10% Na_2SO_4				0.50	2.02	0.68	+1.0
20	0.0020	2	3 ml 10% Na_2SO_4	Cd^{2+}	8	3	0.50	3.98	1.35	−0.5
21	0.0020	2	3 ml 10% Na_2SO_4	Ni^{2+}	6	4	0.60	4.00	1.22	0.0
22	0.0020	2	—	Pb^{2+}	5	4.8	0.40	4.04	1.18	+1.0
23	0.0020	2	3 ml 10% Na_2SO_4	Mn^{2+}	6	4	0.20	4.02	1.32	+0.5
24	0.0020	2	3 ml 10% Na_2SO_4	Cr^{3+}	5	4.8	0.50	4.00	1.35	0.0
25	0.0020	2	3 ml 10% Na_2SO_4	MoO_4^{2-}	5	4.8	0.50	3.06	1.26	−1.0
26	0.0020	2	3 ml 10% Na_2SO_4	WO_4^{2-}	5	4.8	0.50	4.06	1.27	+1.5
27	0.0020	2	3 ml 10% Na_2SO_4	CrO_4^{2-}	8	3	0.50	4.02	1.28	+0.5
28	0.0020	2	3 ml 10% Na_2SO_4	NO_3^-	5	4.8	0.40	4.02	1.32	0.5
29	0.0020	2	3 ml 10% Na_2SO_4	Au^{3+}	1	2.4	0.40	4.00	1.35	0.0
30	0.0020	2	3 ml 10% Na_2SO_4	Pt^{4+}	1	2.4	0.40	3.98	1.42	−0.5
31	0.0020	2	3 ml 10% Na_2SO_4 + 2 ml 1 *M* NaF	Fe^{3+}	2	12	0.50	3.94	1.35	−1.5
32	0.0020	2	3 ml 10% Na_2SO_4 + 2 ml 1 *M* NaF	Fe^{3+}	3	8	0.50	4.00	1.26	0.0
33	0.0020	2	3 ml 10% Na_2SO_4 + 2 ml 1 *M* NaF	Fe^{3+}	5	4.8	0.50	4.02	1.22	+0.5

[a] Foreign anions were added as sodium salts, lead was added as nitrate, and all other cations as sulphates.

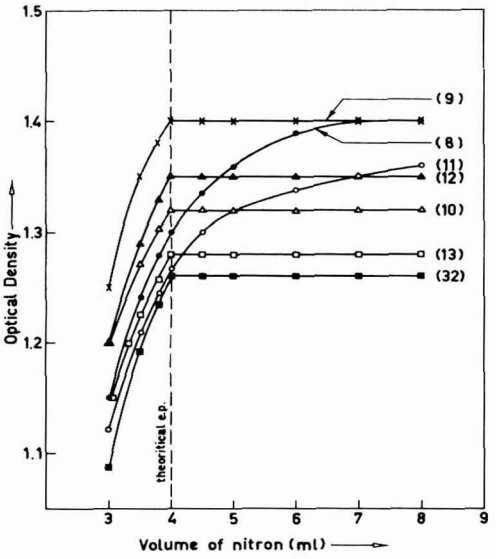

FIGURE 3

Adding protective colloids the end points were characterized by nondifferential points on the titration curves (Experiments 4–7), but they were not clear enough. On the other hand, the addition of a strong electrolyte (Na_2SO_4) gave excellent results; a horizontal maximum density line was obtained after the first maximum density point (=end point). Experiments 8–13 show the effect of sodium sulphate concentration on the titration curves. The optimum thiocyanate concentration was found to be about 0.5 *M*, that of sodium sulphate 2% (Experiment 9). The effect of strong electrolytes is apparently the rapid coagulation of the primary particles.

Effect of foreign ions. As a check on the procedure "unknowns," with and without diverse ions present; were analyzed by one of us (S.K.). The results obtained are shown in Table 1. Cadmium, nickel, lead, manganese, and chromium did not interfere. Molybdate, tungestate, chromate, nitrate, gold (III), and platinum (IV), which are known to form precipitates with nitron, did not interfere up to reasonable ratios; their nitron salts are more soluble than the cobalt complex and the formation of their precipitates is slow at low nitron concentration. Iron (III) could be masked with sodium fluoride (Experiment 32).

Results in all cases were satisfactory. In most cases the error was less than 1%, but exceptionally it amounted to 1.5%.

Zinc interferes; its thiocyanate complex forms a white precipitate before the precipitation of the cobalt complex, and copper interferes by forming a precipitate with thiocyanate.

SUMMARY

Small amounts of cobalt (0.1–0.25 mg in 15 ml solution) were determined heterometrically in the presence of potassium thiocyanate by titration with nitron acetate solution. A strong electrolyte (Na_2SO_4) must be added to obtain a clear end point. The titration can be carried out in the presence of a number of diverse ions, e.g., Cd^{2+}, Ni^{2+}, Pb^{2+}, Mn^{2+}, Cr^{3+}, MoO_4^{2-}, WO_4^{2}, CrO_4^{2-}, NO_3^{-}, Au^{3+}, Pt^{4+} without interference; Fe^{3+} can be masked with NaF. Copper and zinc interfere and must be absent. The titration takes about 2–3 min. and the maximum error was 1.5%.

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Spectrophotometric Determination of Halogens in Water Using Phenophthalin as Reagent

Determination of Residual Chlorine in Tap-Water

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INTRODUCTION

Reduced phenolphthalein, known as phenophthalin, is colorless in alkaline medium. An intense red color is produced immediately on addition of oxidizing agents that can act in alkaline medium (oxidative regeneration of phenophthalein). The reagent was originally proposed for the detection of ferricyanide and hydrogen peroxide (*1*) and recently, Shahine *et al.* have used it for the spot-test detection (2) and the spectrophotometric determination (*3*) of gold.

In the course of investigations in this laboratory, it was observed that halogens do not oxidize phenophthalin, but they readily oxidize ferrocyanide if the latter is present in excess. It was also found that ferricyanide oxidizes phenolphthalin stoichiometrically; the produced color obeys Beer's law. These facts have been made the basis for the spectrophotometric determination of halogens in water. Excess ferrocyanide is added to the sample solution and the produced ferricyanide is estimated spectrophotometrically with phenolphthalin. The method was applied to the determination of residual chloride in tap-water and compared with the orthotolidine method.

EXPERIMENTAL

Apparatus and Reagents

All spectral measurements were made with a "Prolabo" spectrophotometer, Type No. 05 886, using 1-cm cells.

Phenolphthalin solution. This reagent was prepared by mixing 2 g of phenolphthalein, 10 g of sodium hydroxide, 5 g of zinc dust, and 20 ml of water in a 50-ml round bottom flask, and heating for 2 hr under a reflux. After cooling the liquid was passed through a hardened filter paper and the colorless filtrate was made up to 50 ml with distilled water. This solution was kept in a brown bottle contianing a few granules of zinc. Under these conditions it remains colorless for at least 1 week. Before use, 10 ml of this solution were diluted to 100 ml with boiled-out distilled water and the diluted solution used as reagent.

Potassium ferrocyanide. An approximately 0.01 *M* solution was prepared daily from the AnalaR salt.

Standard solutions of halogens. Fresh 0.003 *M* chlorine, bromine, and iodine solutions were used for constructing calibration curves. These were prepared by dilution of 0.03 *M* stock solutions immediately standardized by the iodometric method.

Procedure

Transfer an aliquot of the test solution, containing 0.0002–0.002 *M* equivalent of the halogen, to a 25-ml volumetric flask containing 2 ml of 0.01 *M* potassium ferrocyanide solution, stopper, mix, and after 2 min add 2 ml of phenophthalin reagent. Make up to volume with boiled-out distilled water and measure the absorbance within 2 min at 553 nm against a blank carried through the same process with all reagents but the halogen.

Construct calibration curves using 0.5–5 ml portions of the standard halogen solution treated in the same way as for the sample solution.

Note: For the determination of residual chlorine in tap-water use 20-ml samples and if necessary use 5-cm cells.

RESULTS AND DISCUSSION

Conditions of Reactions

It was found that the reaction between free halogens and ferrocyanide takes place stoichiometrically only when the latter is present in excess. Thus, in a trial to establish a colorimetric method for mixtures of ferrocyanide and ferricyanide using excess halogen to oxidize ferro to ferricyanide the results were somewhat lower than expected. This was attributed to the reversible nature of the reaction in the case of iodine and to the partial decomposition of the complex cyanide (oxidation to cyanogen) in the cases of bromine and chlorine.

The oxidation of phenolphthalin with ferricyanide occurs almost instantaneously, but the color slightly fades after 4 min and the absorbance should be measured within this period. Also the intensity and stability of the color decreases with increasing the final concentration of sodium hydroxide. The optimum concentration was found to be 0.02 *M* (*3*) which is usually attained when the above procedure is followed.

Analysis of "Unknowns"

As a check on the procedure "unknowns" were analyzed by one of us (R.M.). The results obtained are shown in Table 1; the error scarcely exceeds 1%.

Statistical Comparison with the Orthotolidine Method

The present method was found to be applicable to the determination of

TABLE 1

Halogen	Present (μg)	Found (μg)	Difference	Error %
Chlorine	2.84	3.86	+0.02	+0.70
	5.68	5.73	+0.05	+0.88
	8.52	8.52	0.0	0.0
	11.36	11.32	−0.04	−0.34
Bromine	2.40	2.38	−0.02	−0.83
	7.67	7.71	+0.04	+0.52
	11.50	11.43	−0.07	−0.61
	19.18	18.96	−0.22	−1.14
Iodine	9.35	9.38	+0.03	+0.32
	18.70	18.82	+0.12	+0.64
	27.58	27.34	−0.24	−0.87

total residual chlorine in water supplies. It was statistically compared with the standard orthotolidine method (*4*). Replicate analyses by the two methods were carried out on the same sample[1] and in the same period of time. The results are shown in Table 2 arranged in descending order of magnitude. The estimate of variances found for phenolphthalin and orthotolidine are 4.60×10^{-4} and 10.57×10^{-4}, respectively. The precision of the two methods may be compared with the Variance Ratio F test, whence

$$\text{Variance Ratio } (F) = 10.57 / 4.60 = 2.298.$$

TABLE 2[a, b]

Phenolphthalin method			Orthotolidine method		
x	$(x-\bar{x}) \times 100$	$(x-\bar{x})^2 \times 10^4$	x	$(x-\bar{x}) \times 100$	$(x-\bar{x})^2 \times 10^4$
0.63	+3	9	0.66	+4	16
0.62	+2	4	0.66	+4	16
0.62	+2	4	0.64	+2	4
0.62	+2	4	0.63	+1	1
0.60	0	0	0.61	−1	1
0.60	0	0	0.60	−2	4
0.60	0	0	0.58	−4	16
0.59	−1	1	0.58	−4	16
0.58	−2	4			
0.58	−2	4			
0.56	−4	16			

[a] x = chlorine found in ppm.

[b] Mean value = 0.60 ppm. $\Sigma(x-\bar{x})^2 = 46 \times 10^{-4}$, $S^2 = 4.60 \times 10^{-4}$; $S = 2.145 \times 10^{-2}$ ppm. Mean value = 0.62 ppm. $\Sigma(x-\bar{x})^2 = 74 \times 10^{-4}$; $S^2 = 10.571 \times 10^{-4}$, $S = 3.251 \times 10^{-2}$ ppm.

[1] Water supply of Abbassia district.

With 7,10 degrees of freedom the critical value of F at the 5% level is 3.14. The calculated value is below the critical value. Hence there is no evidence that the reproducibility of either method is superior to that of the other.

To compare the accuracy of the two methods, the same sample was analyzed by iodometric titration. The mean result, which may be considered the nearest to the true value, was found to be 0.60 ppm. As this value is equal to that obtained by the phenolphthalin method and smaller than that obtained by the orthotolidine method, it may be stated that the former method is more accurate than the latter. However, this statement can be justified only if the relative bias between the two methods is statistically significant. The 95% confidence limits for relative bias may be found as follows:

$$\text{Relative Bias} = 0.02 \text{ ppm} \cdot$$

As the two variances do not differ significantly, they may be combined to give the common variance

$$S^2_{com} = \frac{(46 + 74)}{(10 + 7)} \times 10^{-4}$$

$$= 7.059 \times 10^{-4}$$

from which the variance and standard deviation of the difference between the two means are

$$S^2_d = S^2_{com} \left(\frac{1}{n_1} + \frac{1}{n_2}\right)$$

$$= 1.524 \times 10^{-4}$$

$$S_d = 1.234 \times 10^{-2} \text{ ppm} \cdot$$

and

Confidence limits may now be set with the help of the table of t; the number of degrees of freedom is that of the combined estimate of variance, i.e., 17.

The 95% confidence limits for relative bias

$$0.02 \pm (2.11)\,(1.23) \times 10^{-2}$$

$$= -0.006 \text{ to } +0.046 \text{ in terms of ppm.}$$

As zero lies within these limits there is no evidence that one method is biased with respect to the other; i.e., the two methods may possess the same accuracy.

SUMMARY

A new spectrophotometric method for the determination of halogens is described. Excess potassium ferrocyanide is added to the test solution followed by phenolphthalin reagent (alkaline solution of reduced phenolphthalein). The ferricyanide produced reacts with phenolphthalin to give an intense red color. The method was statistically compared with the orthotolidine method. It was found that both possess almost the same accuracy and precision.

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MICROCHEMICAL JOURNAL 21, 291–301 (1976)

Lower Limits of the Potentiometric Microtitration of Chloride with Silver Ions[1,2]

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INTRODUCTION

Chloride has been determined by potentiometric titration with silver ions for many years. Nevertheless, papers continue to appear dealing with this determination (*5,8,9*). Our interest in the procedure stems from a paper by Jay *et al.* (*4*) that is not easily obtained. These workers reported preliminary results using acetonic silver perchlorate as titrant in various nonaqueous media and claimed success in differentiating all four halogens—including fluoride—in acetic anhydride. Although we plan further work to quantify their findings, this paper will be limited to the investigation of the lower limits of the potentiometric titration of chloride.

Frazer *et al.* (*1*) have recently described an integrated automated titration system controlled by a PDP-8/I minicomputer. We have used such a system in this work to generate titration curves and to evaluate experimental results for chloride with acetonic silver perchlorate in acetone and acetic anhydride.

EXPERIMENTAL METHODS

Reagent grade chemicals were used throughout. The titrant was 0.001 *N* silver perchlorate (G. F. Smith Chemical Co.,[3] anhydrous) in acetone. The standard chloride solution contained 1 μmol/ml. It was prepared by dissolving 58.4 mg of sodium chloride in 40 ml of water and diluting to 1 liter with acetone. For amounts of chloride <1 μmol, this standard was

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[2] This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Energy Research and Development Administration, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsiblity for the accuracy, completeness or usefulness of any information, apparatus, produce or process disclosed, or represents that its use would not infringe privately-owned rights.

[3] Reference to a company or product name does not imply recommendation of the product by the University of California or the U. S. Energy Research & Development Administration to the exclusion of others that may be suitable.

diluted 1:10, by adding the required amount of distilled water to keep the solution 4% (v/v) and by diluting with acetone. The titration system was controlled by a conventional PDP-8/I minicomputer processor. A summary of its operation and a schematic drawing of the equipment were published elsewhere (*1*). The emf was monitored by a silver sulfide ion-selective electrode (ISE) and a double-junction reference electrode (salt bridge filled with saturated potassium nitrate in methanol or anhydrous lithium perchlorate in acetic anhydride).

Titration endpoints were calculated according to Savitsky and Golay (*10*). A convolute was used for a third-order second derivative using 25 points. The zero-crossing was found by linear interpolation near the sign change.

Stirring was provided by a magnetic stirrer. The stirring motor was separated from the titration vessel by a water-cooling plate and an aluminum plate connected to ground.

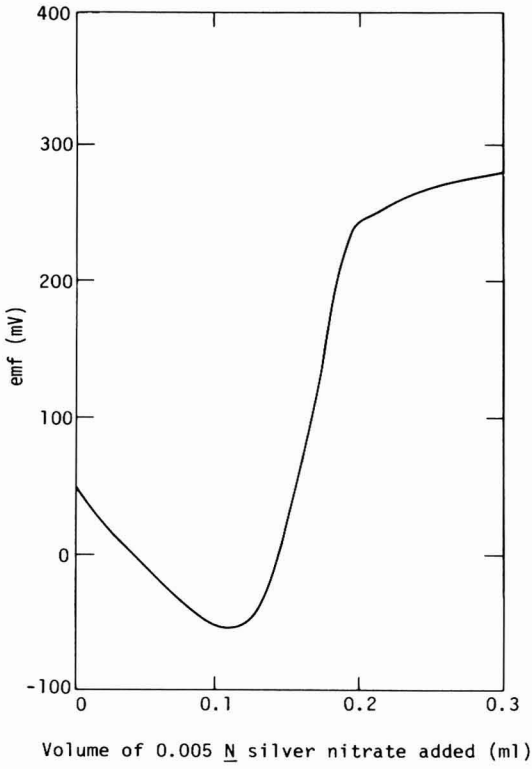

FIG. 1. Titration of 22.25 μg of chloride in 70% methanol with an applied polarization current of -0.4 μA.

RESULTS AND DISCUSSION

Endpoint breaks in potentiometric precipitation titrations can be enhanced by reducing the solubility of the precipitated species or by applying a polarization current to one of the electrodes (*7*). The solubility of the precipitate can be reduced by lowering the temperature of the titration medium, by using a nonaqueous solvent, or by combining the two procedures. This approach has been widely used and will be discussed below. The second approach (application of a polarization current) has been discussed thoroughly by Liteanu *et al.* (*7*). They have noted that the changes in the linearity range and the slope upon application of a small current can be used advantageously for potentiometric titrations and direct potentiometry. This second approach was used by Heistand and Blake (*3*) in the determination of small amounts of sulfate by titration with lead ions using a lead ISE. It has also been used by the author (*14*) in the determination of microamounts of phosphate with lead ions using a lead ISE or with silver ions using a silver sulfide ISE. We also briefly investigated it for the microtitration of chloride (*13*). It was found that use of a partially nonaqueous medium (60–80% methanol) improved the titration curves more than the application of a small polarization current.

Combination of the two (use of a partially nonaqueous medium plus application of a polarization current) yielded further improvement in the steepness and magnitude of the endpoint breaks. Figure 1 shows the

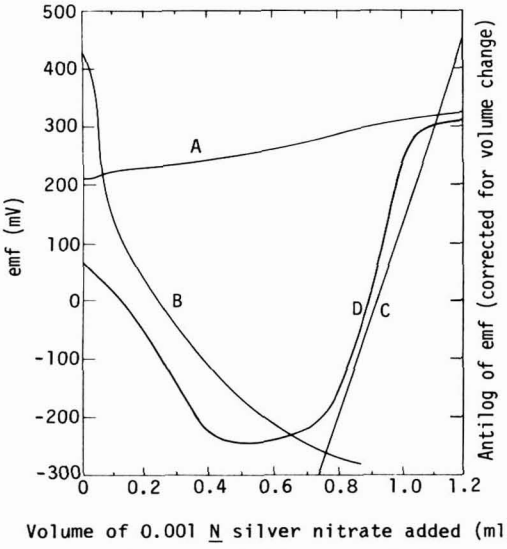

FIG. 2. Titration of 22.25 μg of chloride in 80% methanol with 0.001 *N* silver nitrate. Curve A, titration curve without impressed current; curve B, Gran plot; curve C, linearized and extrapolated Gran plot; curve D, titration curve with —0.4 μ A.

titration curve for 22.3 μg of chloride in 50 ml of 70% methanol (0.63 μmol; 1.26×10^{-5} N chloride) with 0.005 N silver nitrate using a polarization current of -0.4 μA (the sensing electrode was negative or cathodic with respect to the reference electrode). The potentiometric break (defined by the final minus the initial emf) was ~350 mV. Titration curves for the same amount of chloride in 80% methanol with a more dilute titrant, 0.001 N aqueous silver nitrate, are shown in Fig. 2. Although the break is barely discernible (~50 mV) without a polarization current, it is shown that a Gran plot can yield useful data, as previously discussed (*1*). The Gran plot yields a straight line only when silver ions are in excess because we are working near the electrode's limit of detection and in a partially nonaqueous medium. For 22.3 μg of chloride per 50 ml of 80% methanol, with a current of -0.4 μA, the potential break was ~500 mV. Figures 1 and 2 show that in very dilute chloride solutions under an applied current, the titration curves do not follow the familiar sigmoid-shaped pattern: the emf decreases before rising in the endpoint region. We found, however, that if the solutions are conditioned for 5 min by running the stirring motor and the polarization current (before starting the titration), then the curves tend to approach the S-shaped pattern.

With 0.001 N aqueous silver nitrate, the limit for this method is near 22.3 μg of chloride per 50 ml (1.26×10^{-5} N). Although good potentiometric breaks are obtained for smaller amounts of chloride, the curves are not steep enough to yield unequivocal endpoints using the method of Savitsky and Golay (*10*).

The lower theoretical limit for the potentiometric titration of chloride with silver ions can be calculated from the solubility product of silver chloride. The data in Table 1 show that lowering the temperature of the titration medium will reduce the solubility product in an aqueous medium.

TABLE 1
SOLUBILITY PRODUCT OF SILVER CHLORIDE IN VARIOUS MEDIA (*15, 16*)

Temp. (°C)	Medium	$-\log K_{SP}$	Solubility product	Chloride concentration (N)	Chloride (ppm)	Dielectric constant of pure solvent (*11*)
25	aqueous	9.75	1.75×10^{-10}	1.33×10^{-5}	0.47	78.5
5	aqueous	10.595	2.54×10^{-11}	5.04×10^{-6}	0.18	
25	methanol	13.05	8.91×10^{-14}	2.98×10^{-7}	0.01	32.7
25	50% ethanol	11.11	7.76×10^{-12}	2.79×10^{-6}	0.10	24.6
25	acetonitrile	12.9	1.26×10^{-13}	3.55×10^{-7}	0.01	37.5
25	20% dioxane	10.22	6.02×10^{-11}	7.77×10^{-6}	0.28	2.2
25	60% acetone	11.90	1.26×10^{-12}	1.12×10^{-6}	0.04	20.7
25	80% acetone	13.61	2.46×10^{-14}	1.57×10^{-7}	<0.01	

A much more significant reduction can be obtained in nonaqueous, or partially nonaqueous media. To obtain a relatively noise-free titration curve, one should choose a medium of fairly high dielectric constant. All solvents (or mixtures) listed in Table 1 qualify in this regard.

Havacek and Swann (*2*) recommend a titration medium and titrant 95% in methanol. They determined 0.01 mmol (354 μg) of chloride per 100 ml of solution with 0.01 *N* silver nitrate (10 μmol; 1×10^{-4} *N*). The potentiometric break was ~270 mV.

Prokopov (9) used a medium 90% in acetone and a platinum indicator electrode for the titration of 10^{-3} to 10^{-6} *N* chloride and bromide. He found that the platinum electrode produced potential breaks twice as large as that of the silver electrode. In recent work (*12*) we have found the breaks obtained with the platinum electrode to be considerably smaller than those obtained with a silver sulfide ISE. No data or titration curves are given, but Prokopov's experimental conditions produced a break of 100 mV for a 4×10^{-6} *N* chloride solution. Acetone yielded larger breaks than 1-propanol, 2-propanol, or ethanol.

Jay *et al.* (*4*) reported preliminary work on the determination of halides by potentiometric titration with acetonic silver perchlorate. With this titrant in acetic anhydride, all four halides—including fluoride—could be differentiated. No quantitative results were given, but the titration curves showed the magnitude of the breaks for chloride to be:

$$\text{acetic anhydride} > \text{acetone} > \text{DMF} > \text{acetonitrile} > \text{water}.$$

These authors recommend a 0.005 *N* titrant for 0.07 to 1.75 mg of chloride. As little as 60 μg of chloride can be determined, while for smaller amounts a titrant strength of 0.001 *N* is recommended. On the basis of their titration curves, we have calculated the breaks for approximately 2.5 μmol of chloride per 30 ml of solution to be 600 mV in acetic anhydride, 500 mV in acetone, 250 mV in DMF, and 200 mV in acetonitrile.

Lefferts (*6*) used a silver/glass electrode system and a titrant of 0.003 *N* silver nitrate in acetic acid/acetic anhydride. The titration medium was acetic acid. Sample sizes ranged from 0.4 to 5 mg. For 2.57 mg of chloride per 50 ml of acetic acid (72.5 μmol; 1.45×10^{-3} *N*), the break was ~500 mV; for 50 μg of chloride per 20 ml of acetic acid (1.41 μmol; 7.5×10^{-5} *N*), it was ~400 mV. Solvent blanks were 1 to 2 μg of chloride.

Nara *et al.* (*8*) determined 71 μg of chloride (2×10^{-6} *N*) with 0.002 *N* silver nitrate in 50% isopropanol. The titration medium was approximately 75% in isopropanol. They recommend a titration temperature of 10°C for chloride, thus combining the two methods for lowering the solubility of silver chloride. They stress that the delivery speed of the titrant is critical and that it should be <0.2 ml/min. However, for this amount of

TABLE 2

POTENTIOMETRIC MICROTITRATION OF CHLORIDE: SUMMARY OF PARAMETERS

Reference	Chloride taken		Titration medium	Titrant	Potentiometric break (mV)	Polarization current (μA)
	N	μmol				
This work	1.3×10^{-5}	0.63	70% methanol	0.005 N $AgNO_3$, aqueous	~350	−0.4
This work	1.3×10^{-5}	0.63	80% methanol	0.001 N $AgNO_3$, aqueous	~50	
This work	1.3×10^{-5}	0.63	80% methanol	0.001 N $AgNO_3$, aqueous	~500	−0.4
(2)	1×10^{-4}	10	95% methanol	0.01 N $AgNO_3$/95% methanol	~270	
(9)	4×10^{-6}		90% acetone	0.001 N $AgNO_3$, aqueous	~110	
(6)	1.45×10^{-3}	72.5	acetic acid	0.003 N $AgNO_3$/acetic acid/acetic anhydride	~500	
(6)	7×10^{-5}	1.41	acetic acid	0.003 N $AgNO_3$/acetic acid/acetic anhydride	~400	
(8)	2×10^{-6}	2	75% isopropanol	0.002 N $AgNO_3$/50% isopropanol	~100 at 30°C ~150 at 10°C	
(4)	8.3×10^{-5}	2.5	acetic anhydride	0.005 N $AgClO_4$/acetone	~600	
(4)	8.3×10^{-5}	2.5	acetone	0.005 N $AgClO_4$/acetone	~500	
(4)	8.3×10^{-5}	2.5	DMF	0.005 N $AgClO_4$/acetone	~250	
(4)	8.3×10^{-5}	2.5	acetonitrile	0.005 N $AgClO_4$/acetone	~200	
This work	1×10^{-5}	0.5	acetone	0.001 N $AgClO_4$/acetone	~500	
This work	6×10^{-6}	0.3	acetic anhydride/acetone, 4:1	0.001 N $AgClO_4$/acetone	~500	
(5)	1.4×10^{-6}	0.07	75% acetic acid	0.002 N $AgNO_3$, aqueous	not given	

TABLE 3
TITRATION OF CHLORIDE WITH 0.001 *N* ACETONIC $AgClO_4$ IN ACETONE[a]

Taken (μg Cl)	Taken (μmol Cl)	Normality chloride	Cl (ppm)	Titer (μg/ml)	Mean titer	N_{AgClO_4}
284.56	8	1.6×10^{-4}	5.69	38.73		
				39.01		
				39.40	39.05	0.001101
					s 0.34	
213.42	6	1.2×10^{-4}	4.27	38.79		
				38.59		
				38.69		
				38.69	38.69	0.001091
					s 0.08	
142.28	4	8×10^{-5}	2.85	38.84		
				39.16		
				38.73		
				39.38	39.03	0.001101
					s 0.30	
106.71	3	6×10^{-5}	2.13	39.05		
				39.51		
				39.51		
				39.05	39.28	0.001108
					s 0.27	
71.14	2	4×10^{-5}	1.42	37.47		
				37.71		
				37.59		
				38.19	37.74	0.001065
					s 0.32	
35.57	1	2×10^{-5}	0.71	40.93		
				41.22		
				41.50		
				40.87	41.13	0.001160
					s 0.29	
17.785	0.5	1×10^{-5}	0.36	39.94		
				41.39		
				40.29	40.54	0.001143
					s 0.76	
10.671	0.3	6×10^{-6}	0.21	40.19		
				39.61		
				40.19	40.00	0.001128
					s 0.33	
7.114	0.2	4×10^{-6}	0.14	48.06		
				43.99		
				43.98	45.34	0.001279
					s 2.35	

[a] The blank was about 0.27 ml of titrant, equivalent to 9.5 μg or 0.19 ppm of chloride.

chloride, the potentiometric break was only ~100 mV at 30°C, and ~150 mV at 10°C.

Krijgsman *et al.* (*5*) described the automatic argentometric determination of 2.5 to 50 μg of chloride in 50 ml of 75% acetic acid with 0.002 *N* aqueous silver nitrate. They used a silver sulfide ISE as an indicator electrode, pretitrated approximately 10 μg of chloride prior to sample addition, and titrated automatically to a preselected end point. This eliminates in an elegant way the necessity for a blank correction. To our knowledge 2.5 μg is the lowest amount of chloride ever determined by potentiometric titration. No titration curves are given in their paper.

We have summarized in Table 2 the results of the above experiments. The amounts of chloride determined are near or at the lower limits re-

TABLE 4
TITRATION CHLORIDE WITH ACETONIC $AgClO_4$ IN ACETIC ANHYDRIDE[a]

Taken (μg Cl)	Taken (μmol Cl)	Normality chloride	Titer (μg/ml)	Mean titer	N_{AgClO_4}
142.28			39.50		
			39.94		
	4	8×10^{-5}	40.28	39.91	0.001126
				s 0.39	
71.14			37.49		
			38.22		
			37.97		
	2	4×10^{-5}	38.09	37.94	0.001070
				s 0.32	
35.57			39.09		
			39.48		
			38.58		
	1	2×10^{-5}	39.09	39.06	0.001102
				s 0.37	
17.785			36.98		
			38.07		
			37.29		
	0.5	1×10^{-5}	42.98	38.83	0.001095
				s 2.80	
10.671			37.35		
			39.69		
	0.3	6×10^{-6}	38.83	38.62	0.001089
				s 1.18	
7.114			42.01		
			39.08		
	0.2	4×10^{-6}	39.51	40.20	0.001134
				s 1.58	

[a] The blank was about 0.12 ml of titrant, equivalent to 4 μg or 0.08 ppm of chloride.

ported by the various workers, and potentiometric breaks were estimated from the titration curves given. The media yielding the largest breaks are acetic anhydride, acetone, and glacial acetic acid. For our work we have chosen a titration medium of 50 ml acetone, or 40 ml acetic anhydride plus 10 ml acetone. Chloride samples were dissolved in acetone after adding the minimum amount of water to keep the sample in solution, approximately 4% (v/v). We have chosen acetonic silver perchlorate as titrant in view of its ability to differentiate all four halides.

A summary of our results in acetone is presented in Table 3, and in acetic anhydride:acetone in Table 4. Representative titration curves are shown in Fig. 3 for 17.8 μg of chloride in acetone, and in Fig. 4 for 10.7 μg of chloride in acetic anhydride:acetone. The breaks at those levels of chloride were ~500 mV and are also cited in Table 2. It is of interest to note that in acetone two potentiometric breaks were obtained for chloride, the first one corresponding to the species $AgCl_2^-$. The 500 mV potentiometric break in acetone is the sum of the two breaks, for $AgCl_2^-$ and AgCl. The total water content per 50 ml of solution was <0.8% (v/v). If the water content is increased, the break for $AgCl_2^-$ becomes less pronounced until it finally disappears.

The lowest amount of chloride which still produced useful results was 7 μg (0.2 μmol; 4×10^{-6} N). At this low concentration, however, the standard deviation was, as expected, larger than at higher levels. The titration curves for the blanks were similar to those shown in Fig. 3 and 4. In acetone, the blank titration was ~0.27 ml of 0.001 N titrant, equivalent to 9.5 μg or 0.19 ppm of chloride. In acetic anhydride:acetone it was ~0.12 ml of 0.001 N titrant, equivalent to 4 μg or 0.08 ppm of chloride. No

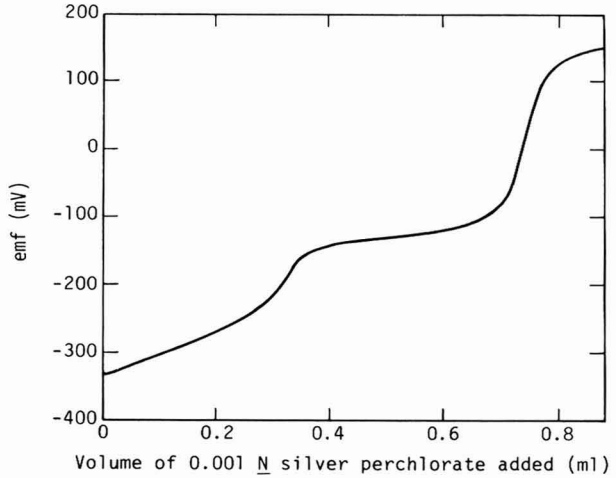

FIG. 3. Titration of 17.8 μg of chloride in acetone.

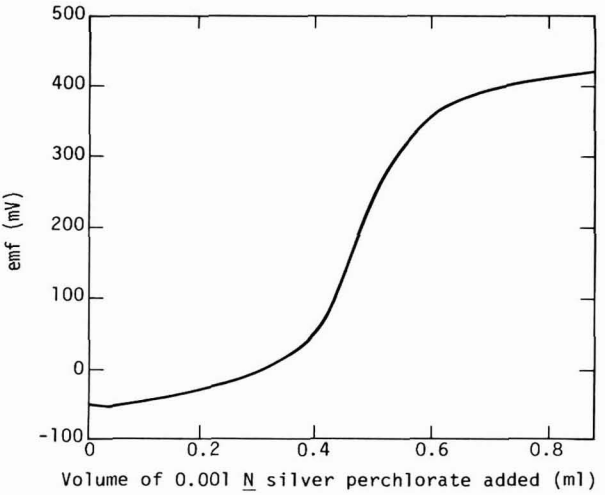

FIG. 4. Titration of 10.7 μg of chloride with $AgClO_4$ in acetic anhydride/acetone (40/10 ml).

special precautions were taken to free the reagents from traces of chloride. It is thus quite likely that with special care lower blank values can be obtained. The acetic anhydride medium, because it removes traces of water, is particularly suitable for titrations were water impairs the titration curve. It is more convenient than acetone, in which some evaporation occurs because of the rapid stirring of the solution. Because the blanks still produced well-defined titration curves (acetone ~475 mV; acetic anhydride:acetone ~400 mV), we believe that the potentiometric titration of chloride with silver ions is limited only by the amount of chloride in the reagent blank.

As previously discussed (*1*), the Gran function past the equivalence point, where silver ions are in excess, may also be used to evaluate the end points.

SUMMARY

The lower levels of the potentiometric titration of chloride with silver ions were investigated. The titrant was 0.001 *N* acetonic silver perchlorate. The titration media were acetone and acetic anhydride:acetone (4:1). A silver sulfide ion-selective indicator electrode and a double-junction reference electrode were use to monitor emf's. This titration is limited only by the trace amounts of chloride in the reagents used. Satisfactory results and well-defined titration curves were obtained down to 7 μg of chloride per 50 ml of solution (0.2 μmol; 4×10^{-6} *N*).

A small polarization current can be used to enhance the potentiometric breaks of this titration. In an 80% methanolic medium with 0.001 *N* aqueous silver nitrate and a polarization current of -0.4 μA, the lower practical limit of this titration was near 22.3 μg of chloride (1.26×10^{-5} *N*).

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A Spectrophotometric Method for the Determination of Nitrite

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INTRODUCTION

In the past 20 years, a wide variety of techniques spectrophotometric (*6*), colorimetric (*4*), titrimetric (*5*), polarographic (*1, 7*), chromatographic (*3*), gasometric (*2*), have been recommended for the determination of nitrite. Most of them, however, are suitable only for the microdetermination of nitrite and suffer disadvantages such as color instability and interference from extraneous ions.

In this paper, a method is described which is based on the reduction of chromium (VI) to chromium (III) by nitrite, the latter then being determined by the measurement of chromium (III) spectrophotometrically at 580 nm.

EXPERIMENTAL METHODS

Chemicals and apparatus. Sodium nitrite (E. Merck, Analytical Reagent Grade), Potassium dichromate (Analar, B.D.H.). All other reagents used were of reagent grade.

Spectra were measured on a Beckman DK2A Recording Spectrophotometer in 2 cm^3 spectrophotometric cells.

Preliminary investigations. Investigations were carried out to determine the optimal temperature and acid concentration for the reduction of chromium (VI).

It was found that if the temperature of a solution containing nitrite, dichromate and sulphuric acid was allowed to rise above 0°C, nitrite decomposed to oxides of nitrogen. In all subsequent work, therefore, the temperature was maintained below 0°C until the reaction was complete, i.e., about 10–15 min.

To determine the optimal acid concentration (H_2SO_4), the optical densities of solutions containing nitrite, dichromate and sulphuric acid of varying concentrations were measured at 580 nm. A selection of the results obtained are shown in Table 1.

The optical density of solutions in 1 mol dm^{-3} H_2SO_4 was found to approach that of the 2 mol dm^{-3} H_2SO_4 with time, while the latter did not

TABLE 1
OPTICAL DENSITIES AT 580 nm FOR NITRITE–DICHROMATE SOLUTIONS[a]

Volume of 0.0728 M NO_2^- solution (cm^3)	Concn of H_2SO_4 (mol dm^{-3})	Optical Densities			
		Initial	2 hr	3 hr	24 hr
20	1	0.235	0.390	0.470	0.470
20	2	0.470	0.470	0.470	0.470
40	2	0.850	0.850	0.850	0.850

[a] In different concentrations of sulphuric acid as a function of time.

change even after 24 hr. In 4 mol dm^{-3} H_2SO_4, a brown precipitate appeared on standing. In subsequent work, 2 mol dm^{-3} H_2SO_4 was used.

Procedure. Aliquots of a standard 7.28×10^{-2} mol dm^{-3} nitrite solution were transferred to conical flasks. Solid potassium dichromate was added and dissolved and the mixture placed in an ice-bath at 0°C. Sufficient cold sulphuric acid was added to make an acid concentration of 2 mol dm^{-3}.

After 15 min, the solutions were removed from the ice-bath, transferred to a volumetric flask and, after adjusting the volume, the optical density determined at 580 nm.

Calibration curve. A calibration graph for the nitrite-dichromate reaction at 0°C in 2 mol dm^{-3} H_2SO_4 was prepared. A Beer's law plot was found to exist for nitrite concentrations in the range 7.28×10^{-3} to 3.64×10^{-3} mol dm^{-3} with a calculated standard deviation of 0.5%.

Effect of extraneous ions. The optical density at 580 nm of solutions containing various extraneous ions was measured and compared with that of a control. Results are shown in Table 2. From the reuslts, it may be concluded that nitrate in large excess, VO_3^-, PO_4^{3-}, and Fe^{3+} do not interfere at equimolar concentrations. Cl^-, Co^{2+}, Cu^{2+}, and Ni^{2+} show only slight interference, while Ce^{4+}, Ce^{3+}, Sn^{2+}, Fe^{2+}, Mn^{2+}, F^-, Br^-, and I^- interfere seriously.

DISCUSSION

In 2 mol dm^{-3} H_2SO_4, it was clear that the reaction is fast and complete. In 4 mol dm^{-3} H_2SO_4, the brown compound precipitating appeared to be a chromium (VI) oxide and it is suggested that in acid of this concentration, results are low because insufficient chromium (VI) is present to react completely with nitrite.

Although the absorption peak at 580 nm for the chromium (III) species has a small extinction coefficient, it is free from interference by the dichromate. The sensitivity of the nitrite determination can be increased by measuring the decrease in absorbance at 348 nm due to a dichromate absorption band which has a molar extinction coefficient of 3220. This

TABLE 2
EFFECT OF EXTRANEOUS IONS ON DETERMINATION OF NITRITE

Ion	NO_2^- Concn (Control 2.91×10^{-2})	% Error
NO_3^-[a]	2.91×10^{-2}	0.00
VO_3^-	2.91×10^{-2}	0.00
PO_4^{3-}	2.91×10^{-2}	0.00
Fe^{3+}	2.91×10^{-2}	0.00
Ce^{4+}	2.25×10^{-2}	−22.70
F^-	2.74×10^{-2}	− 5.84
Cl^-	2.86×10^{-2}	− 1.72
Co^{2+}	3.02×10^{-2}	3.78
Cu^{2+}	3.05×10^{-2}	4.81
Ni^{2+}	3.08×10^{-2}	5.84
Sn^{2+}	3.18×10^{-2}	9.28
Fe^{2+}	3.41×10^{-2}	17.20
Mn^{2+}	3.45×10^{-2}	18.60
I^-	3.72×10^{-2}	27.80
Br^-	3.76×10^{-2}	29.20
Ce^{3+}	3.83×10^{-2}	31.60

[a] There was no interference from NO_3^- up to a 100-fold excess.

procedure, however, cannot be applied in the presence of large concentrations of nitrate, because of possible interference from the nitrate absorption band at 300 nm.

From the study on the effect of extraneous ions on the nitrite determination, it was clear that those ions which are present in their highest oxidation states and are unable to be reduced by nitrite show no interference.

Nitrate does not interfere up to a 100-fold excess which makes the method very suitable for the determination of nitrite in the presence of nitrate.

The reaction stoichiometry was investigated and the dichromate–nitrite molar ratio determined. A linear relationship was found to exist between dichromate concentration and optical density at 348 nm over the concentration range 6.51×10^{-4} to 3.25×10^{-3} mol dm^{-3} for dichromate. This was then used to determine the remaining dichromate after a known amount of nitrite had reacted with a known excess of dichromate. A mole ratio of 3 was found for nitrite:dichromate. The reaction may therefore be written as

$$Cr_2O_7^{2-} + 3NO_2^- + 8H^+_{aq} \rightarrow$$

$$3NO_3^- + 2Cr^{3+}_{aq} + 4H_2O$$

SUMMARY

Nitrite reacts with dichromate quantitatively under suitable conditions of temperature and acid concentration. A linear relationship was found to exist between nitrite concentration and the absorbance at 580 nm of the chromium (III) species produced. This was used to determine the nitrite. The influence of a number of ions on the determination of nitrite was investigated; up to 100-fold excess nitrate has no influence on the determination of nitrite.

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MICROCHEMICAL JOURNAL 21, 306 - 308 (1976)

Determination of Free Sulfur in Chemical Reagents by Means of Thin-Layer Chromatography

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INTRODUCTION

Determination of free sulfur in both natural and manufactured products is usually based on oxidation of the sulfate ion or on reduction of the sulfide ion and the consequent quantitative determination of these ions with the help of a method that is the most suitable regarding all the substances accompanying sulfur in the given sample.

Davies and Thuraisinham *(2)* attempted to quantitatively determine free sulfur in several vulcanizates of rubber by means of the adsorptive TLC, and established that the components of the examined vulcanizates caused no disturbance in these measurements.

Banaszkiewicz and Sliwiok *(1)* used TLC for determination of free sulfur in different natural and manufactured products and stated its applicability with no regard for the accompanying contaminations.

The purpose of this paper is to define the applicability of the adsorptive TLC technique to the quantitative determination of traces of free sulfur in those chemical reagents, that include sulfur in their structure.

EXPERIMENTAL

The detailed information concerning the performance of TLC analysis was given in our previous paper *(1)*. Silicagel (Kieselgel G, E. Merck, Darmstadt) was applied as an adsorbent, and *n*-heptane was used as a mobile phase. Free sulfur spots were developed on the chromatographic glassplates and evoked with the 0.1 *N* solution of iodine in a mixture of water and ethanol (3:7, v/v), with an addition of 3% of sodium azide.

Dependence between the area of the evoked chromatographic spots and the amounts (in μg) of the determined free sulfur is given in Table 1.

The areas of the chromatographic spots y show the linear dependence of the microgram amounts of the determined sulfur x in the range of 5–35 μg of sulfur. Therefore, the discussed parameters can exchange their positions and the equation can be established, which presents the microgram amounts of sulfur y as a linear function of the chromatographic spot areas x. This equation was established by method of the average:

TABLE 1
DEPENDENCE BETWEEN THE CHROMATOGRAPHIC SPOT AREAS AND THE μg AMOUNTS OF ANALYZED SULFUR[a]

Amount of sulfur (μg)	Average spot area[b] $\bar{X}$ (mm²)	Standard deviation S (mm²)	Relative standard deviation $S/\bar{X}$	Distribution of the obtained chromatogr. spot areas (mm²)
5	38	3.89	0.105	33–42
10	64	4.00	0.062	60–71
15	92	4.77	0.051	84–97
20	112	6.71	0.060	109–122
25	133	6.42	0.048	125–145
30	158	5.29	0.039	151–168
35	185	4.91	0.026	179–198

[a] Chromatograms evoked with a solution of iodine and sodium azide.
[b] The presented number values are the mean values obtained from 20 determinations.

$$y = 0.21x - 3.79.$$

This equation enables one to calculate the μg amount values of sulfur y in the examined sample on the basis of the chromatographic spot area x.

Determination of free sulfur in thiourea. The 27-g sample of thiourea was dissolved in a 750-ml conical flask with 315 ml of water. Then 30 ml of toluene was added. The process of extraction was carried out three times. Each extraction was run with the efficient mechanical stirring for 90 min. After the separation of the toluene and water layer 27 ml of a sulphur solution in toluene was obtained from each extraction. Three extracts were combined and evaporated at 70 ± 1°C to the volume of 2 ml. The 5 μl amount of this solution were applied in TLC. After developing of the

TABLE 2
PERCENTAGE AMOUNTS OF FREE SULFUR IN THE SELECTED CHEMICAL REAGENTS[a]

Reagent	Purity	Chromatographic spot area[b] (mm²)	Percentage amount of free sulfur
Thiourea	chemical grade	47.35	9.1160×10^{-3}
Sodium naphthionate	chemical grade	45.35	2.8667×10^{-3}
Carbon disulfide	chemical grade	57.50	4.3720×10^{-4}
Thiophene	chemical grade	39.05	4.1436×10^{-4}

[a] Determined by means of the adsorptive thin-layer chromatography.
[b] The presented number values are the mean values obtained from 20 performed determinations.

chromatograms in *n*-heptane, they were dried at room temperature and evoked with a solution of iodine with an addition of sodium azide.

Determination of free sulfur in sodium naphthionate. The 40-g sample of sodium naphthionate was dissolved in a 750-ml conical flask with 300 ml of water. Then 30 ml of toluene was added. Extraction was carried on as with the thiourea. Three extracts were collected and evaporated at 70 ± 1°C to the volume of 1 ml. The 5 μl amounts of this solution were applied in TLC. From here the procedure was analogical.

Determination of free sulfur in carbon disulfide. The 300 ml solution of carbon disulfide (d = 1.2632 g/cm^3) was evaporated at 40 ± 1°C to the volume of 1 ml. The 5-μl solution of this sample was used in TLC, and from here the procedure was analogical.

Determination of free sulfur in thiophene. The 200-ml solution of thiophene (d = 1.0644 g/cm^3) was evaporated at 70 ± 1°C to the volume of 1 ml. The 5-μl volume of this sample was used in TLC and from here the procedure was also analogical.

The results are presented in Table 2.

SUMMARY

The applicability of adsorptive TLC technique was established to determine free sulfur traces in chemical reagents which include sulfur in their structure.

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MICROCHEMICAL JOURNAL 21, 309 - 314 (1976)

Micromethod for Phosphorus Determination in Research Laboratories

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INTRODUCTION

The majority of methods for the determination of inorganic phosphorus in serum and other biological fluids are based on the formation of phosphmolybdate complexes followed by reduction to molybdenum blue in a variety of ways. Some *(2, 5)* require protein removal; these, however, require relatively large serum volumes. Direct methods *(1, 3, 4)* still use samples greater than 25 μl, are more expensive and time consuming.

A micromethod for inorganic phosphate determination is useful for clinical and research laboratories, especially because of the practical difficulty in obtaining blood samples in volumes large enough for conventional techniques. This is a particular problem faced by microchemical laboratories which must serve neonatal nurseries, infant wards, and small animal research.

A micromethod should retain or improve upon the accuracy of macromethods, be reproducible, use few stable reagents, have a linear standard curve, and not require major equipment changes. The method described fulfills these criteria.

MATERIALS AND METHODS

Reagents

Stock phosphorus (20 mg%). Dissolve 219 mg potassium dihydrogen phosphate in 1000 ml. water.

Working phosphorus standard (5 mg%). Dilute 25 ml of stock standard to 100 ml with water.

Ammonium molybdate (1.25%). Dissolve 1.25 g ammonium molybdate in distilled water and dilute to 100 ml. This is stable at room temperature.

Ascorbic acid (5.0%). Dissolve 5 g L-ascorbic acid in distilled water and dilute to 100 ml. This solution is stable for approximately 6 weeks when refrigerated.

Working reagent. Mix together equal parts of ammonium molybdate and L-ascorbic acid solutions. This working reagent is stable for 5 min. Using a reagent blank this mixture has a 2 hr stability.

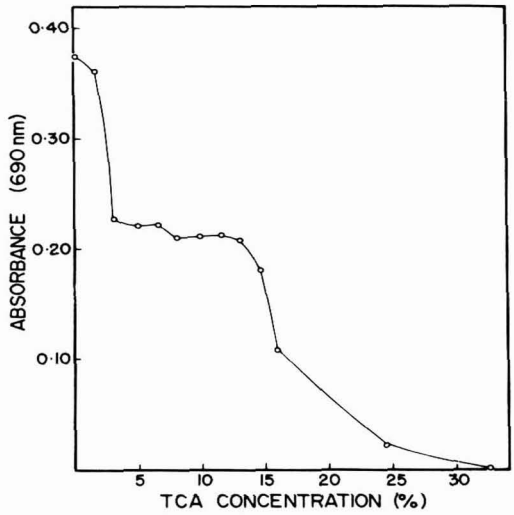

FIG. 1. Plot of one concentration of inorganic phosphorus with varying concentrations of trichloroacetic acid.

Trichloroacetic acid (10% TCA). Dissolve 100 g low phosphate, trichloroacetic acid in distilled water and dilute to 1000 ml. The distilled water and TCA should be checked for detectable amounts of phosphorus. Glassware should be acid washed or disposable.

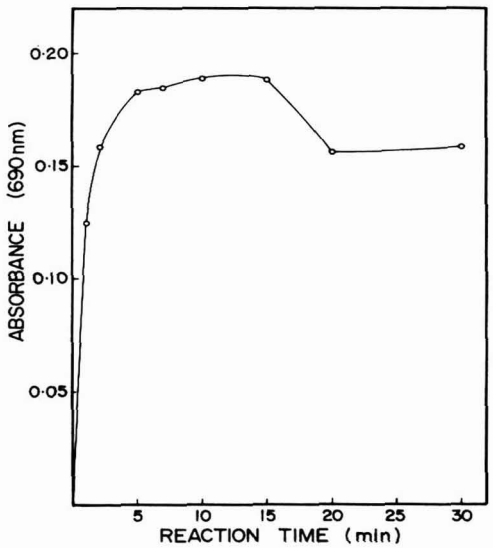

FIG. 2. Time of incubation of reaction mix at 80°C with one concentration of inorganic phosphorus.

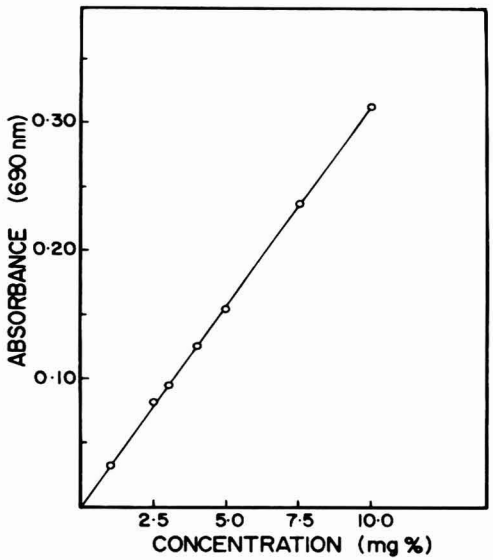

FIG. 3. Profile of varying concentration versus the absorbance of reactions (standard curve).

PROCEDURE

Pipette 12 μl of serum into 1.2 ml 10% TCA (ice cold). This should remain on ice for 5 min and then be centrifuged at 3000 rpm for 10 min in a clinical centrifuge. When measuring urinary phosphorus predilution of 1/10 is usually necessary. Into a 12 × 75 mm tube pipette 1 ml of supernate. Add 0.1 ml of working reagent and mix immediately. Place in a water bath at 80°C for 10 min, cool to room temperature and read absorbance of 690 nm versus reagent blank using a 1 ml cuvette (1 cm light path).

TABLE 1
COMPARISON OF PHOSPHORUS RECOVERY (mg/dl) BETWEEN TCA AND KIT MICROMETHODS

Std. serum	TCA	% Recovery	KIT	% Recovery
2.7	2.9	107	3.0	111
4.2	4.3	102	4.3	102
6.2	6.2	100	6.4	103
12.4	12.4	100	12.3	99
13.9	14.1	101	13.9	100
15.9	15.9	100	15.6	98
		avg. 101.6		102.2

RESULTS

Using a CARY 111 spectrophotometer to scan the reaction a peak was present between wavelengths 690 and 720 nm. As many spectrophotometers have 700 nm as a maximum, 690 was used as a convenient wavelength. Since the absorbance of the reagent blank was between 0.004 and 0.006, a water blank may be substituted. If the working reagent is to be used longer than 5 min after mixing, a reagent blank must be used. The concentration of TCA for protein precipitation is critical because of pH dependency and must be in the range of 3 to 12% *(5)*. There is uniform color development between 3 and 12%. Above 12% there is a reduced color and at 32% there is no color development, with or without the presence of phosphorus (Fig. 1).

When the mixture is heated at 80°C the reaction is complete after 7 min and stable to 13 min, however at that temperature denaturation occurs

TABLE 2

COMPARISON OF RESULTS OF IDENTICAL SERA USING AN AUTOMATED PROCEDURE, DIRECT METHOD, AND THE PROPOSED METHOD

AA II	TCA		KIT	
3.6	3.7	3.8	3.5	3.7
2.5	2.3	2.3	2.5	2.4
3.4	3.6	3.7	3.6	3.4
3.4	3.4	3.4	3.4	3.6
5.3	5.2	5.2	5.0	5.3
2.9	3.1	3.3	3.1	3.2
3.7	3.6	3.6	3.7	4.0
4.3	4.5	4.5	4.2	4.7
5.8	5.8	5.8	6.1	5.8
3.7	3.9	3.9	4.1	4.1
3.6	3.3	3.3	3.9	3.7
3.9	3.8	3.8	3.7	3.6
3.3	3.6	3.4	3.4	3.4
3.7	3.9	4.0	3.8	3.7
4.3	4.1	4.3	4.4	4.8
3.2	3.2	3.3	3.1	3.2
4.1	4.2	4.3	3.9	4.0
6.7	6.2	6.1	6.5	6.2
4.4	4.5	4.6	4.5	4.5
MEAN 4.0		4.0		4.05

TABLE 3

ANALYSIS OF VARIANCE BETWEEN SAMPLES AND METHODS[a]

SOURCE	*df*	*ss*	*ms*
Methods	2	0.036	0.018
KIT vs TCA	1	.01621	0.01621
AA2 vs TCA & KIT	1	.01979	0.01979
Samples	18	81.487	4.527
Methods × samples	36	1.183	0.0328
Determinations within			
methods within samples	38	0.534	0.01405
determinations within KIT	19	.454	0.02389
determination within TCA	19	.080	0.004211
Corrected Total	94	83.241	

[a] Calculations made using a version of the Statistical Package for the Social Sciences from the Vogelback Computing Center, Northwestern University, on the CDC 6400 at McMaster University.

after 15 min (Fig. 2). After the color develops the reaction is stable for 1–1/2 hr at room temperature and there is an increase of absorbance of only 5% after 24 hr. The plot of concentration versus absorbance is linear to 20 mg% (Fig. 3). The readings are identical with the Gilford 2400 or the Unicam 1800 using reagents made up at different times.

In Table 1 the data from recovery experiments demonstrate the consistency of this micromethod (TCA) compared with that of the Pierce "Phosphorus Stat Reagent Kit" (KIT). The results of comparing identical serum samples done using an AutoAnalyzer II, our method and the KIT are shown in Table 2. These three methods appear to have the same means, i.e., there is no significant difference between them, however there is more variation in the KIT than the TCA method. From the analysis of variance Table 3 the ratio of the determinations within methods within samples is highly significant ($F = 0.02389/0.004211 = 5.67$, $p < 0.0001$).

DISCUSSION

The micromethod described has several advantages. It is a true microtechnique using 12 μl of serum. This is of value where sample size is limited, such as, in neonatal nurseries and small animal research laboratories. The absorbance is read on standard spectrophotometers in 1 ml cuvettes at a convenient wavelength (690 nm). It is inexpensive, consistent and accurate and is subject to less "random" error than the commercial micromethod tested.

SUMMARY

A simple micromethod for serum inorganic phosphorus determination is described. Its reproducibility, accuracy, and nominal cost make its use particularly valuable in research laboratories where small sample size is mandatory. There is less variation in this method than a comparable commercial micromethod.

ACKNOWLEDGMENTS

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MICROCHEMICAL JOURNAL 21, 315 - 324 (1976)

A Rapid Method for the Microdetermination of Nitrogen in Organic Compounds Using a Flushed-Oxygen Combustion Tube

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INTRODUCTION

Microdetermination of nitrogen in organic compounds has been carried out by the Dumas method in which samples are oxidized by cupric oxide and reduced by reduced copper filled in a combustion tube (1–4). The method has been highly improved by a number of microchemists *(5)* and is still considered to be the most reliable method, although the electronic C–H–N microanalyzer has come into wider use these days. Since the Dumas method employs carbon dioxide as a carrier gas, the rate of oxidation is relatively slow, and at the same time there remains a problem, i.e., the partial retention of nitrogen oxides, which will be formed during the decomposition of nitrogen containing samples, may be encountered in contact with the oxidation catalyst of cupric oxide resulting in an uncertain factor of the analytical error. Another source of error comes from the nitrogen in the air absorbed in the cupric oxide used for mixing with the organic sample, which temporarily fills the combustion tube before every analysis.

In a previous report *(1)* the author introduced a method of carbon and hydrogen determination of organic compounds, using a "flushed-oxygen combustion tube," in which oxygen flushed through the two-stage nozzles fused into the combustion tube, and it is known that the rapid combustion of the sample was possible eliminating the presence of oxidation catalyst. Excess oxygen was transported by nitrogen gas and removed by reduced copper heated at 500–600°C, nitrogen oxides being reduced to nitrogen at the same time. The flushed oxygen method could be readily modified to Dumas nitrogen determination by replacing nitrogen with carbon dioxide. In this connection the author has attempted some experimental studies about the flushing nozzle configurations, flow rate of gases, and other necessary parameters for optimization in the modified Dumas method.

EXPERIMENTAL

Apparatus. A schematic diagram of the apparatus is shown in Fig. 1.

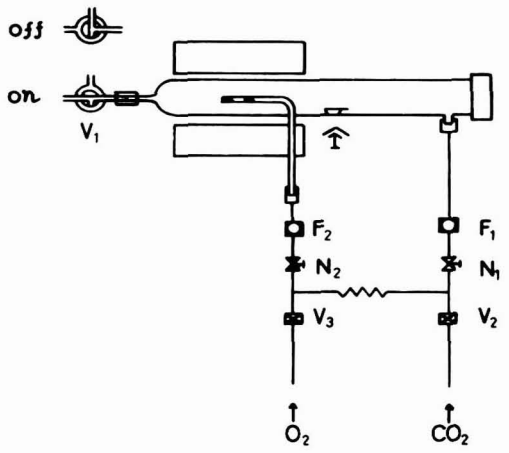

FIG. 1. Schematic diagram of combustion system.

The tail end of the combustion tube is connected to a reduced copper tube via a solenoid valve V_1 and the end of the latter tube is further connected to a nitrometer via a small dilution volume made up of two adjacent glass bulbs.

The combustion tube (Fig. 2) is made of silica, 12 mm in diameter and 170 mm long. It is fused with a L tube as a flushing nozzle of oxygen and the tube has two silica filters, one at the end and the other in the middle, the filters having there mesh grade of No. 2. The combustion tube fused with the flushing nozzle is wound around with nichrome wire on which ceramic fiber covers up for keeping the temperature inside at 850°C. The oxygen gas to be introduced into the combustion tube from a needle valve N_2 and a flow meter F_2 via a solenoide valve V_3 in Fig. 1 is supposed to be preheated to 850°C in the L tube, and to be flushed onto the gasified sample through the silica filters. The gas is mainly flushed through the

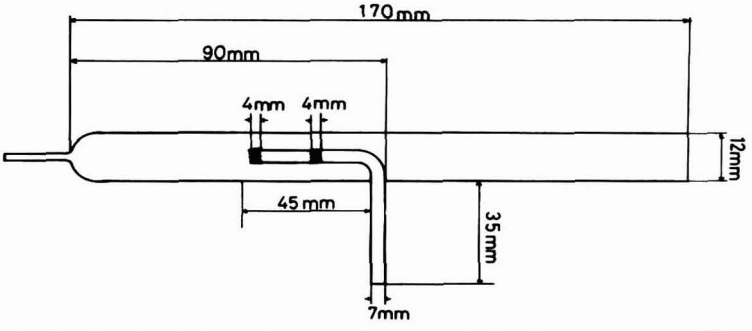

FIG. 2. Combustion tube with one piece nozzle having two quartz filters.

first filter from the entrance of the L tube, diffusing generally towards rectangular directions against the tube axis, which contributes more effective consumption of oxygen by the organic vapor, while a small portion of oxygen flushing straight forwards from the second filter at the end will mainly consume the reduced copper installed after the solenoid valve V_1.

Reagents. Reduced copper, granular, made by Coleman Co. for the elemental analysis. Sulfix (Kishida Chemicals Co., Osaka). Oxygen, high purity, cylindered.

Procedure. The CO_2 cylinder is adjusted to flow the gas at 4–8 ml/min beforehand by regulating a needle valve not indicated in Fig. 1. The solenoid valve V_1 in Fig. 1 is switched off, and the combustion tube is set to flush out the gas. A measured sample in a platinum boat is put into the combustion tube and the tube is closed. The valves V_2 and V_3 are switched on and opened so that oxygen begins to flow. At this moment the needle valve adjusting the flow rate of the CO_2 cylinder remains set as before and allows the gas to flow. The flow rate is adjusted to 10 ml/min from the entrance of combustion tube and 100 ml/min from the nozzle by adjusting the needle valve N_1 and N_2. The valve V_1 is switched on and the gas in the combustion tube flows towards the reduced copper. At the same time the sample is heated up by a burner and decomposed. After 10 sec the valve V_2 is switched off and CO_2 ceases to flow. After being kept open for 5 sec more, the valve V_3 is also switched off so that the oxygen stops flowing. The heating goes on just as before for 45 sec more with the stagnant oxygen inside. After 1 min from the beginning, the heating of the sample is stopped, then the valve V_2 is switched on again. N_1, N_2 is adjusted, so that from the entrance flow 5–8 ml/min of CO_2 and 2–3

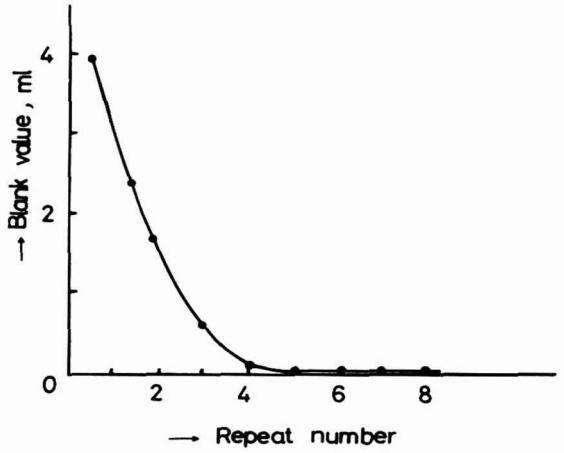

FIG. 3. Blank tests after reduction of copper.

TABLE 1
BLANK VALUE AT DIFFERENT FLOW CONDITION OF OXYGEN

Oxygen flow (ml/min)	Duration time (min)	Blank value (ml)
97	5	0.200
39	5	0.110
30	5	0.120
115	0.5	0.015
33	0.5	0.014
10	0.5	0.014

ml/min from the nozzle. After it comes into the state of microbubbles, the valve V_1 is switched off and CO_2 stops flowing to the nitrometer. The N% will be calculated out by deducting the blank value from the volume of the nitrogen accumulated in the nitrometer.

RESULTS AND DISCUSSION

Blank value. In the combustion method using nozzles, a comparatively large amount of oxygen is flushed out. In consequence of this the most crucial problem is whether the oxygen gas can be absorbed completely into the reduced copper within a short time. Therefore the author attached the nitrometer to the apparatus, and examined the volume of the gas not absorbed by alkali. A microcombustion tube was filled with reduced copper 30 cm long and heated up to 500–600°C. When high purity oxygen was used, the result was as follows.

(1) As is shown in Fig. 3, the blank value under the proposed procedure appeared as quite high at the first two or three trials, but it became a stable low level for microanalysis after several trials.

(2) As is shown in Table 1, in case the oxygen continued to flow for 5 min, the blank value was large, but when the time was reduced to 30 sec, it had a favorable value.

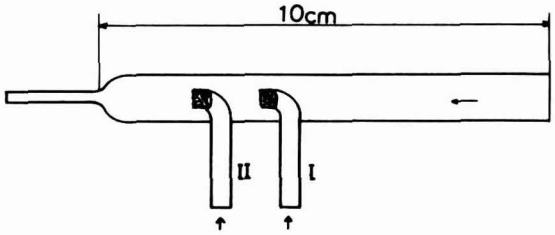

FIG. 4. Combustion tube with two nozzles.

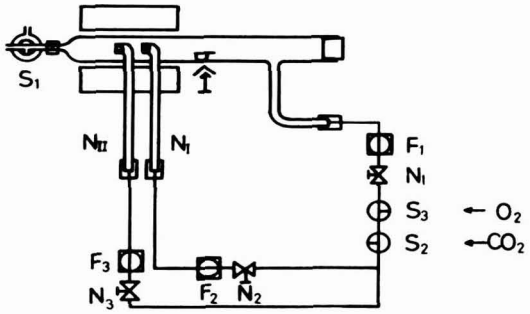

FIG. 5. Schematic diagram of combustion system with two nozzles combustion tube.

Flushing-nozzle configuration. The author tried to shorten the combustion tube to mix the sample vapor with oxygen more effectively and to diminish the consumption of reduced copper at the same time. The tube used in *(1)* was the one having two nozzles, each consisting of bundles of capillaries, so that the length of the nozzles and the distance between them could be shortened only within certain limits.

The author therefore replaced the capillary nozzles with silica filters as shown in Fig. 4, and set it as in Fig. 5, samples having been analyzed with flushed oxygen in a manner similar to *(1)*, with the carrier gas replaced by carbon dioxide. It was found, however, that the gasified samples sometimes back-diffused towards the entrance of the combustion tube, forming lightly colored deposition on the inside wall of the tube, and the analytical values were consequently lower than the theoreticals. The following reason for such behavior has been conjectured: In the previous report the oxygen gas flushed straight forwards to the end of the tube as the nozzles consisted of bundles of capillaries, but in the present experiment the porous silica filter flushed oxygen towards random directions, so that the flowing oxygen at the same rate as described in the previous report, namely, 4 ml/min from the entrance, could not prevent the backward diffusion of the organic vapor. The author therefore increased the flow

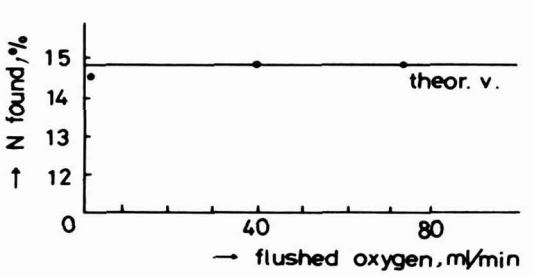

FIG. 6. Recovery of nitrogen in antipyrine using combustion system of Fig. 5.

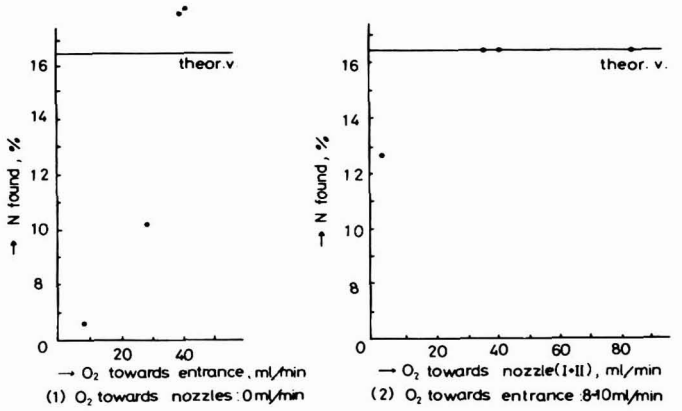

FIG. 7. Recovery of nitrogen in sulfathiazole.

rate of oxygen up to 8–10 ml/min and examined the analytical value. The result supported the effectiveness of the increased flow rate of oxygen by which analysis at the same size, ranging from 0.7–1.5 mg, became feasible.

The effect of the varying flow rate of the oxygen flushed from the nozzles is shown in Figs. 6–8. In the case of antipyrine, as shown in Fig. 6, there was no obvious effect whether the oxygen was flushed from the nozzles or not. In the case of sulfathiazole, however, the recovery of nitrogen in the sample was significantly dependent on the flow rate of

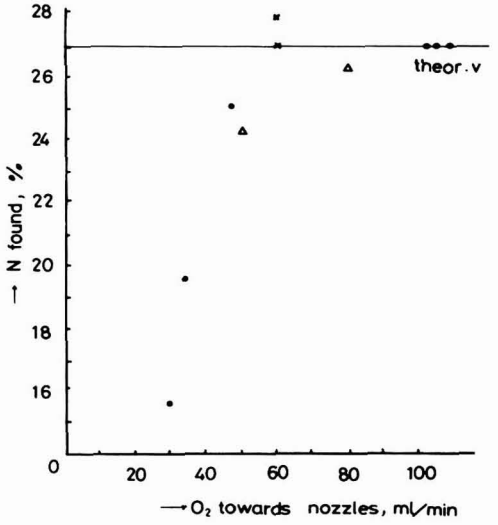

FIG. 8. Recovery of nitrogen in cyclohexanone semicarbazone.

oxygen flushing from the nozzles as shown in Fig. 7. Figure (1) shows that when the oxygen was supplied only from the tube entrance the analytical value was very unreliable because of imperfect oxidation in the combustion tube, while Fig. 7 (2) shows that the value of the gas being flushed from the nozzles approachs the theoretical. In the case of cyclohexanone semicarbazone, as illustrated in Fig. 8, the effect of the oxygen from the nozzles is more obvious.

The influence of the weight of samples under the same conditions was also examined. The result was, as shown in Table 2, that about 3 mg of antipyrine could be determined with 10 ml/min of oxygen flowing from the tube entrance, 30 ml/min from nozzle I and 5 ml/min from nozzle II, but that cyclohexanone semicarbazone showed a large negative value, although less than about 2 mg of the sample could show the correct analytical result.

By the same method several standard samples were determined and the results were examined. The conditions and the results of the analysis are shown in Table 3. Of several compounds, cyclohexanone semicarbazone demanded the highest flow rate of oxygen from the nozzles for its decomposition, while caffeine exhibited a scatter of the analytical value, and *m*-Dinitrobenzene showed the value with a slightly positive bias.

One piece flushing nozzle. The author has designed another type of flushing nozzle configuration, as shown in Fig. 2, in which two silica filters were fused in a L tube as one piece flushing nozzle. The two silica filters had their mesh grade of No. 2 with 4 mm thickness, being located 12 mm apart each other. With this configuration, it has been supposed that the flushing oxygen mainly goes out through the filter closer to the entrance and the remaining oxygen through the other filter at the end of the tube. The whole apparatus was set as shown in Fig. 1. Considering the fact that the complete combustion of cyclohexanone semicarbazone could

TABLE 2
ANALYTICAL RESULTS AT VARIED SAMPLE SIZES

Sample	Amount (mg)	Oxygen towards (ml/min)[a]			N%		
		E	N_I	N_{II}	Theor.	Found	Δ
Antipyrine	3.212	10	30	5	14.88	14.86	−0.02
Cyclohexanone semicarbazone	3.210	10	30	5	27.08	19.83	−7.25
	2.495	10	60	40		27.02	−0.06
	1.224	8	45	15		27.38	+0.30

[a] E = entrance, N_I = nozzle I, N_{II} = Nozzle II.

TABLE 3
ANALYTICAL RESULTS OF NITROGEN IN ORGANIC COMPOUNDS BY TWO NOZZLE COMBUSTION TUBE

Compound	Amount (mg)	Oxygen towards (ml/min)[a]			N%		
		E	N_I	N_{II}	Theor.	Found	Δ
Antipyrine	1.870	10	30	25	14.88	14.84	−0.04
	1.942	10	30	25		14.29	−0.59
	3.212	10	30	25		14.86	−0.02
Caffeine	1.128	10	30	25	28.85	29.22	+0.37
	1.578	10	30	25		32.92	+4.07
	4.030	10	30	25		28.50	−0.35
Cyclohexanone semicarbazone	3.245	10	30	0	27.08	15.76	−11.32
	3.210	10	30	5		19.83	−7.25
	3.732	10	40	8		25.28	−1.80
	2.711	10	40	8		21.39	−5.69
	4.735	10−20	50	20−25		22.33	−4.75
	2.495	10	60	40		27.02	−0.06
	1.224	8	45	15		27.38	+0.30
p-Nitroaniline	1.543	8	50	10	20.28	2026	−0.02
	1.283	8	50	15		20.42	+0.14
Thiourea	0.7682	8−25	45−70	10−30	36.80	36.71	−0.09
	1.0385	8	50	10		36.56	−0.24
s-Benzylthiuroniumchloride	0.7070	8	50	10	13.82	15.66	+1.84
	0.8150	8	50	10		14.10	+0.28
	0.8553	15	40	3		13.50	−0.32
m-Dinitrobenzene	1.5440	10	40	15	16.66	17.85	+1.19
	1.4580	8	50	15		19.90	+3.24
Fluoroacetanilide	1.0530	10	60	20	18.18	18.64	+0.46

[a] E = entrance, N_I = nozzle I, N_{II} = nozzle II.

TABLE 4
ANALYTICAL RESULTS OF NITROGEN IN ORGANIC COMPOUNDS USING COMBUSTION SYSTEM OF FIG. 1[a]

Compound	Amount (mg)	N%	
		Theor.	Found
Cyclohexanone semicarbazone	0.7755	27.08	29.47
	0.8660		26.62
m-Dinitrobenzene	2.5535	16.66	51.45
	1.1150		106.97
	2.8440		21.97
	1.7990		39.70

[a] CO_2 was not supplied during combustion.

be achieved when the ratio (oxygen flow rate from the nozzles/oxygen flow rate from the entrance of the combustion tube) was more than nine, 100 ml/min of oxygen from the two nozzles would be in the safety zone when 10 ml/min of oxygen was supplied from the entrance. In order to save the consumption of reduced copper, the oxygen gas was flushed only for 15 sec, so that each sample was heated for 30 sec including the time needed to flush the oxygen, and the sweeping by CO_2 was for 5 min with a flow rate of 10 ml/min. The analytical results were not very good in every case as shown in Table 4, however *m*-Dinitrobenzene had a exceptionally large positive bias, probably because the sample burned explosively with this method.

The author has also attempted to optimize the analytical conditions, including those of the samples as above, and has come to the conclusion that the combustion using oxygen mixed with carbon dioxide was most effective. The method is as described under in "Procedure" above. Several standard samples were analyzed after this method and the results are shown in Table 5. They look satisfactory except for one or two examples. At each trial 35 ml of oxygen was consumed. The combustion tube has been considerably shortened with the one piece flushing nozzle, so that the stationary furnace also has been shortened with lower power consumption.

TABLE 5
ANALYTICAL RESULTS OF NITROGEN USING MODIFIED FLOW PROGRAM IN FIG. 1[a]

Compound	Amount (mg)	N%		
		Theor.	Found	Δ
Antipyrine	1.1860	14.88	14.91	+0.03
	1.4480		15.07	+0.19
m-Dinitrobenzene	2.8460	16.66	16.51	−0.15
	0.8885		16.69	+0.03
Caffeine	1.1695	28.85	28.76	−0.09
	1.0345		29.01	+0.16
s-Benzylthiuroniumchloride	1.1450	13.82	13.80	−0.02
	1.3285		13.46	−0.36
Cyclohexanone semicarbazone	1.1450	27.08	26.96	−0.12
	1.1175		27.50	+0.42
p-Nitroaniline	0.8200	20.28	20.48	+0.20
	0.8690		20.59	+0.31
Fluoroacetanilide	1.8280	18.18	18.01	−0.17
	2.0520		17.84	−0.34
Thiourea	1.5000	36.80	36.71	−0.09
	2.6505		36.37	−0.43

[a] CO_2 was supplied towards entrance during combustion.

The author compared the case where two filter-type nozzles were separated with each having its individual flow rate of gas (Fig. 4) with the case where a one piece flushing nozzle used oxygen exclusively for the combustion (Fig. 2) and found that the latter had a larger positive bias with an explosive sample like *m*-Dinitrobenzene. But at the same time the former had two separate nozzles so that it needed more complicated operation for the individual flow control. The latter method, which used oxygen mixed with carbon dioxide, is therefore preferred for simpler operation.

Although this method is restricted by the sample size for ordinary microanalysis, it enables one to carry out rapid combustion and eliminates the problem of the retention of nitrogen oxides in the oxidation catalyst which has suffered poorly reproducible blank value in the conventional Dumas method. There is another merit of durable oxidation efficiency, i.e., it is free from poisoning of the oxidation catalyst. In addition, the remaining ash in the boat may be determined by reweighing.

SUMMARY

A micromethod to determine nitrogen in organic compounds of 1–1.5 mg has been studied by the combustion method where oxygen is flushed into an empty combustion tube heated at 850°C. The flushing nozzle is consisted of two silica filters in a L-tube and oxygen [illegible] out of the nozzle rapidly oxidizes the organic vapor gasified in a sample heater, oxygen and nitrogen oxides are absorbed and reduced with reduced copper [illegible] 500–600°C.

ACKNOWLEDGMENT

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Ultramicrodetermination of Nitrogen in Organic Compounds

Centimilligram Determination of Nitrogen with Sealed Tube Method

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Nitrogen determination is often necessary for samples as small as 50 μg in practical work, the nitrogen content being between 2 and 20 μl. In order to measure accurately the volume of nitrogen collected in a combustion tube the collected nitrogen is expanded under reduced pressure and titrated with a micropiston burette (*7*). However, this method is too time-consuming for routine use in an analytical laboratory.

Recently, one of us introduced a new nitrometer for measuring the collected nitrogen in the capillary tube (*5, 7*), found that the actual rise in pressure of the gas in the nitrometer due to capillary action agrees with results calculated by a theoretical formula, and showed that the analytical results corrected accordingly agree well with the expected results (*6*).

We attempted the exact determination of smaller amounts of nitrogen in organic compounds using a new modified nitrometer, and describe here a few solutions to difficult procedure problems in ultramicroanalysis.

APPARATUS AND REAGENTS

Electrolytic oxygen generator. An apparatus of this type used in our laboratory has been described recently (*4*). The 700-ml electrolysis cell is equipped with a water jacket and filled with 2 *N* potassium hydroxide. In order to keep a minimal and constant blank, a small electrolytic current was continuously passed through the cell by a dry cell overnight.

Nitrometer. The nitrometer with a centimilligram scale is illustrated in Fig. 1. It consists essentially of three parts: a water jacket, A; a calibrated measuring tube, B; and a receiving chamber, C. To make the nitrometer as short as possible, the measuring tube was made up of two parts: a subtraction volume, D, with a capacity of about 5.0 μl, and a graduated glass capillary tube of about 0.23 mm inside diameter. The graduation starts from about 20 mm below the upper orifice. The capillary tube is about 36 cm long with a capacity of about 15 μl, and is marked off in

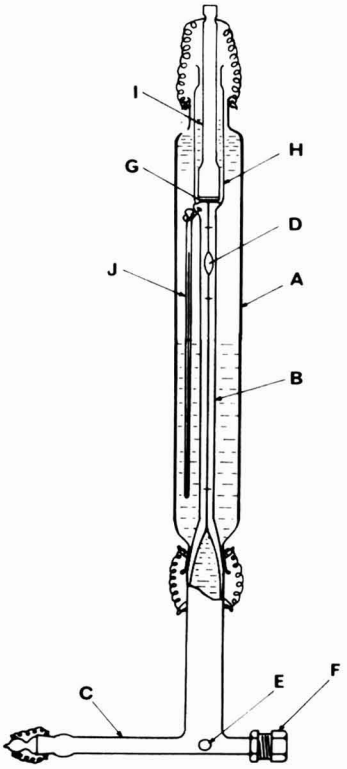

FIG. 1. Nitrometer with centimilligram scale. A, water jacket; B, calibrated measuring tube; C, receiving chamber; D, subtraction volume; E, side arm (connected with Tygon tubing to levelling bulb); F, stuffing-box joint; G, nozzle; H, upper glass bulb; I, plunger; J, thermometer.

0.05-μl graduations. The bottom of the capillary tube widens out into stems which are joined to the receiving chamber, a short glass side-arm, E, and a tube which is blocked with a self-sealing rubber cap held on by a metal stuffing-box type joint, F, as described previously (*5*).

Combustion furnace. The combustion furnace is the same as that described elsewhere (*2*). We recommend the use of a potter's cylinder in which eight combustion tubes are placed in a circle concentric with the center of the furnace.

Combustion tube. A Miracron-PH-3-P tube with an inside diameter of about 5 mm was used for the combustion tube and is economically available from Nippon Gaishi Co., Ltd., Nagoya (formerly known as Heatron-P). Supremax and Pyrex 1720 glass were described previously (*1*). The glass tubes were cut into 10-cm sections, immersed in Scat 20-X solution, then washed with distilled water heated in an electric furnace at 700°C for

1.5 hr or immersed in a chromic acid mixture, washed with distilled water then dried in an oven. Every tube was swept with oxygen then sealed off, and had an internal volume of 1 ml, as illustrated in Fig. 2(a) and 2(b).

Copper boat. A simple device for making copper boats is illustrated in Fig. 3. A piece of copper foil was cut out or punched into the required size as shown in (a) and (b). We used a copper clad laminate with a thickness of 0.035 mm. The cut-out foil was placed on a polyurethane foam plate 1, then block 2 in the form of a boat was pressed against the foil 3 and moved so as to knead the foil around the block. The resulting copper boat (d) weighted about 26 mg. It was immersed in warm acetic acid, washed with distilled water, dried in an oven, then heated in a current of nitrogen at 800°C for 1 hr in an electric furnace, and finally cooled under nitrogen.

50% KOH solution. Five hundred milligram of analytically pure potassium hydroxide was added to 500 ml of distilled water.

METHOD OF ANALYSIS

Procedure. Samples of between 20 and 70 μg were weighed in a copper boat and burned in a sealed tube filled with pure oxygen. The nitrogen was

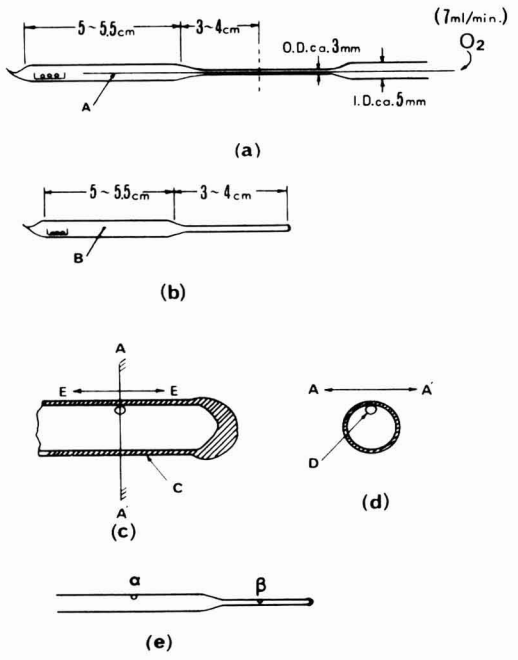

FIG. 2. Combustion tube and measurement of blank value. A, stainless steel capillary with an outer diameter of 1.5 mm; B, combustion tube with a volume of about 1 ml; C, capillary of the combustion tube; D, gas or mercury granule; α and β, position of gas or mercury granule in the combustion tube.

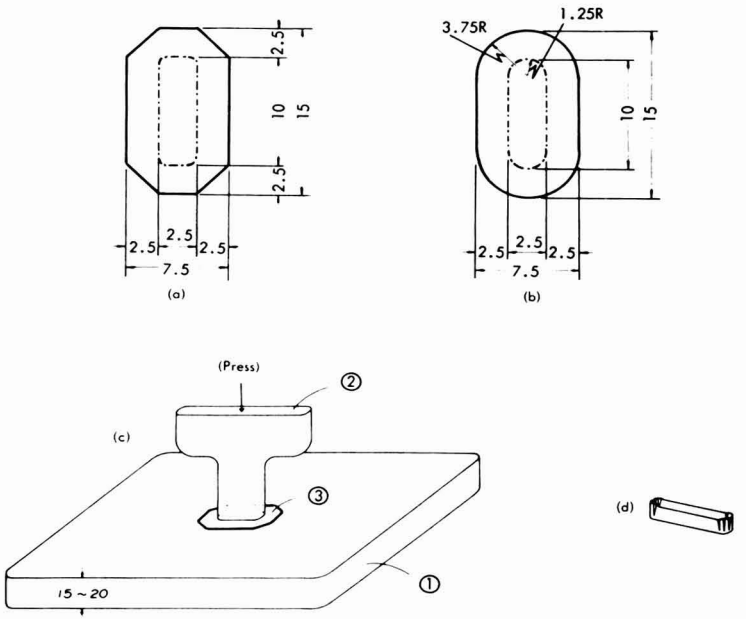

FIG. 3. Simple device for making a boat of copper foil (scale in mm). (a) A piece of copper foil cut out into the required size, (b) a piece of copper foil punched into the required size, (c) forming a boat from the piece of copper foil, (d) copper boat (Wt: Ca 26 mg, t:0.03 mm). 1, polyurethane foam plate; 2, metal block; 3, copper foil.

collected with 50% potassium hydroxide as described in previous papers (*1*, *4*). Nitrogen oxides are reduced to elemental nitrogen by the copper boat and the excess of oxygen is absorbed by it. Therefore copper gauze is not used here. The capillary of the combustion tube was cut about 20 mm below the meniscus, and placed in the horizontal receiving chamber of the nitrometer with the centimilligram scale (see Fig. 1 (c)). Nitrogen in the capillary was displaced into the measuring tube by water injected through a hypodermic syringe introduced through a rubber sealing cap at the end of the receiving chamber as described previously (*5*). The nitrogen volume was read after 5 min. The determination was repeated using a number of tubes.

Calculation. The percentage of nitrogen present in the sample was calculated using the formula:

$$\% \text{ nitrogen} = V \cdot F / S \times 100,$$

$$V = f[V' - (C_1 + C_2)]$$

where F = weight of 1 ml of nitrogen under the analytical conditions; S =

weight of the sample, μg; f = factor from Table 1; V' = volume of nitrogen read, μl; C_1 = blank; C_2 = calibration correction of the nitrometer.

DISCUSSION

Blank value. Since the gas volume for the blank test of this method was very small, the gas granule collected in the capillary was observed with a micrometer microscope and evaluated.

TABLE 1

THE "f" FACTOR, INCLUDING (1) CORRECTION FOR ADHESION OF DILUTE POTASSIUM HYDROXIDE SOLUTION,[a] (2) TEMPERATURE CORRECTION OF BAROMETER READING, AND (3) CORRECTION FOR VAPOR PRESSURE OF WATER

f	Temp. (°C) at 760 (mm Hg)	Temp. (°C) at 770 (mm Hg)	Temp. (°C) at 780 (mm Hg)	f	Temp. (°C) at 760 (mm Hg)	Temp. (°C) at 770 (mm Hg)	Temp. (°C) at 780 (mm Hg)
0.976[b]	9.9	10.1	10.3	0.961	26.7	26.9	27.1
0.975	10.9	11.1	11.3	0.960	27.1	27.4	27.6
0.974	11.9	12.1	12.3	0.959	27.6	27.8	28.1
0.973	12.8	13.0	13.2	0.958	28.0	28.3	28.5
0.972	13.7	14.0	14.2	0.957	28.9	29.1	29.4
0.971	14.5	14.8	14.9	0.956	29.3	29.5	29.8
0.970	15.5	15.6	15.8	0.955	29.7	30.0	30.2
0.969	16.1	16.4	16.5	0.954	30.1	30.3	30.6
0.968	16.9	17.1	17.3	0.953	30.5	30.7	31.0
0.967	17.6	17.8	18.0	0.952	30.9	31.1	31.4
0.966	18.3	18.5	18.7	0.951	31.2	31.5	31.7
0.965	19.0	19.2	19.4	0.950	31.6	31.9	32.1
0.964	19.7	19.9	20.0	0.949	32.0	32.2	32.5
0.963	20.3	20.5	20.7	0.948	32.3	32.6	32.8
0.962	20.9	21.1	21.3	0.947	32.7	32.9	33.2
	21.5	21.7	21.9		33.0	33.3	33.5

Continued

TABLE 1—*continued*

f	Temp. (°C) at 760 (mm Hg)	770 (mm Hg)	780 (mm Hg)	*f*	Temp. (°C) at 760 (mm Hg)	770 (mm Hg)	780 (mm Hg)
0.946	22.1	22.3	22.5	0.935	33.4	33.6	33.9
0.945	22.7	22.9	23.1	0.934	33.7	34.0	34.2
0.944	23.2	23.4	23.7	0.933	34.0	34.3	34.5
0.943	23.7	24.0	24.2	0.932	34.4	34.6	34.9
0.942	24.3	24.5	24.7	0.931	34.7	34.9	35.2
0.941	24.8	25.0	25.2	0.930	35.0	35.3	35.5
0.940	25.3	25.5	25.7	0.929	35.3	35.6	35.8
0.939	25.8	26.0	26.1	0.928	35.6	35.9	36.1
0.938	25.8	26.0	26.1	0.927	35.9	36.2	36.4
0.937	26.2	26.5	26.7	0.926			
0.936							

[a] 1.0% from Table 3.

[b] For example, $f = 0.976$ corresponds to the temperature range 9.9–10.9°C at 760 mm Hg, 10.1–11.1°C at 770 mm Hg, and 10.3–11.3°C at 780 mm Hg.

The gas granule looks just like an ellipsoid with a major axis, a, of view of *E–E'*. Its apparent size varies according to its position, α or β, in the combustion tube as shown in Fig. 2(c)–2(e).

A mercury granule, accurately weighing 200.4 mg at 22.2°C, is about the size of the gas granule. It was placed in the capillary tube to estimate the volume of the latter. The true volume at this time was 0.0148 μl.

In Table 2, *Vt*, *Vs*, and *Ve* are the true volume of the mercury granule, the volume of a sphere with a minor axis, *b*, the volume of a ellipsoid of revolution having a major axis, *a*, and a minor axis, *b*, respectively, and α and β are positions of the granule in the combustion tube.

We found that the ratio of *Vt*/*Vs* is larger than *Vt*/*Vc*, and the repeatability of these ratios is the same (see data in Table 2). Now, the accurate volume of a gas granule can be determined by multiplication with the coefficient, *Vt*/*Vs*. We found a blank of ≤ 0.02 μl in our case.

Correction for adhesion of water and diluted alkali solution. The ap-

TABLE 2
MEASUREMENT OF THE VOLUME OF A MERCURY GRANULE SEEN THROUGH GLASS TUBE AND CAPILLARY

Position	Major axis a (mm)	Minor axis b (mm)	Volume of sphere Vs^a (μl)	Volume of ellipsoid Ve^b (μl)	Ratio of Vt^c/Vs	Ratio of Vt/Ve
α	0.17112	0.15535	0.015695	0.017290	0.943	0.856
	0.17070	0.15464	0.015481	0.017090	0.956	0.866
	0.17616	0.15311	0.015025	0.017290	0.958	0.856
	0.17466	0.15749	0.016354	0.018137	0.905	0.816
β	0.17712	0.15405	0.015305	0.017598	0.967	0.841
	0.17407	0.15680	0.016140	0.017918	0.917	0.826
	0.17791	0.15557	0.015761	0.018027	0.939	0.821
	0.18143	0.15675	0.016122	0.018663	0.918	0.793

[a] $Vs = 4/3\pi b^3$ corresponds to estimation by micrometer microscope.
[b] $Ve = 4/3\pi ab^2$ corresponds to estimation by micrometer microscope.
[c] Vt: true volume of mercury granule, 0.0148 μl at 22.2°C. Standard deviation of Vt/Vs = 0.0275. Standard deviation of Vt/Ve = 0.0247.

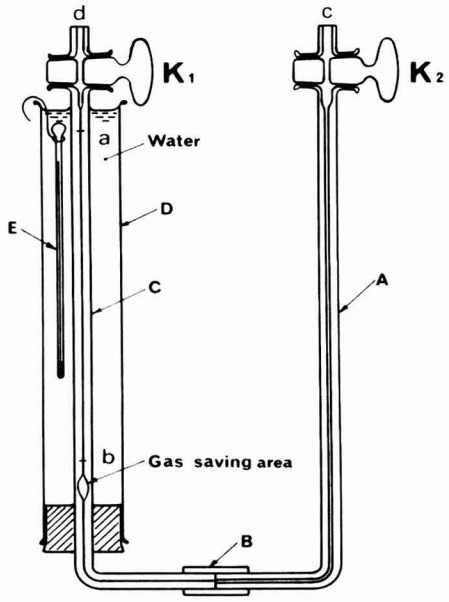

FIG. 4. Measurement of water adhesion in capillary tube. A, levelling tube with a bore of about 1 mm; B, Tygon tubing; C, graduated column with a bore of 0.23 mm; D, water jacket; E, thermometer; K_1 and K_2, stopcocks; a and b, zero and lowest points of graduation.

paratus for measurement of water adhesion in a capillary tube is illustrated in Fig. 4.

We found that the level of the meniscus gave rise to a large vertical movement, no matter how little a shock was applied, when the levelling bulb was first adjusted to the lowest graduation. Therefore, instead of using flexible tubing to connect the water reservoir, a glass levelling tube of about 1.0 mm internal diameter was used (see Fig. 4).

The glass levelling tube A was filled with water. Stopcock K_2 was closed, then connected by Tygon tubing B to a graduated column C which had been fitted with water jacket D. Stopcock K_1 was closed. The internal bore of the graduated column is the same as that of the centimilligram nitrometer. The levelling tube was turned at the position of the Tygon tubing, and upper orifice c was adjusted to the level of the lowest graduation b of the graduated column. We then allowed the water in the levelling tube to rise to a position a little higher than b, and open stopcocks K_1 and K_2. Next, stopcock K_2 was closed and water was injected with a syringe through stopcock K_1 into upper orifice d. The upper meniscus of the water in the capillary was now drawn down a little lower than the zero line of the graduation. Stopcock K_1 was closed and K_2 opened, then the levelling tube turned until its water level coincided with that in the graduated column. The gas volume was determined and its temperature simultane-

TABLE 3

CORRECTION FOR ADHESION OF DISTILLED WATER AND DILUTE ALKALI SOLUTION[a]

Time (min)		Vol. (μl)	Temp. (°C)	Vol. (μl) at 27.1°C
Dry	0	12.61	25.7	12.67
	0	12.63	25.8	12.69
	0	12.64	25.9	12.70
				(Mean: 12.685)
Wet	1	12.76	26.0	12.81
	2	12.76	26.0	12.81
	3	12.77	26.1	12.82
	4	12.77	26.1	12.82
	5	12.77	26.1	12.82
	10	12.77	26.2	12.81
	15	12.77	26.3	12.81
	20	12.79	26.4	12.82
	30	12.75	26.5	12.79
	60	12.77	26.8	12.78
	120	12.84	27.1	12.84
				(Mean: 12.812)

[a] $[(12.812-12.685)/12.812] \times 100 = 0.991\%$; mean of four determinations with distilled water: 0.989%; mean of four determinations with 0.018% KOH solution: $1.025\% \doteqdot 1.0\%$.

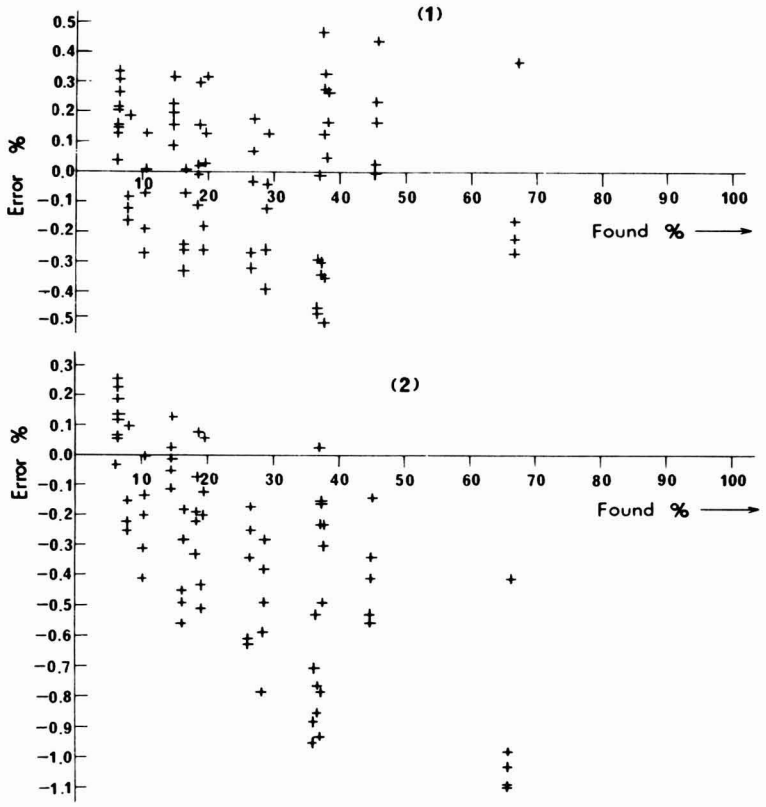

FIG. 5. Distribution of error on results obtained with a series of suitable organic compounds. (1): Values corrected for the rise in pressure due to capillarity. Mean error, −0.008%; standard deviation of error, 0.240%; number of determinations, 75. (2): Values not corrected for the rise in pressure due to capillarity. Mean error, −0.325%; standard deviation of error, 0.343%, number of determinations, 75.

ously measured with thermometer E. Next, the gas was drawn down into the gas saving area by slightly turning the stopcock K_1, and the inner wall of the capillary tube wetted with water.

Once again the gas was adjusted to above the zero line, and its volume measured as it decreased with time. The temperature was simultaneously measured. The volume correction of 0.989% for adhesion of water was necessary (see data in Table 3), and the drainage time was 3 min. The correction factor changed as repeated measurements were made with the same water and the concentration of potassium hydroxide increased as recently described (5). The actual concentration of potassium hydroxide in the water was 0.018% and the correction factor was then increased to

1.025% (see data in Table 3). Hence, a correction of 1.0% in the measured volume was made. This value depends on the ratio of the area of the inner surface of the capillary to its volume, and also on the nature of the glass surface. Accordingly, it should be measured for each nitrometer used.

Correction for pressure rise due to capillary action. The question of rise in pressure due to capillarity was recently described by Miyahara (*6*). In

TABLE 4
RESULTS FOR SYNTHESIZED COMPOUNDS CONTAINING INTERFERING ELEMENTS

Exp.	Sample[a]	Wt of sample (μg)	Temp. (°C)	Press. (mm Hg)	Vol. of nitrogen (μl)	Found (%)	Error (%)	Diff. of error (%)
1	$C_{18}H_{33}O_4N_2P$	66.4	25.1	P_u[b]766.9	4.25	7.40	−0.12	−0.09
				P_c[c]776.3	4.25	7.49	−0.03	
2		42.1	25.2	P_u767.0	2.68	7.37	−0.15	−0.09
				P_c776.4	2.68	7.46	−0.06	
3		49.1	25.2	P_u767.2	3.19	7.51	−0.01	−0.10
				P_c776.6	3.19	7.61	0.09	
4		41.5	23.1	P_u768.2	2.58	7.24	−0.28	−0.10
				P_c777.6	2.58	7.34	−0.18	
5		32.2	23.2	P_u768.2	2.04	7.39	−0.13	−0.10
				P_c777.6	2.04	7.49	−0.03	
6	$C_7H_{17}O_3N_2Cl_2P$	52.5	22.8	P_u766.3	4.60	10.18	0.14	−0.14
				P_c775.7	4.60	10.32	0.28	
7		49.0	22.8	P_u766.3	4.18	9.91	−0.13	−0.13
				P_c775.7	4.18	10.04	0	
8		53.0	23.5	P_u767.0	4.54	9.95	−0.09	−0.13
				P_c776.4	4.55	10.08	0.04	
9		54.1	23.5	P_u767.2	4.50	9.67	−0.37	−0.14
				P_c776.6	4.51	9.81	−0.23	
10		48.8	23.5	P_u767.2	4.23	10.08	0.04	−0.15
				P_c776.6	4.24	10.23	0.19	
11	$C_{16}H_{27}O_4N_4SP$	57.0	24.1	P_u771.4	6.69	13.68	−0.24	−0.20
				P_c780.8	6.70	13.88	−0.04	
12		69.5	24.1	P_u771.5	8.12	13.63	−0.29	−0.18
				P_c780.9	8.12	13.81	−0.11	
13		71.7	24.1	P_u771.5	8.33	13.56	−0.36	−0.17
				P_c780.9	8.33	13.73	−0.19	
14		42.4	24.0	P_u771.7	4.95	13.62	−0.30	−0.18
				P_c781.1	4.95	13.80	−0.12	
15		40.4	23.9	P_u771.7	4.68	13.54	−0.38	−0.16
				P_c781.1	4.68	13.70	−0.22	

continued

TABLE 4—*continued*

Exp.	Sample[a]	Wt of sample (μg)	Temp. (°C)	Press. (mm Hg)	Vol. of nitrogen (μl)	Found (%)	Error (%)	Diff. of error (%)
16	$C_{18}H_{22}O_4N_2S_4Br_2Hg$	53.4	24.5	P_u770.4	1.62	3.52	0.10	−0.05
				P_c779.8	1.62	3.57	0.15	
17		32.4	24.5	P_u770.4	0.97	3.46	0.04	−0.05
				P_c779.8	0.97	3.51	0.09	
18		25.7	24.5	P_u770.4	0.75	3.37	−0.05	−0.05
				P_c779.8	0.75	3.42	0	
19		32.8	24.4	P_u770.4	0.94	3.32	−0.10	−0.05
				P_c779.8	0.94	3.37	−0.05	
20		26.2	24.4	P_u770.6	0.75	3.32	−0.10	−0.04
				P_c780.0	0.75	3.36	−0.06	
21	$C_{26}H_{16}O_8N_4S_4F_6$	32.6	23.2	P_u770.2	2.08	7.46	0.04	−0.10
				P_c779.6	2.08	7.56	0.14	
22		33.2	23.3	P_u770.2	2.05	7.21	−0.21	−0.09
				P_c779.6	2.05	7.30	−0.12	
23		28.3	23.2	P_u770.2	1.67	6.89	−0.53	−0.09
				P_c779.6	1.67	6.98	−0.44	−0.07
24		52.6	23.3	P_u770.2	3.12	6.91	−0.51	−0.09
				P_c779.6	3.12	7.00	−0.42	
25		64.5	22.7	P_u758.8	3.75	6.71	−0.71	−0.08
				P_c768.2	3.75	6.79	−0.63	
26	$C_{33}H_{36}N_3B_3$	74.4	22.9	P_u758.7	5.96	9.23	−0.43	−0.11
				P_c768.1	5.96	9.34	−0.32	
27		56.5	22.9	P_u758.7	4.53	9.23	−0.43	−0.11
				P_c768.1	4.53	9.34	−0.32	

[a] The formulas were established by other analyses carried out in routine work.
[b] Value not corrected for the rise in pressure due to capillarity
[c] Value corrected for the rise in pressure due to capillarity. Standard deviation of error calculated from P_c: 0.208%.

this case, the correction for the rise in pressure of a gas in a nitrometer due to capillary action was 9.4 mm Hg, according to a previous calculation from a theoretical formula (*6*).

Analysis results. Results with suitable organic compounds ranging in nitrogen content between 5.94−66.4% are shown in Fig. 5. Considering the weighing error of 0.1 μg, $\Delta\% = 0.10 \cdot R/S$, where $\Delta\%$ = error of result, R = nitrogen content, S = sample weight. In our case the sampling error $2\hat{\sigma}$

is about 0.1% since the precision of the Oertling ultramicrobalance Model QO1 used is 0.03–0.04 μg. Therefore a standard deviation of 0.240% may be permissible.

Our results, obtained by a method which considers the rise in pressure produced by capillarity, agree fairly well with expectations (see Fig. 5). Table 4 shows the results of analyses of synthesized compounds containing interfering elements. The results are satisfactory at the centimilligram level. We found that the fine tip end is frequently broken due to the force of explosive combustion of a compound containing a boron. In such a case, care must be taken not to leave the sample at a tip end of the combustion tube (see Fig. 2(a)).

SUMMARY

A new improved nitrometer was developed for centimilligram determination of nitrogen by the sealed-tube method. A combustion tube having an internal volume of 1 ml was used and the sample was weighted in a boat made of copper foil, which was made by a simple device. The accurate volume of a gas granule of the blank was evaluated by observation with a micrometer microscope. A standard deviation of 0.240% was rapidly and conveniently obtained with organic compounds having a range of 5.94–66.4% nitrogen.

ACKNOWLEDGMENT

The authors are grateful to Mr. Tetsuro Kadono of this laboratory for the glass work used to construct the nitrometer.

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MICROCHEMICAL JOURNAL 21, 337—343 (1976)

BOOK REVIEWS

Some Fundamentals of Analytical Chemistry (ASTM Special Technical Publication 564). American Society for Testing and Materials, Philadelphia, Pa., 1974, vi + 81 pp. $7.75.

This pamphlet consists of six papers presented at the 1973 ASTM symposium of the same title. The work can be commended to technical libraries having substantial holdings for the Library of Congress class "Chemistry, Analytic."

F. P. Byrne (5 pages) delineates the nonexpertise of analysts in statistics and error treatment in terms of the "syndrome of standard reference materials" (the value reported is within one or two integers of the certified value regardless of the limited precision of the latter value) and the "syndrome on replicate values" (all replicates tend to be the same, often to the last significant figure). J. P. McKaveney (17 pages, 33 references) reviews the status of gravimetric analysis, especially in relation to ASTM gravimetric procedures for key elements. J. Penkrot (17 pages) presents basic information on volumetric ware and calibration and delineates different types of titrations, partially via annotated periodic tables. T. C. Rains (18 pages, 37 references) highlights considerations in applying atomic absorption spectrometry (including an understanding of the instrument, production of an atomic vapor, sample preparation, and calibration and data assessment). E. S. Hobart and S. Kallman (five pages) consider logical approaches to the dissolution of metals as the initial step to their analysis. C. L. French and S. N. Tuthill (six pages, one reference) review the activities of the Committee on Analytical Reagents of the American Chemical Society.

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The Laboratory Recorder. By GALEN W. EWING AND HARRY A. ASHWORTH. Plenum Press, New York, 1974. vii + 129 pp. $18.50

An almost universal piece of scientific equipment, familiar to anyone who has worked in a laboratory, is the laboratory recorder; however most laboratory workers have only limited knowledge of how recorders work and how to choose the best recorder for specific applications. In this concise volume, the authors have presented a comprehensive review of the physical and electronic fundamentals of recorders, with the aim of facilitating both the selection of recorders and the full utilization of the equipment on hand.

The book includes 10 chapters, an Appendix, and a brief Introduction to the subject of the remaining chapters. Chapters 2, 3, and 4 discuss four types of recorder: the deflection recorder, the servo recorder, the X–Y recorder, and the oscillograph. Chapter 6 focuses on the paper feed and writing mechanism. Chapter 7 discusses shielding and grounding, an area of critical importance in recorder operation. Chapter 8 presents specifications and explanations of specifications for recorders. Chapter 9 presents important information on trouble shooting of recorder operation. The last chapter discusses a number of different recorder accessories. The Appendix includes a list of commercial suppliers of recorders and most importantly compares the characteristics of the 44 recorders listed. The chapters are filled with a number of excellent photographs and descriptive line drawings which are quite useful.

This volume is the first in a series on "Laboratory Instrumentation and Techniques"

edited by Dr. Ewing. The authors have provided a useful discussion of the laboratory recorder. Most laboratory personnel will gain information of value from a review of this book.

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Principles and Techniques of Electron Microscopy. Vol. 5. Biological Applications. Edited by M. A. HAYAT. Van Nostrand Reinhold, New York, 1975. xvii + 250 pp. $19.95.

The fifth volume of the famous Hayat's comprehensive series of treatises on the "Principles and Techniques of Electron Microscopy (EM)" discusses five major EM techniques employed in the study of the structure, composition, and location of cellular components. It is intended to serve as an international authoritative source in the field, and is designed to cover important new developments systematically. This volume consists of five chapters. It has been developed through the joint efforts of six distinguished author–scientists. Each chapter has an exhaustive list of references with complete titles, and full author and subject indexes are included at the end of the book.

The quantitative mapping with the EM by P. Sterling serves as the beginning chapter. It discusses the problems encountered in constructing accurate quantitative maps with the EM. Two broad categories, those of sampling and those of record-keeping, are considered. The particular practical problems described are: tissue sampling with the EM, selection of blocks, selection of sections, sampling of sections, and recording the distribution of elements within the section, etc. However, the difficulties in choosing appropriate blocks and sections remain as challenges to the ingenuity and patience of each investigator. For illustration, seven well-reproduced electron micrographs drawn from neuroanatomy are shown. References cited to 1974 are given.

The second chapter by G. C. Farnell and R. B. Flint deals with the photographic aspects of EM. This chapter considers the essentials of physical and chemical properties of emulsion and its interaction with electrons. The relation between density and exposure for electrons, granularity of electron exposures, electron response and emulsion properties, signal/noise ratio and detective quantum efficiency, and electron image spread are critically and extensively demonstrated and illustrated. In addition, some practical considerations, such as choice of photographic support, choice of emulsion, processing, and alternative methods of photographically recording the electron image are also concisely provided. Some 32 of the most current references are cited.

Chapter 3 by D. L. Allinson reviews the recent development of environmental devices in EM. The environmental devices are defined as the special specimen holders which sustain a nonvacuum environment about the specimen. These kinds of studies play an important role in obtaining very useful information from the specimens by EM. Two distinct types are discussed in detail: (1) with small electron transparent "windows," or membranes, above and below the specimen, which can withstand a static pressure difference; (2) the use of very narrow bore throttling apertures above and below the specimen to maintain dynamically the high pressure region. Some 21 well-designed figures and clearly reproduced electron micrographs serve as excellent illustrations. A total of 65 references from 1934 to 1974 are available.

The following chapter by B. V. Johansen introduces the optical diffraction of electron micrographs. A brief introduction to the basic diffraction theory (the necessary principles) with illustration is first given. Then, lens qualities and dimensioning of the diffractometer are emphasized. Alignment and operation of a standard diffractometer with an image recon-

struction system are subsequently described. The final demonstration with a few examples from various fields of application and three available commercial systems enhances the reader's understanding of the principles and usefulness of this powerful technique. A list of up-to-date literature (1975, 56 references) is provided.

The last chapter by B. A. Weavers offers the readers detailed information on the instrumentation of the analytical electron microscope, EMMA-4. The EMMA-4 is the first commercially available instrument to combine conventional high-resolution transmission EM with full facilities for X-ray microanalysis. It has an electron optical resolution of 1 nm and a selected area for analysis of better than 10^{-15} g. The special topics discussed here include: description, operation, and sensitivity of EMMA-4; wavelength and energy dispersive spectrometry; generation of X-ray quanta; collection and interpretation of X-ray signals; tissue preparation; and special applications to physiological, histochemical, and pathological investigations; etc. In addition, 32 high-quality figures and micrographs are provided for illustration. Some 80 references cited to 1975 are given. Since EMMA-4 possesses revolutionally technical importance and potential analytical applications, the readers are urged to pay great attention to this interesting chapter.

In summary, the book is well written and the material is carefully selected and up-to-date. Methods given are complete, self-explanatory, and applicable to both conventional and high-resolution EM. In addition, advantages and disadvantages of each technique are pointed out and the potential research areas are indicated. With no doubt this volume, as were the previous volumes in the series, will be widely accepted by biologists, biochemists, biomedical scientists, biophysists, cytologists, biomicro-analytical chemists, scientific researchers, and laboratory technicians as a most useful reference book.

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Archaeological Chemistry, Advances in Chemistry Series No. 138. Edited by CURT W. BECK. American Chemical Society, Washington, D. C., 1974. ix + 254 pp. $22.50.

Each year, new physical and chemical analytical methods are applied to old archaeological problems, and new types of archaeological materials are investigated by established analytical methods—micro or, ideally, completely nondestructive methods. Both of these trends are presented in this particular volume: "Archaeological Chemistry." The volume collects the proceedings of the Fifth Symposium sponsored by the Division of the History of Chemistry held at the 165th National Meeting of the American Chemical Society, Dallas, Texas, April 9–10, 1973. It surveys and compiles the historical knowledge of chemistry obtaining from the comprehensive application of versatile analytical techniques. Thus, this collection eventually confirms the view that archaeological chemistry is emerging steadily into a discipline of its own.

The volume contains 13 papers discussing analytical methods used on a diverse array of archaeological objects. Each paper has been contributed by an expert or a group of specialist scientists, and each deals specifically with a sort of archaeological artifact. All articles follow the usual format of the individual scientific papers; that is, each includes an abstract, introduction, experimental procedures and analytical techniques, results and discussion, conclusion, acknowledgment, literature cited and bibliography, and so forth. Prints, tables, and figures are quite clear and paper materials are of high quality. Throughout the text there are no significant typographical errors. The treatment of the subject matter is comprehensive, comparative, and yet pleasantly readable and understandable. A complete index is also available making use of the book more convenient and effective.

The first paper by I. L. Barnes, W. R. Shields, T. J. Murphy, and R. H. Brill concluded, from a precise mass spectrometric isotopic analysis of Laurion lead ores, that the lead isotope data closely match leads from archaeological objects found in Greece.

The second article, by D. F. Gibbons, K. C. Ruhl, and L. S. Staikoff, reports the use of X-ray fluorescence, electron microprobe, and thermal neutron activation analyses to the analysis of copper, gold, and lead contents in the Sasanian silver objects. It demonstrates that the accuracy is directly related to the uniformity of the metallographic structure. The following paper by P. Meyers, L. V. Zelst, and E. V. Sayre is also concerned with the application of thermal neutron activation analysis to the determination of Ag, Cu and Au, three major elements, and Na, K, Sc, Cr, Mn, Fe, Ni, Co, Zn, As, Br, Sn, Sb, Se, Ir, and Hg, 16 trace elements in Sasanian silver objects. Two microsampling techniques, drilling and rubbing, are critically described in detail. Only gold and iridium seem to indicate the silver source used.

The fourth paper, by F. R. Matson, describes and discusses the use of a thermal gradient furnace for archaeological ceramic studies. The results aid in a better understanding of the ancient Egyptian potters' problems of working with clays and in establishing criteria for classifying archaeological ceramic materials.

The next four articles consider the powerful utilization of thermal neutron activation analysis in the investigation of ancient pottery, medieval stained glass, and silver coins. Paper 5 by D. Brooks, A. M. Bieber, Jr., G. Harbottle, and E. V. Sayre treats the biblical studies of a group of 225 sherds of the Persian period ceramics from Tell el-Hesi, Israel. It was revealed, through correlations between pairs of elemental composition patterns, that a large fraction of the collection fits the characteristic local pattern, a few of the Hesi sherds matched that near Jerusalem, and some importation from the Aegean and Cypriote areas. In Paper 6, R. Abascal-M, G. Harbottle and E. V. Sayre indicate that the Teotihuacan ceramics (from Valley of Mexico) and preclassic Terra Cotta Figurines "Tlatilco" have been locally manufactured. In Article 7, J. S. Olin and E. V. Sayre conclude, from an extensive survey of 94 specimens from three groups of glass, that the compositions of glasses from different workshops tend to be distinct from one another and consistent within themselves. Paper 8 by A. A. Gordus and J. P. Gordus summarizes the analytical data of gold impurity levels from over 8000 coins and metallic art objects from Sasanian coins and silver art objects, Umayyad and Byzantine coins. Streak method of sampling for analysis is clearly demonstrated here.

Paper 9, "Comparative Analysis of Archaeological Bronzes," by W. T. Chase is a very interesting one. It describes in detail the comparative use of 10 different analytical methods by 21 laboratories in analyzing bronze samples from the same specimen. Two bronze objects, a Shang Dynasty Chinese bronze *ku* (or beaker) and a Luristan spear point, were used. Forty-eight elements were analyzed from 500-mg samples. The analytical techniques used cover optical emission spectrography, cathode ray polarography, atomic absorption spectroscopy, wet chemistry, nondispersive X-ray fluorescence spectrometry, neutron activation anaylsis, spark source mass spectrometry, coulometry, photon activation, and electrolysis. Chemists who are interested in sophisticated analytical procedures and confident experimental results should study this paper very carefully.

In Article 10, B. Keisch provides a brief review of Mössbauer effect spectroscopy without sampling (surface analysis) technique and its application to the study of art and archaeology, especially with regard to iron and its compounds. The following paper by J. Winter discusses the preliminary investigations on Chinese ink (pine wood soot and lampblack inks) in Far Eastern paintings by scanning electron microscopy (by identifying the particle size distributions of carbon particles).

Paper 12 by C. W. Beck, C. A. Fellows, and E. MacKennan reports, by nuclear magnetic resonance spectrometry, that an oil sample of the sixth–fourth century B. C. consists largely of oleic acid, while a solid fat of the third century A. D. contains principally myristic and

palmitic acids. Both samples were at least 95% hydrolyzed. The final article by N. S. Baer and N. Indictor summarizes the results of chemical investigations of ancient Near Eastern archaeological ivory artifacts. The changes in collagen content were measured by combustion analyses and micro-Kjeldahl methods. Excavated specimens of provenance were studied.

In summary, owing to the value of the objects analyzed and the versatile, sensitive micro-analytical methods used, this volume should be found very interesting not only to archaeological chemists, but also to broad areas of chemists, particularly analytical chemists. Sociologists, historians, metallurgists, and other cultural researchers will also find it an invaluable reference book.

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Chemical Analyses for Medical Technologists. By CLIVE I. WYNTER. Charles C Thomas, Springfield, Ill., 1975. xvi + 217 pp. Clothbound, $14.75, paperbound, $9.95.

The objectives of writing this book are bifunctional, as mentioned in the Preface by the author: (1) to familiarize the medical technologist with modern chemical instrumentation and wet analysis applicable to the field; and (2) to provide a working knowledge of theoretical principles involved in obtaining results from data collected by these analytical techniques. It is therefore divided into two major parts. The first section reviews some fundamental chemical and physical concepts, and the second part emphasizes practical training in laboratory work. The text is thus heavily weighted with laboratory methods, concepts, and techniques. Other materials supplemented to the text include a periodic chart of elements, atomic weights (based on carbon-12), a selected bibliography, four Appendixes, answers to some numerical problems, and an Index.

The first part consists of 12 chapters. The simple statistical methods including frequency distribution, accuracy, precision, significant figures, quality control charts, and normal values opens the first section. The second chapter reviews briefly some fundamental chemistry of solutions with the aid of several examples of numerical calculations. There are some obvious typographical errors that need to be corrected. The next chapter deals with stoichiometry of volumetric and gravimetric analyses with illustrations. However, gravimetric and chemical factors have been neglected. Then, the useful theoretical considerations of acids, bases, and gases in relation to the blood gas analyzer are given in Chapter 4. Unfortunately, there are quite a few mathematical errors which confuse the reading. Also, there is no discussion of the typical acid–base titration curves.

The review of the fundamentals of organic chemistry including the essentials of some sorts of functional group derivatives and nomenclatures is given in Chapter 5. The characteristic properties and reaction mechanisms of the related organic compounds are not available here, however. In Chapter 6, some classes of biochemically important substances such as proteins, common amino acids, nucleic acids (DNA and RNA), porphyrins, adenosine triphosphate (ATP), and biochemical processes in the cell are functionally described. The following chapter is concerned primarily with the clinical chemical aspects of biological fluids and specimen variables. A valuable table summarizes the popular constituents analyzed in blood, and methods for their determination are provided.

Technically, the chromatographic theory and separation techniques including paper, thin-layer (tlc), column, gas–liquid (glc), gas–solid (gsc) chromatography, and zone electrophoreses (paper and gel) are concisely described in Chapter 8. Following this, the powerful spectrophotometric methods (including uv, visible, ir, atomic absorption, fluorescence, and flame photometry) of the clinical laboratory are conceptually discussed. Regretfully, the

description of Beer's law is not sufficient. The Beer–Lambert laws should be discussed together.

Chapter 10 demonstrates the isolation and identification of drugs from blood, urine, water, beer, minerals, spirits, tablets, capsules, and crystalline powders. Chapter 11 reviews the sensitive radiochemical (isotopes) methods. Finally, the last chapter discusses an important and interesting technique, automation in clinical chemistry.

Experimentally, the second part presents 15 well-selected laboratory works. These are volumetric analysis of chloride in blood; the screening of sickling hemoglobin by AAII system; electrophoretic analysis of A, S, and C hemoglobins; the determination of blood urea nitrogen (BUN), glucose, chloride, and carbon dioxide analyses by AAII system; separation and identification of drugs by tlc; glc analysis of drugs in serum; the stepwise separation and identification of thyroxine by ion-exchange chromatography and spectrophotometry; the determination of pH, P_{CO_2}, and P_{O_2} of blood by employing a Corning Model 165 Blood Gas Meter; further blood hemoglobin assay by colorimetry; the determination of total proteins in serum by the biuret method; the fluoremetric analysis of plasma II—hydroxycorticosteroids; ir spectrophotometric determination of pellet drugs; the measurement of calcium content in serum by atomic absorption; flame photometric determination of sodium and potassium in serum using lithium as internal standard; and the practice of radiochemical analytical tenchiques; etc. Each experiment is provided with a brief introduction, apparatus required, reagents list, sample preparation, experimental procedure, calculation and results, and data report sheet. The description of each subtitle is straightforward, complete, and self-explanatory. Students or laboratory technicians could perform each unit easily and successfully without other help.

In spite of several typographical errors and some discrepancies in mathematical derivations found in the text, this book deserves serious consideration as a potential candidate for a laboratory text for medical technologists, lab technicians, and nonchemistry major students. It is sincerely suggested that the author or the publisher compile an errata for distribution to users as soon as possible. Furthermore, if a new revised edition is planned, it would be useful to add some other new analytical methods such as chemical kinetics, enzymatic approach, immunological techniques (i.e., immunodiffusion, immunoelectrophoresis, and radioimmunodiffusion, etc.), complexometric methods, and oxidation–reduction procedure, since these techniques are equally important and are sensitive analytical methods generally and effectively used in clinical laboratory and biomedical research areas. In addition, selective addition of an up-to-date bibliography for each specific technique would be worthwhile.

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Inorganic Chromatographic Analysis. By JAN MICHAL, Van Nostrand Reinhold Ltd., London, New York, 1974. x + 217 pp. $24.95.

In general, inorganic chemists have lagged behind their colleagues in organic chemistry in their applications of chromatographic methods. This may be due to the intrinsic differences between the substances dealt with, such as the general similarity of most organic materials and the extremely difficult separation problems which occur. It may also be due in part to the lack of good reference material on inorganic problems. Books such as this one will go a long way toward correcting the latter situation.

The book is divided into several sections: principles, techniques, methods of detection and determination, systematic qualitative analysis, and two very large (ca. 60% of the book) review chapters on separations and quantitative analysis. The section on principles is par-

ticularly well done, with a clear delineation between adsorption and partition methods. The section on techniques is rather sparse and quite out of date. The most valuable portions are the review chapters, which are well worth the price of the book.

In a more critical vein, there is little consideration of the separation of inorganic coordination compounds, no discussion of attempts to separate chiral materials, and no discussion of gas chromatography, which is becoming quite important in inorganic work.

In summary, the book is an outstanding review of most of the chromatography of inorganic ions and should be available to any research group in general or inorganic chemistry.

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