Volume 17, Number 5, October 1972

Microchemical Journal

devoted to the application of microtechniques in all branches of science

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ORD and CD in Chemistry and Biochemistry: An Introduction

By PIERRE CRABBÉ

Syntex, S. A.
Universidad Nacional Autonoma de Mexico and
University Iberoamericana
Mexico, D. F., Mexico

CONTENTS:

This monograph provides a basic introduction to Optical Rotatory Dispersion (ORD) and Circular Dichroism (CD) and their applications to structural and stereochemical problems. These techniques furnish precise and accurate stereochemical data quite rapidly, and they are the only ones that can be used to study chiral molecules by analyzing their Cotton effects. The book emphasizes those Cotton effects encountered in organic molecules, although ORD and CD techniques can also be applied to inorganic molecules.

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Microchemical Journal, Vol. 17, No. 5

Briefs

The Microdetermination of Sulfur with an Automatic Elemental Analyzer. ROBERT F. CULMO, *Microanalytical Department, The Perkin-Elmer Corporation, Norwalk, Connecticut 06852.*

A method is described which permits the rapid microdetermination of sulfur in the presence of hetero-elements which normally interfere in conventional methods. Use is made of an automatic elemental analyzer (CHN–O). Halogens are removed by means of 8-hydroxyquinoline, oxygen by means of copper, and sulfur absorbed as sulfur dioxide between the two sides of a differential thermal conductivity detector which thus produces a signal proportional to the sulfur content. The analysis time is approximately 13 min.

Microchem. J. **17**, 000 (1972).

Determination of Ketoses by Ultraviolet Absorption Following Thermal Hydrolysis. M. A. PFEIFER AND R. J. DOYLE, *Department of Microbiology, University of Louisville School of Medicine, Louisville, Kentucky 40201.*

The hydrolysis of a number of ketoses in hydrochloric acid results in the production of ultraviolet absorbing materials, forming the basis of a sensitive method for the quantitative determination of these sugars.

Microchem. J. **17**, 000 (1972).

Direct Titrimetric Microdetermination of Glycine. Direct Determinations of Glycine–DL-Alanine, Glycine–DL-Valine, and Glycine–L-Arginine in One Solution Without Separating. O. C. SAXENA, *Chemical Laboratories, University of Allahabad, Allahabad, India.*

Glycine is quantitatively determined in micro amounts separately and also in combination with DL-alanine, DL-valine, and L-arginine in one solution without separation. The reaction between beryllium sulfate and glycine results in the formation of a complex in the ratio of 1 : 2.

Microchem. J. **16**, 000 (1971).

ICN and BrCN as Volumetric Oxidants. RAM PARKASH, RAJ KUMAR CHAUHAN, AND JAROSLAV ZÝKA, *Department of Analytical Chemistry, Charles University, Prague, Czechoslovakia.*

Redox potential and polarographic behavior of iodine cyanide and bromine cyanide have been studied and electrometric titrations with potassium propyl xanthate, vanadium(II) chloride, and tin(II) chloride performed to investigate their potentialities as volumetric oxidants and their mode of reduction.

Microchem. J. **17**, (1972).

Microchemical Tests for Hydantoins and Barbiturate–Hydantoin Mixtures. EDWARD F. RHODES, AND JOHN I. THORNTON, *School of Criminology, University of California, Berkeley, California 94720.*

Substituted hydantoins, which are often prescribed compounded with barbiturates, were observed to give reactions commonly used to identify the latter type of compounds. Consequently there is a possibility of confusion resulting while testing for the former type. Identifications of the barbiturate–hydantoin mixtures may be effected by means of a judicious selection of appropriate microcrystalline tests.

Microchem. J. **17,** (1972).

Benzoylacetanilide as a Spectrophotometric Reagent for Iron(III). K. P. SRIVASTAVA AND A. D. TANEJA, *Chemistry Department, Birla Institute of Technology and Science, Pilani, Rajasthan, India.*

Benzoylacetanilide (BAA) forms violet colored complex $Fe(BAA)_3$ with Fe(III) in 60% alcohol with λ_{max} at 540 nm. The stability constant (log K) and free energy of formation of the complex at 20°C are log $K = 7.8 \pm 0.1$ and $\Delta F = -10.53$ kcal/mole, respectively. The molar adsorptivity, sensitivity, effect of diverse ions in spectrophotometric determination of iron have also been investigated.

Microchem. J. **16,** 000 (1971).

Determination of Traces of Free Acid by the Polarographic Reduction of Quinones. K. TAKAMURA AND Y. HAYAKAWA, *Tokyo College of Pharmacy, 1-10-19, Uenosakuragi, Taito-ku, Tokyo 110, Japan.*

The effects of proton donors on the polarographic reduction of methyl-*p*-benzoquinone (MQ) and some examples of analytical application of the experimental results have been presented. The presence of a small amount of acid gives rise to a new reduction wave which precedes the original reduction wave of MQ. The new wave is affected by the strength of the added acid and the basicity of the solvent. The new wave is diffusion controlled and attributed to the protonation of MQ at the electrode surface prior to the electron transfer.

The new wave is proportional to the concentration of acid present and therefore can be used for the determination of minute quantities of free acid in reagents.

Microchem. J. **17,** (1972).

Automation in Organic Analysis. II. Simultaneous Microdetermination of Oxygen and Nitrogen. KAREL UBIK, *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague 6, Czechoslovakia.*

Simultaneous determination of oxygen and nitrogen is described in the presence of halogens. The method consists of the pyrolytic decomposition of the compound in a current of hydrogen at 1000–1100°C and passing the products through nickel and platinized carbon at 1050°C. After removal of the halogens, carbon monoxide

and nitrogen are separated on a chromatographic column and then determined by means of a catharometer. The determination requires 25 min. Sulfur interferes with the determination.

Microchem. J. **17**, (1972).

The Microdetermination of Soluble Iodides in Biological Fluids. M. Z. BARAKAT, M. BASSIONI, AND MAMDOUH EL-WAKIL, *Biochemistry Department, Faculty of Medicine, Azhar University, Madina Nasr, Cairo, U.A.R.*

A modified titrimetric method for the microdetermination of soluble iodides, e.g., potassium iodide or sodium iodide is described. The mechanism of the reaction between *N*-bromosuccinimide and potassium iodide in aqueous solution is discussed. The determination is done within the range of 10 mg to 50 μg for soluble iodides. The experimental error does not exceed $\pm 2\%$. Comparative analysis of potassium iodide by the proposed method and the official potassium iodate method is reported. The method has been applied to determine potassium iodide in biological fluids within the limits of 10 to 1 mg giving satisfactory results.

Microchem. J. **16,** 000 (1971).

Chromatographic and Spectroscopic Investigations of the Oxidation Process in Methyl Oleate and Methyl Elaidate. JÓZEF SLIWIOK AND WITOLD J. KOWALSKI, *Department of Organic Chemistry, Institute of Chemistry, Silesian University, Szkolna Street 9, Katowice;* AND ANNA WASIELEWSKA, *Institute of Polymers, Polish Academy of Sciences, Zabrze, Poland.*

The purpose of the work was to compare the initial stage of autoxidation of methyl oleate and methyl elaidate (methyl esters of *cis*- and *trans*-9-octadecenoic acids, respectively. Chemical and physical data are presented.

Microchem. J. **17,** 000 (1972).

Design and Construction of a Spectrophotometer Accommodating Long-Path Microcells. H. FLASCHKA AND R. BARNES, *School of Chemistry, Georgia Institute of Technology, Atlanta, Georgia 30332.*

A simple single-beam, single-cell instrument is described that accommodates 40-cm cells with less than 0.8 ml solution required for complete filling and showing an effective path length of about 48 cm. The instrument can be built from readily available components and material at a reasonable effort and cost. An increase in sensitivity of spectrophotometric measurements results by the use of small volumes of more concentrated solutions.

Michrochem. J. **17,** (1972).

Macro- and Microdetermination of Mercury(I) Using Potassium Permanganate as an Oxidant in Acid Medium in Presence of Fluoride Ions. I. M. ISSA, A. S. MISBAH, AND M. H. HAMDY, *Chemistry Department, Assiut University, Assiut, U.A.R.*

Mercury(I) can be titrated potentiometrically and visually with potassium permanganate in the presence of fluoride and nitric acid. The optimum conditions are 0.2–1.2 *N* HNO_3 in the presence of 1% sodium fluoride at temperatures not exceeding 50°C.

Microchem. J. **16**, 000 (1971).

Oxidation of Glycerol, Mannitol, and some Saccharides with Co(III). M. Asghar Ali Butt, Riaz Ul Malik, and Jaroslav Zýka, *Department of Analytical Chemistry, Charles University, Prague 2, Czechoslovakia.*

Oxidations of glycerol and various sugars have been carried out at various temperatures. Stepwise oxidation was observed at well-defined periods. The number of electrons exchanged is discussed.

Microchem. J. **17**, 000 (1972).

MICROCHEMICAL JOURNAL 17, 499–507 (1972)

The Microdetermination of Sulfur with an Automatic Elemental Analyzer

ROBERT F. CULMO

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Received May 5, 1972

A review of the literature shows various techniques for the determination of sulfur. Many of these employ the Pregl combustion method in which the sample is burned in a stream of oxygen and the quantitation effected by microgravimetric or electrochemical means. Modifications of this method have been developed which permit the simultaneous determination of several elements including sulfur (*1, 6, 7*). This general method has been considered impractical because it is time-consuming and tedious. In another method the sample is oxidized in a closed flask filled with oxygen (*10*) and, after several additional steps, quantitation is achieved by either titrometric or gravimetric means. When interfering anions or cations are present, the sample solution must be passed through an ion-exchange column or an additional reagent added to reduce their effect.

A fast, automated technique for the determination of sulfur has been developed using a commercial elemental analyzer (Perkin-Elmer Model 240) normally used to determine carbon, hydrogen, nitrogen and oxygen. This method permits the determination of sulfur directly in the presence of common interferences.

The basic principle of this analyzer was originally developed by Simon, Sommer, and Lissy (*11*) and later refined to a commercial product by Condon (*2*). The design features include a combustion system which very closely approximates the classical techniques and a thermal conductivity detection system. The combustion products are mixed and equilibrated in helium and then differentially monitored by three independent bridges to produce steady-state signals proportional to the nirogen, carbon dioxide and water concentrations. Later developments included the selection of combustion packings and oxidation aids which

[1] Because magnesium perchlorate is listed as a potential hazard when subjected to heat, the designed combustion tube shown in Fig. 1A with its 3 mm indentation and glass extension stops is mandatory.

extended the application of the analyzer to virtually every class of organic compound. Most significantly, the microdetermination of oxygen was effected (*4*). This involved changing the chemistry in the combustion zone of the analyzer where the oxygen by-products of reaction are first reduced to carbon monoxide over platinized carbon (at 950°C) and then oxidized to carbon dioxide over cupric oxide (at 650°C). The latter is measured by the detection system in the same manner as the CO_2 in the CHN mode of operation. In this technique, however, the CO_2 is quantitated as oxygen.

In the sulfur method described below the same basic analyzer is used but with a particular choice of reagents in the combustion and detection area and particular operating conditions which have been found suitable for sulfur determinations within aceptable limits of accuracy for a wide variety of organic and inorganic materials.

The reagents include tungstic oxide (*8*) used as the combustive oxidative catalyst, magnesium perchlorate,[1] or calcium chloride to remove water; 8 hydroxyquinoline to remove residual halogen by-products; copper to remove excess oxygen and halogens; and silver oxide (at 220°C) to absorb sulfur as sulfur dioxide between the two sides of a differential thermal conductivity detector which thus produces a signal proportional to sulfur content.

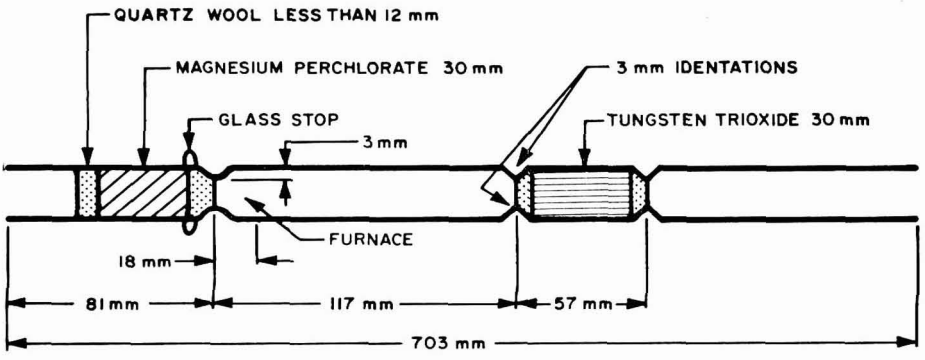
EXPERIMENTAL METHODS

Apparatus. Automatic CHN analyzer (Perkin-Elmer Corp., Norwalk, CT, Model 240). Modified as follows: The standard combustion and reduction tubes are removed and replaced with (a) a combustion tube designed to contain tungstic oxide in the combustion zone and magnesium perchlorate at the exit end of the combustion zone ouside the furnace (Fig. 1A), (b) a reduction tube containing free copper (Fig. 1B), and (c) a U-tube containing 8-hydroxyquinoline (Fig. 2) connecting the exit of the combustion tube with the entrance of the reduction tube and connecting fittings (Fig. 3). The water absorber is removed and replaced with a tube surrounded by a small furnace and containing silver oxide (Fig. 1C). The operating temperatures of the furnaces are 950–1000°C for the combustion tube, 820–880°C for the reduction tube and 200–240°C for the sulfur dioxide absorber.

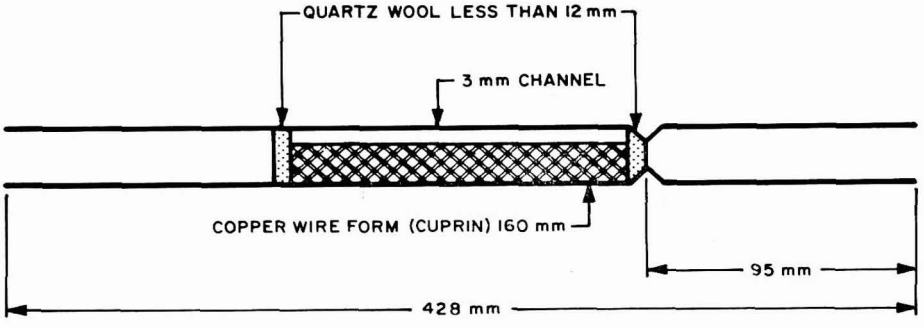
The sulfur dioxide absorber tube furnace is the preheater type with a cylindical chamber. It is 110 mm long by 13 mm i.d. and was obtained from the A. H. Thomas Co., Philadelphia, PA (Part No. 5676-A).

A powerstat (Variable Autotransformer 115 V) was used to adjust the temperature of the sulfur dioxide absorber furnace.

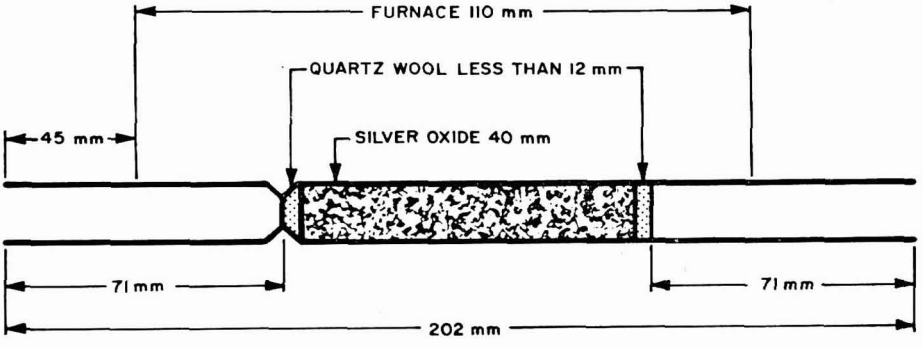
Reagents. Anhydrous magnesium perchlorate, copper reagent (cuprin), tungstic oxide (all from Perkin-Elmer Corp., Norwalk, CT).



(A) COMBUSTION TUBE



(B) REDUCTION TUBE



(C) SULFUR DIOXIDE ABSORBER

FIGURE 1

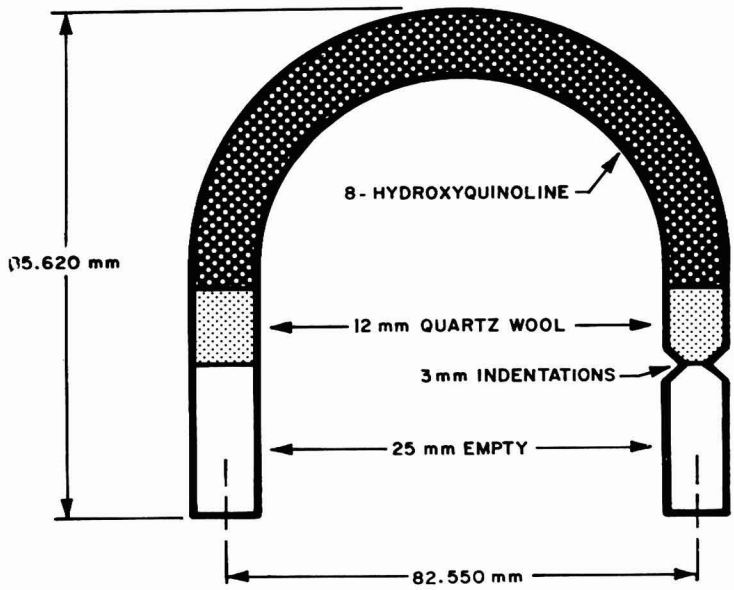


FIGURE 2

The silver oxide reagent was obtained from the K and K Laboratories, Inc., Plainview, NY, or Hollywood, CA.

The 8-hydroxyquinoline and calcium chloride were obtained from the Fisher Scientific Co., Fair Lawn, NJ.

PRECONDITIONING OF REAGENTS

Preconditioning in bulk of several of the reagents used in the method described reduced the down time otherwise realized if these reagents were installed directly into the analyzer.

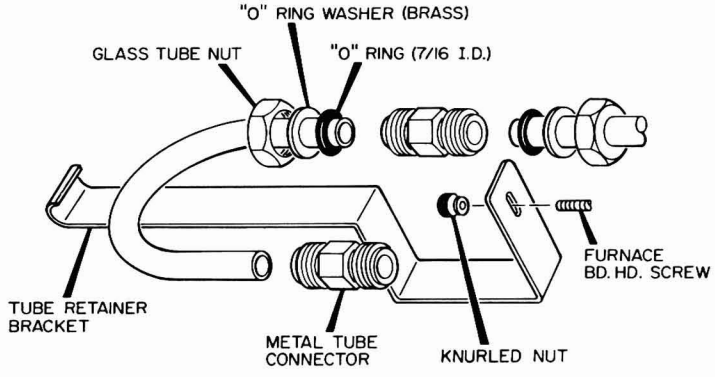


FIGURE 3

The reagents include the silver oxide which was conditioned by heating 100 g at 200°C for 48 hr in a muffle furnace and stored in a desiccator. Tungstic oxide (200 g) was heated at 900°C for 48 hr in a muffle furnace, allowed to cool to room temperature and stored in a desiccator. Copper (cuprin) (200 g) was loosely packed in a large bore Pyrex tube. The tube was installed in a large bore furnace (1 in. bore Lindberg/Hevi-Duty furnace). The tube was heated to 400°C for 2 hr while purging with helium. The reagent was allowed to cool and then stored in a desiccator.

Packing the combustion tube. Quartz wool (10 mm) was firmly packed to the second set of indentations 515 mm from the entrance end of the combustion tube (Fig. 1A). The preconditioned tungstic oxide (30 mm) was added and quartz wool was firmly packed to the first set of indents. Quartz wool (10 mm) was packed to the first indentation 81 mm from the exit end. Magnesium perchlorate (30 mm) was then added and firmly packed in place with quartz wool as before.

Packing the halogen gas scrubber U-tube. Quartz wool (10 mm) was packed to the indent 30 mm from one end of the U-tube as shown in Fig. 2. 8-Hydroxyquinoline was added to within 40 mm of the other open end. After closing with 10 mm of quartz wool, 30 mm of empty tube remained.

Packing the reduction tube. Quartz wool (10 mm) was packed to the indent 333 mm from the entrance end and 160 mm of copper reagent were added in such a way as to leave a 3 mm channel. A final 10 mm of quartz wool was packed in place leaving 163 mm of empty tube upstream of the packing.

Packing the sulfur dioxide absorption tube. Quartz wool (10 mm) was packed 131 mm from the entrance end. The preconditioned silver oxide (40 mm) was then added followed by an additional 10 mm of quartz wool leaving 71 mm empty.

Gas flow adjustments. The helium pressure was adjusted to obtain a filling time from 30 to 60 sec. The oxygen pressure was adjusted to achieve a flow of 75–86 ml/min, measured at the exit end of the combustion tube.

Course of the combustion. Before any sample runs are made, the system is flushed of any residual water by making several blank runs until the blank values obtained for the carbon and sulfur bridges are less than 300 μV.

When metals are present in the sample material, 10 mg of tungstic oxide are added to a platinum boat. A 1 to 3 mg sample is accurately weighed over the donor, 70 mg of tungstic oxide are added over the sample.

When coal or phosphorus compounds were analyzed, a 1 to 3 mg sample is accurately weighed in a platinum boat. Then 60–80 mg of tungstic oxide are added over the sample.

With the setup as described, the procedure for sample analysis is as follows: A 1–3 mg sample is accurately weighed into a platinum boat, the boat is placed in a quartz ladle, and the ladle is inserted into the cool zone of the combustion tube. The program is started. When the "Inject" panel lamp lights, (the "program" lamp will remain on due to the high input helium pressure) coal samples and materials containing metals were injected into the heated combustion zone. All other sample types were injected at program index setting 4. From this point the analysis is fully automatic and follows the same sequence as in the analysis for CHN with the Model 240. The sulfur signal is read from the bridge normally used to detect hydrogen.

Known compounds are first analyzed to calibrate the detector voltage in terms of micrograms of sulfur. This calibration factor is then used to measure the unknown samples.

RESULTS

The sample results listed in Table 1 demonstrate the system's ability to analyze the various oxidation states of sulfur in organic and inorganic species. These include sample materials which contain potassium, sodium, silver and phosphorus. When these elements were present in the sample, the donor tungstic oxide was sandwiched over the sample in the platinum boat. The liquid samples analyzed were sealed in aluminum capsules (*5*) using the Perkin-Elmer volatile sample sealer (Part No. 219-0061). The coal samples analyzed were received from the Department of Energy, Mines and Resources in Ottawa, Can. The theoretical values shown for these samples were determined by this agency using conventional macromethods.

DISCUSSION

The primary objective of this effort was to develop a technique for the microdeterminatoin of sulfur using an automatic analyzer. The analyzer employed is designed to detect carbon, hydrogen, and nitrogen in three independent bridges. In the technique described here, the first bridge is modified to quantitate sulfur in place of hydrogen, leaving the carbon and nitrogen bridges unchanged, therefore permitting quantitation of carbon and nitrogen with sulfur although with less accuracy. The conditions described above were deliberately determined to be optimum for the analysis of sulfur. While many analyses yielded excellent

TABLE 1

SULFUR

	Theory	Observed
Phenylthiourea	21.07	21.27
Sulfathiazole	25.12	24.99
Cystine	26.69	26.68
L-Cysteine	26.47	26.64
Dibenzyl disulfide	26.03	26.26
Sulfanilamide	18.62	18.71
Phenyl sulfone	14.66	14.89
N-Acetyl-*N*(*p*-nitrophenyl)-sulfanilamide	9.64	9.63
Polysulfone	7.15	7.42
Sulfur	100.00	100.08
Malathion	19.41	19.63
S-Benzylthiuronium chloride	15.82	16.00
4-Homosulfanilamide hydrochloride	14.40	14.20
p-Toluenesulfonyl chloride	16.82	16.56
p-Diphenylaminesulfonic acid sodium salt [a]	11.82	11.64
Sodium sulfate [a]	22.57	22.41
Potassium sulfate [a]	18.40	18.32
Silver sulfate [a]	10.28	10.19
Coal (Camp Sussex)[a]	6.72	6.81
Coal (Springhill Mines)[a]	7.18	7.08

[a] Mixed with WO_3.

results for all three elements N, C and S (7), the values for N and C are occasionally found to fall outside 0.3% limits of accuracy.

Since the success of this method requires that the sulfur arrive at the detector quantitatively converted to SO_2, there are at least three areas of concern with respect to the chemistry of reactivity of sulfur oxides.

The first is the possibility of reaction with the flow path of the analyzer. It was found that when water was removed immediately at the exit of the combustion tube that no loss of sulfur by reaction or absorption in the lines and fittings of the instrument was detectable. Evidently the reactivity of sulfur oxides on such materials depends upon the coexistence of water.

The second concern was the well-known reaction $2SO_2 + O_2 \rightleftarrows 2SO_3$. Although data exists on the temperature dependence of the equilibrium constants and the rates of the forward and reverse reactions, the rather complex temperature profile through the flow path of the gases in the Model 240 required an empirical approach to find conditions which prompted complete conversion to SO_2.

The third problem was the effect of contact of the gaseous mixture

with the copper in the reduction tube at 800°C. The reduction tube very effectively removes oxygen and reduces nitrogen oxides to nitrogen in the normal operation of the instrument but its effect on the sulfur oxides and the above equilibrium was not predictable.

The manner of packing both the combustion and reduction tubes which has been described and the recommended helium pressure and oxygen flow rate was arrived at after much experimentation in varying these conditions. Since the tightly packed tungstic oxide in the combustion tube acts as a restrictor whereas the "channeled" copper in the reduction tube offers little resistance to the flow, these apparently optimum conditions will cause a long residence time in the combustion zone, efficient percolation of the gases through the tungstic oxide packing and a relatively short residence time in the reduction zone. All of these conditions appear to be necessary for efficient and quantitative conversion of sulfur compounds to the single species, SO_2.

SUMMARY

A method has been developed to permit the rapid microdetermination of sulfur in the presence of hetero-elements which normally interfere in conventional methods, using an automatic elemental (CHN–O) analyzer.

The time required to convert the analyzer over to the sulfur capability requires no more than 20 min. However, since the change involves the detection area of the analyzer, it is recommended that this conversion be made at the end of the day. Conversion back to CHN showed no ill effects. The analysis time is 13 min.

ACKNOWLEDGMENT

The author expresses his thanks to Drs. D. S. Montgomery and B. N. Nandi for providing the coal samples and its collaborating data for this study.

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MICROCHEMICAL JOURNAL 17, 508–511 (1972)

Determination of Ketoses by Ultraviolet Absorption Following Thermal Hydrolysis

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Received May 30, 1972

INTRODUCTION

Several sugars can be quantitated by measuring the ultraviolet absorbance of their thermal hydrolysis products. Usually, thermal hydrolysis is carried out in mineral acid solution at 80–100°C. Recently, Doyle and Pfeifer (*1*) found that 2-deoxyhexoses could be determined by absorbance readings at 217 nm after the sugars had been subjected to 4 *N* HCl at 80°C. Previously, Seydel and Garrett (*5*) quantitated 2-deoxyribose by use of a similar method. In addition, Garrett, Blanch and Seydel (*3*) found that ribose and 2-deoxyribose could be quantitated individually or in a mixture by ultraviolet absorbance following thermal hydrolysis in HCl. Garrett and Blanch (*2*) showed that when fructose was degraded at 80°C in 1 *N* HCl, an ultraviolet chromophore appeared at 283 nm, the absorbance of which was proportional to the fructose concentration. This procedure was extended to the determination of sucrose. In this communication, we show that several ketoses can be determined by ultraviolet measurements following hydrolysis in 4 *N* HCl at 80°C.

MATERIALS AND METHODS

Mannoheptulose, fructose, and sorbose were purchased from Pfanstiehl Laboratories, Waukegan, IL. Tagatose and sedoheptulose anhydride monohydrate were obtained from Sigma Chemical Company, St. Louis, MO. Other sugars used in the interference experiment were purchasd from Pfanstiehl, Sigma or Calbiochem, Los Angeles, CA.

Ketose stock solutions were prepared as before (*1*) and diluted to the proper concentration before hydrolysis. The sample size and conditions for hydrolysis were the same as those used previously (*1*).

Ultraviolet spectra of the hydrolysis products were recorded with a Cary Model 15 spectrophotometer using 1-cm silica cuvettes. Absorb-

ance readings at single wavelengths were made on a Beckman Model DU spectrophotometer.

RESULTS AND DISCUSSION

Ketose solutions, including fructose, sorbose, tagatose, mannoheptulose, and sedoheptulose were hydrolyzed in 4 *N* HCl at 80°C. The spectra of the hydrolysis products are shown in Fig. 1. In agreement with Garrett and Blanch (*2*) it is seen that fructose gave a λ_{max} at 283 nm. Mannoheptulose and tagalose also absorbed maximally at 283 nm while sorbose gave a λ_{max} at 281 nm and sedoheptulose produced a λ_{max} at 285 nm.

Experiments in which the HCl concentration and temperature were varied showed that 4 *N* HCl and 80°C were optimal conditions for chromophore production. Under these conditions, fructose could be determined after 75 min (Table 1). In 1.0 *N* HCl at 80°C, over 9 hr were required to achieve maximal absorbance for fructose (*2*). Table 1 summarizes some of the conditions necessary for the quantitative determination of ketoses by the ultraviolet method. As shown, all of the ketoses give rise to products with extinction coefficients high enough to make the method amenable for quantitative determinations of the sugars. Some disadvantages are apparent, however. For example, 300

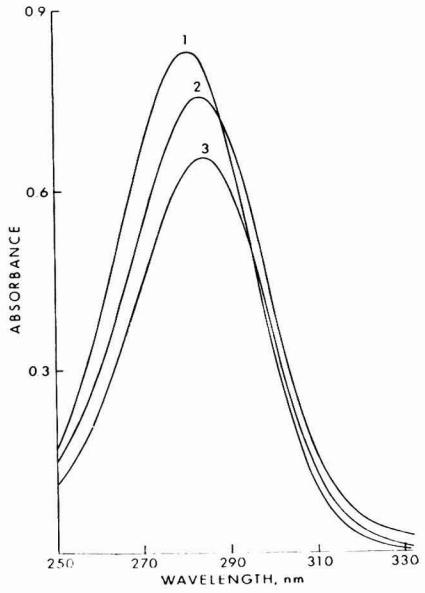

FIG. 1. Ultraviolet absorption spectra of degraded ketoses. (1) sorbose; (2) fructose; (3) sedoheptulose. Conditions were 4 *N* HCl at 80°.

TABLE 1

DETERMINATION OF KETOSES FOLLOWING ACID HYDROLYSIS AT 80°C

Ketose	λ_{max} (nm)	Time [a] (min)	Molar extinction coefficient [b]	Stability [c] (min)
Fructose	283	75	5150	45
Sorbose	281	90	6160	60
Tagatose	283	45	7483	15
Sedoheptulose	285	300	2054	300
Mannoheptulose	283	150	4650	60

[a] Time required for chromophore absorbance to reach a maximum. Conditions were 80 ± 1°C, 4 *N* HCl.

[b] Absorbance units/mole.

[c] Time during which absorbance was a maximum.

min are required to achieve maximal hydrolysis of sedoheptulose. Moreover, in the case of tagatose, the chromophore is relatively transient.

The effects of unrelated sugars and sugar derivatives on the determination of fructose by the ultraviolet method are shown in Table 2. The only sugar with significant interference is D-ribose. This is probably because D-ribose produces a highly absorbing chromophore at 277 nm following thermal hydrolysis in HCl (*3*). It would be expected that other aldopentoses, such as xylose, lyxose and arabinose would also

TABLE 2

EFFECT OF SUGARS AND SUGAR ALCOHOLS ON THE ULTRAVIOLET DETERMINATION OF D-FRUCTOSE

Sugar or derivative [a]	D-Fructose (μg)	
	Added	Found
D-Glucose	200	199
D-Galactose	200	201
D-Fucose	200	200
D-Mannitol	200	200
D-Rhamnose	200	205
D-Ribose	200	246
D-Mannose	200	200
Methyl-D-glucopyranoside	200	202
D-glucoheptose	200	201
2-deoxy-D-glucose	200	203
D-Ribitol	200	198

[a] The concentrations of saccharides or sugar alcohols added to the D-fructose solutions were 100 μg. Total volume was 8.0 ml. Final concentration of HCl was 4.0 *N*. Hydrolysis was for 90 min at 80°C. The blank was 4.0 *N* HCl.

interfere (*4*). Aldohexoses, such as glucose, mannose and galactose do not interfere (Table 2). This enhances the applicability of the ultraviolet method for ketoses, particularly ketohexoses, since it is often necessary to determine these sugars in the presence of aldohexoses.

SUMMARY

The hydrolysis of fructose, sorbose, tagatose, mannoheptulose and sedoheptulose in 4 *N* HCl at 80°C results in the production of ultraviolet absorbing materials. This forms the basis of a sensitive method for the quantitative determination of these sugars. Optimum conditions for the determination of the individual ketoses were found. Aldohexoses, 6-deoxyhexoses, 2-deoxy-D-glucose, D-glucoheptose and sugar alcohols do not interfere with the method. Ribose produced significant interference.

ACKNOWLEDGMENTS

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MICROCHEMICAL JOURNAL 17, 512–518 (1972)

Direct Titrimetric Microdetermination of Glycine. Direct Determinations of Glycine–DL-Alanine, Glycine–DL-Valine, and Glycine–L-Arginine in One Solution Without Separating

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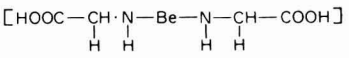
INTRODUCTION

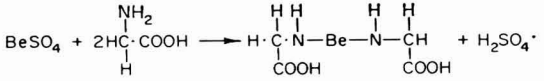
In the literature there is no direct method available for the determination of glycine. Indirect methods are, of course, available for its separate determination, but not in mixture without separating. However, glycine has been determined spectrophotometrically (*1*); potentiometrically (*2*); by adding cupric chloride and disodium hydrogen phosphate (*3*); cerimetrically (*4*); with dichromate heat of dilution method (*5*); by oxidizing with gold chloride in alkaline medium (*6*), hypochlorite (*7*), and potassium permanganate (*8, 12*); in protein hydrolyzate (*13*); and with pyridine and *p*-nitrobenzoyl chloride (*14*).

The aim of the present work was to provide a simple direct method for the quantitative estimation of glycine. Glycine has been determined in micro amounts by directly titrating against beryllium sulfate solution using either Congo red or xylenol orange or chromazural red S as indicators separately or in the presence of DL-alanine, DL-valine, or L-arginine without separating. It is observed that the reaction between beryllium and glycine results in the formation of a complex,



in the ratio of 1 : 2.

Probably the reaction and the tentative structure is,



EXPERIMENTAL METHODS

Reagents used. Glycine, beryllium sulfate, DL-alanine, DL-valine, L-arginine, chromazural red S, and potassium tellurite (E. Merck grade);

gold chloride (Apex, Indian grade); ferrous ammonium sulfate (AnalaR, B.D.G. grade); Congo red and xylenol orange (B.D.H., grade).

Apparatus used. Micropipettes and microburettes had least count = 0.01 ml.

Standard glycine and DL-alanine, DL-valine, L-arginine and ferrous ammonium sulfate (in 0.1 *N* H_2SO_4) solutions were prepared by dissolving the exactly weighed amount in distilled water. Gold chloride and potassium tellurite solutions were prepared by dissolving in distilled water and were further standardized (*10*) and (*11*), respectively.

Xylenol orange and Congo red solutions were prepared by dissolving 0.1 g in 100 ml of distilled water. Chromazural red S solution was prepared by dissolving 0.05 g in 100 ml of distilled water.

PROCEDURE

I. Determination of Glycine Separately

(i) Using Congo red as indicator. A known volume of a known standard glycine solution was placed in a beaker, through a micropipette, and diluted to 30 ml by adding distilled water. Few drops of congo red solution were added to the beaker containing glycine solution, which became red color. This solution was titrated by running in, through a microburette, standard beryllium sulfate solution until the brown color appeared sharply, at the end point.

(ii) Using xylenol orange as indicator. After placing glycine in the beaker and diluting to 30 ml, few drops (2 to 3) of xylenol orange solution were added, and the whole solution became light purple color. Titration was done as usual until the purple changed to light rose color at the end point.

(iii) Using chromazural red S as indicator. Few drops (2 to 3), of chromazural red S solution were added to the beaker containing glycine solution, and it became light yellow color. Standard beryllium sulfate solution was run in the beaker until the appearance of deep purple at the end point.

II. Microdetermination of Glycine and DL-*Alanine in One Solution Without Separating*

(i) Using Congo red solution as indicator. Known volumes of standard glycine and DL-alanine solutions were placed in a beaker, through micropipettes, and diluted to 30 ml by adding distilled water, which formed a solution mixture. A few drops (2 to 3) of Congo red solution were added to the beaker containing solution mixture, which became red. Glycine was first titrated against standard beryllium sulfate solution until orange brown color appeared at the end point. Then, in the same

mixture DL-alanine was titrated against standard gold chloride solution (without adding any other indicator) until the orange color changed to violet at the end point.

(ii) Using xylenol orange solution as indicator. A few drops (2 to 3) of xylenol orange solution were added to the beaker containing the solution mixture, which became light purple. Glycine was, first, titrated as usual to rose color at the end point. Then, in the same solution mixture, after titrating glycine, and without addition of any other indicator, DL-alanine was titrated against gold chloride solution to light yellow color at the end point.

(iii) Using chromazural red S solution as indicator. To the solution mixture, a few drops (2 to 3) of chromazural red S solution were added, and it became light yellow. Glycine was first titrated as usual to purple at the end point. In the same solution mixture, without adding any other indicator, DL-alanine was titrated as usual but the color changed from purple to pink at the end point.

Using chromazural red S and Congo red solutions as indicator. To the solution mixture, first, a few drops (2 to 3) of chromazural red S solution were added, and it became light yellow. Glycine first was titrated, as already mentioned, to purple at the end point. At this stage, DL-alanine was titrated in the same solution by adding a few drops (2 to 3) of Congo red solution and the whole solution became red. Gold chloride solution was run until the red changed to pink purple at the end point.

III. Determination of Glycine and DL-*Valine Together In One Solution Without Separating*

Using chromazural red S and Congo red solution as indicator. Known volumes of standard glycine and DL-valine solutions were placed, through micropipettes, in a beaker and diluted to 30 ml by adding distilled water, which formed solution mixture of the two. A few drops (2 to 3) of chromazural red S solution were added to the solution mixture, and it became light yellow color. At first, glycine was titrated, as mentioned already, until purple appeared at the end point. Then, in the same solution DL-valine was titrated against potassium tellurite solution using Congo red solution as indicator, until the color changed from deep brownish red to organge red at the end point.

IV. Determination of Glycine and L-*Argine in a Mixture Without Separating*

Using chromazural red S solution as indicator. Known volumes of standard glycine and L-argine solutions were placed in a beaker and

diluted to 30 ml with distilled water, which formed a solution mixture. A few drops (2 to 3) of chromazural red S solution were added to the solution mixture which became light yellow color. First glycine was titrated against beryllium sulfate, until purple appeared at the end point. Then, in the same solution mixture L-arginine was estimated, without adding any indicator, by titrating against standard ferrous ammonium sulfate (in 0.1 N H_2SO_4) solution until the purple color changed to violet at the end point.

RESULTS AND DISCUSSION

Results are given in Tables 1,2,3, and 4. Glycine, DL-alanine, DL-valine and L-arginine have been estimated in ranges at 1.53×10^{-4} to 61.25×10^{-4} mg/liter; 9.32×10^{-4} to 23.30×10^{-4} mg/liter; 11.69×10^{-4} to 29.24×10^{-4} mg/liter; and 3.41×10^{-4} to 13.99 10^{-4} mg/liter, respectively.

Results in Tables 1–4 show that glycine has been separately titrated and, also, in combinations with DL-alanine, DL-valine, and L-arginine against beryllium sulfate, gold chloride, potassium tellurite, and ferrous ammonium sulfate, respectively. It is peculiar that in each combination glycine has been first estimated. Maximum error for glycine is 2.0% Since the reaction between beryllium sulfate and glycine results in the formation of a complex in the ratio of 1 : 2, the observed experimental values were multiplied by 2 in the calculations.

In the case of separate determination of glycine, any of the three indicators, viz., Congo red, xylenol orange, or chromazural red S, may be used: but very good color changes are observed when Congo red or chromazural red S is sued as indicator. Congo red has been used as indicator when glycine was titrated in combination with DL-alanine, in the first instance for two color changes; in the second instance, xylenol orange has been used as indicator where both glycine and DL-alanine

TABLE 1

MICRODETERMINATION OF GLYCINE

Glycine 0.04 M (ml)	$BeSO_4$ 0.0102 M (ml)	Amount of glycine ($\times 10^4$ mg/liter)		Error (%)
		Taken	Found	
0.05	0.10	1.50	1.53	
0.10	0.20	3.00	3.06	
0.30	0.60	9.00	9.18	2.0
1.00	2.00	36.02	30.62	
2.00	4.00	60.05	61.25	

TABLE 2

MICRODETERMINATION OF GLYCINE AND DL-ALANINE TOGETHER

Glycine 0.04 *M* (ml)	$BeSO_4$ 0.0102 *M* (ml)	Amount of glycine ($\times 10^4$ mg/liter) Taken	Found	DL-Alanine 0.04 *M* (ml)	$HAuCl_4$ 0.0078 *M* (ml)	Amount of DL-alanine ($\times 10^4$ mg/liter) Taken	Found
0.1	0.2	3.00	3.06	0.5	0.85	23.43	23.30
0.2	0.4	6.00	6.12	0.4	0.70	18.14	18.62
0.4	0.8	12.01	12.25	0.3	0.52	14.06	14.25
0.5	1.0	15.01	15.31	0.2	0.34	9.37	9.32

have been estimated for different color changes, but in this case it is necessary to train in the eye to perceive the correct color change before taking the final sets; in the third instance, chromazural red S has been used as indicator for both and shows prominent color changes; and in the fourth instance chromazural red has been used as indicator for titrating glycine and Congo red for DL-valine in the same solution, after titrating glycine.

Chromazural red S and Congo red solutions were used for titrating glycine and DL-valine, for sharp color changes, respectively, in one solution without separating.

In the titration of a solution mixture containing glycine and L-arginine, chromazural red S solution has been used as indicator for sharp color changes in both cases.

Reproducible results have been obtained in separate estimations of glycine and also in combinations with DL-alanine, DL-valine, and L-arginine. The method is unique in its quickness and reproducibility.

TABLE 3

MICRODETERMINATION OF GLYCINE AND DL-VALINE TOGETHER

Glycine 0.04 *M* (ml)	$BeSO_4$ 0.0102 *M* (ml)	Amount of glycine ($\times 10^4$ mg/liter) Taken	Found	DL-Valine 0.01 *M* (ml)	K_2TeO_3 0.01 *M* (ml)	Amount of DL-valine ($\times 10^4$ mg/liter) Taken	Found
0.1	0.2	3.00	3.06	2.5	0.60	29.29	29.24
0.2	0.4	6.00	6.12	2.0	0.48	23.43	23.39
0.3	0.6	9.00	9.10	1.5	0.36	17.57	17.54
0.5	1.0	15.01	15.31	1.0	0.24	11.71	11.69

TABLE 4

MICRODETERMINATION OF GLYCINE AND L-ARGININE TOGETHER

Glycine 0.04 *M* (ml)	$BeSO_4$ 0.0102 *M* (ml)	Amount of glycine ($\times 10^4$ mg/liter) Taken	Found	L-Arginine 0.02 *M* (ml)	$FeSO_4 \cdot (NH_4)_2SO_4$ 0.0098 *M* (ml)	Amount of L-arginine ($\times 10^4$ mg/liter) Taken	Found
0.1	0.2	3.00	3.06	0.4	0.41	13.93	13.99
0.2	0.4	6.00	6.12	0.3	0.31	10.45	10.58
0.3	0.6	9.00	9.19	0.2	0.20	6.97	6.83
0.4	0.8	12.01	12.25	0.1	0.10	3.48	3.41

SUMMARY

Glycine is quantitatively determined in micro amounts separately and also in combinations with DL-alanine, DL-valine, and L-arginine in one solution without separation. The reaction between beryllium sulfate and glycine results in the formation of a complex in the ratio of 1:2. Maximum error for glycine is 2.0%. In all combinations, glycine is titrated first.

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ICN and BrCN as Volumetric Oxidants

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INTRODUCTION

Halogen cyanides which are predominantly covalent in character behave as the halides of cyanogen rather than the cyanides of halogen in alkaline medium and are thus commonly known as cyanogen halides. It has been concluded only recently that the halogen atom exists as the positive end of the dipole of ICN and BrCN molecules in acidic medium (*2–4*). These compounds have, therefore, been termed as halogen cyanides (XCN) and their solutions used as volumetric oxidants for the estimation of some ions (*2, 3*) and dithiocarbamates (*4*). In the present paper, the work has been extended to the study of some of their basic properties such as the stability of the solutions, redox potential, and polarographic behavior. In addition a few typical electrometric titrations were performed with a view to understanding the mode of their reduction in the absence and presence of a reductant and to assess their potentialities as volumetric oxidants.

MATERIALS AND METHODS

Apparatus

The pH meter "Radiometer" Copenhagen (Denmark) and an electronic potentiometer "Multoscop V" (Laboratorní přistroje n.p., Czechoslovakia) were used for potential measurements. A bright platinum wire electrode and a saturated calomel electrode were used as indicator and reference electrodes, respectively.

Polarographic studies were carried out with Polarograph LP 60 (Czechoslovakia) using a saturated calomel electrode and a platinum wire indicator electrode of approximately 0.5 mm diameter and 4 mm length extending horizontally from a vertical glass tubing rotated con-

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stantly at 200 rpm by means of a synchronous motor designed by Radiometer, Copenhagen (Denmark).

The amperometric titrations were carried out using the rotating platinum indicator electrode at a potential of +0.15 V (vs SCE). A microammeter (Metra Blansko, Czechoslovakia) was used for measuring the current intensity. A pair of identical platinum electrodes was employed in biamperometric and bipotentiometric titrations.

Reagents

Iodine cyanide (*3*), bromine cyanide (*2*), and vanadium (II) chloride (*1*) were prepared and purified as reported in the literature. All the other chemicals used were of analytically pure grade.

The solutions (0.001–0.1 *M*) of the oxidants (*2, 3*) and various reductants (*1*) were prepared and standardized by the usual methods.

General Procedure

The stability of the solutions of iodine cyanide and bromine cyanide in different media was studied by following the change in their potential and determining the titer value with thiosulfate (*2, 3*) from time to time.

Redox potential of iodine cyanide and bromine cyanide was determined by titrating them potentiometrically with potassium propyl xanthate at pH 3 maintained by appropriate dilution with water. The dependence of redox potential of iodine cyanide on acid concentration was studied by titrating with tin (II) chloride (Fig. 1).

The polarographic studies were carried out in 1*M* hydrochloric acid using 0.2 ml of 0.5% gelatin as a maxima suppressor. The oxygen-free pure nitrogen was used for deaerating the solutions.

For direct and reverse amperometric titrations of potassium propyl xanthate, vanadium (II) and tin (II) with iodine cyanide and bromine cyanide, 50 ml of titer solution (0.001–0.01 *M*) was taken in the cell and a potential of +0.15 V (vs SCE) was applied to the rotating platinum electrode. The deaeration of the solutions with nitrogen was

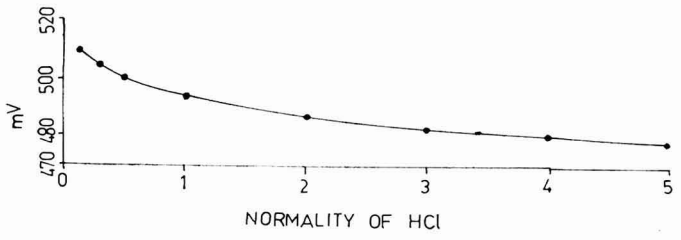

FIG. 1. Dependence of redox potential of ICN on normality of hydrochloric acid.

thus not needed except in case of vanadium (II). Potassium iodide was added to the titer solution in the titrations of tin (II) and vanadium (II) with bromine cyanide. An appropriate concentration of potassium chloride was added in case the amount of the already present hydrochloric acid was not enough. The observed values of current intensity after correction for dilution effect, whenever necessary, were plotted as a function of volume of the titrant and the end points located graphically (Figs. 2 and 3). The biamperometric and bipotentiometric titrations were carried out under the conditions mentioned above at a potential of +0.15 V and a constant current of 1μ A respectively, using a pair of identical platinum electrodes (Fig. 4). The results have been summarized in Table 1. All the investigations were carried out at 20°C.

RESULTS AND DISCUSSION

Stability of Solutions

Iodine cyanide and bromine cyanide are white solids and dissolve in dilute mineral acids or donor nonaqueous solvents giving colorless solutions. The function of the acid is only to liberate the halogen cation and there is no other reaction resulting in the formation of corresponding salts. The solutions in 0.1 *M* hydrochloric acid, 0.1 *M* sulfuric acid, 0.05 *M* nitric acid, glacial acetic acid, ethanol, and acetonitrile are stable and do not undergo decomposition or change in reactivity with time. The titer does not change more than 1% in any of these media even after 2 months, indicating their suitability for redox estimations.

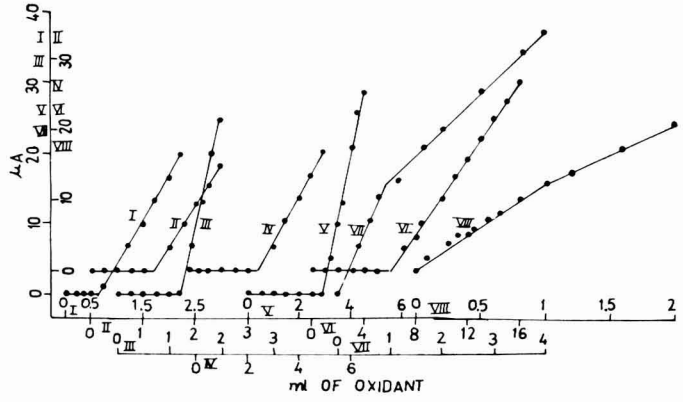

FIG. 2. Direct amperometric titrations of 0.0025 *M* potassium propyl xanthate (I, II), 0.005 *M* V^{2+} (III,IV), 0.000625 *M* Sn^{2+} (V,VI), 0.002 *M* I^- (VII,VIII), with 0.1 *M* ICN (I,III,V,VII) and 0.05 *M* BrCN (II,IV,VI,VIII). Iodide was added to titer solution in titrations of Sn^{2+} and V^{2+} with BrCN.

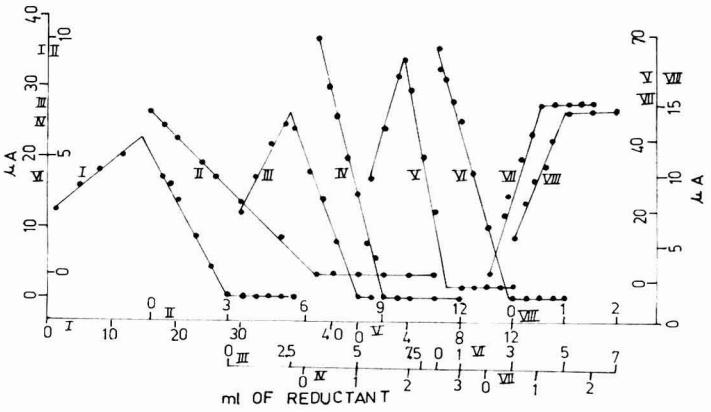

FIG. 3. Reverse amperometric titrations of 0.0308 *M* potassium propyl xanthate with 0.0086 *M* ICN (I) and 0.002 *M* BrCN (II); 0.05 *M* V^{2+} with 0.0025 *M* ICN (III) and 0.00075 *M* BrCN (IV); 0.167 *M* Sn^{2+} with 0.01 *M* ICN (V) and 0.02 *M* BrCN (VI); 0.2 *M* I^- with 0.004 *M* ICN (VII) and 0.002 *M* BrCN (VIII). Iodide was added to titer solution in titrations of V^{2+} and Sn^{2+} with BrCN.

However, it is recommended that their strengths be checked once a week.

Redox Potential

Unlike iodine cyanide, bromine cyanide has been found to act as a volumetric oxidant only in the presence of iodide in acidic aqueous

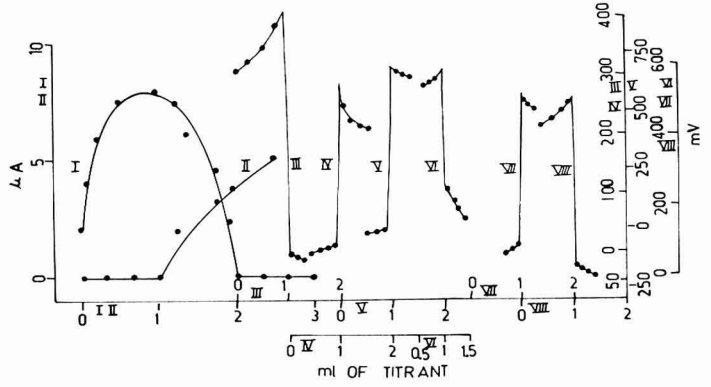

FIG. 4. Biamperometric titrations: 0.004 *M* ICN with 0.1 *M* Sn^{2+} (I), 0.002 *M* Sn^{2+} with 0.1 *M* ICN (II); Bipotentiometric titrations: 0.002 *M* Sn^{2+} with 0.1 *M* ICN (III), 0.02 *M* ICN with 0.1 *M* Sn^{2+} (IV) and 0.2 *M* xanthate (V), 0.004 *M* xanthate with 0.1 *M* ICN (VI) and 0.1 *M* BrCN (VIII), and lastly, 0.002 *M* BrCN with 0.2 *M* xanthate (VII).

TABLE 1

DETERMINATION OF REDUCTANTS WITH IODINE CYANIDE AND BROMINE CYANIDE

Reductant [a]	Direct titration with ICN Taken	Direct titration with ICN Found	Direct titration with BrCN Taken	Direct titration with BrCN Found	Reverse titration ICN Required	Reverse titration ICN Found	Reverse titration BrCN Required	Reverse titration BrCN Found	Method [b]
C_3H_7OCSK (S)	8.70	8.68	17.40	17.42	13.05	13.04	13.05	13.08	b
	4.35	4.36	13.05	13.02	8.70	8.71	10.78	10.83	c
	3.42	3.45	5.13	5.15	3.76	3.72	6.84	6.90	d
	3.42	3.42	—	—	4.35	4.30	—	—	e
	3.42	3.40	13.05	13.00	3.42	3.43	8.70	8.70	f
VCl_2	3.82	3.80	3.82	3.85	5.73	5.70	4.78	4.75	b
	5.10	5.14	3.82	3.80	3.82	3.85	7.65	7.69	c
	3.82	3.83	5.10	5.14	3.40	3.42	3.82	3.86	d
$SnCl_2$	7.40	7.40	14.80	14.82	3.70	3.70	5.90	5.92	c
	1.77	1.75	17.70	17.70	1.18	1.20	5.90	5.94	e
	3.54	3.52	7.40	7.42	1.77	1.75	3.70	3.70	f
KI	2.54	2.53	5.08	5.09	2.54	2.54	5.08	5.10	c

[a] All the amounts are in milligrams.

[b] b—visual; c—amperometric; d—potentiometric; e—biamperometric; and f—bipotentiometric.

medium (*2, 4*). In the present investigations, however, potassium propyl xanthate is directly titrated electrometrically with bromine cyanide without adding iodide, indicating thereby that the reaction does not essentially proceed through iodine formation but the bromine cation reacts as such and is directly reduced to bromide. The titration is reversible, quantitative, and as little as 1.2 mg of potassium propyl xanthate in 50 ml solution can be accurately estimated. A white suspension of dixanthogen is formed subsequent upon the reaction of xanthate with iodine cyanide or bromine cyanide in 2:1 molar ratio.

$$2\ C_3H_7OCSK + X^+ \rightarrow (C_3H_7OCS)_2 + 2\ K^+ + X^-.$$

The titration of bromine cyanide with potassium propyl xanthate is of importance as it has rendered possible not only the determination of the redox potential of the system Br^+/Br^-, but also its comparison with that of the corresponding I^+/I^- redox system. The values obtained are +0.51 and +0.40 V, respectively. Thus it is evident that the halogen atom is

the positive end of the dipole of these molecules with decreasing moment in the order ICN>BrCN.

The dependence of redox potential of bromine cyanide on acid normality could not be studied because its reaction with potassium propyl xanthate is not quantitative at pH values other than 3. Furthermore, the xanthate is decomposed by higher acid concentrations. However, it has been possible in case of iodine cyanide by titrating it with tin (II) chloride and the results are presented in Fig. 1.

It is interesting that unlike in the corresponding amperometric titrations (Fig. 3), there is usually only one abrupt fall in potential in the potentiometric titrations of iodine cyanide with various reductants and it represents the completion of the reaction. If the solutions are fairly concentrated and the acid concentration is high, only then the two different potential falls are clearly noted. One such titration of iodine cyanide with tin (II) chloride in 8*N* hydrochloric acid was utilized in the present investigations to determine the redox potentials for the systems I^+/I_2 and I_2/I^-, and the values found are +0.56 and +0.42 V, respectively. The determination of redox potentials for the systems Br^+/Br_2 and Br_2/Br^- could not be possible because there is no intermediate formation of bromine in the titration of bromine cyanide with potassium propyl xanthate. It is in keeping with the fact that the indicators, such as methyl orange, indigo carmine, crystal violet, and malachite green, which mark the end point due to their destruction by the bromine formed by first drop in excess of the titrant, could not be used in the visual titration. In the absence of a suitable indicator, iodide has to be added for determining the end point using starch as an indicator.

Polarographic Behavior

Iodine cyanide and bromine cyanide depolarize the rotating platinum electrode in dilute hydrochloric acid medium giving cathodic waves which are concentration dependent. The dependence of

$$\log i/({}^{i}d^{-i})$$

on the potential is a straight line and corresponds to reversible and two-electron reduction process in either case. The half-wave potentials of iodine cyanide and bromine cyanide in 1 *M* hydrochloric acid are +0.49 and +0.37 V, respectively. The values are too positive to use a dropping mercury electrode. The half-wave potential is independent of the strength of the supporting electrolyte indicating the absence of formation of complexes of the type $IC1_2^-$. Popov and Geske (*5*) have obtained three waves during voltammetric studies of iodine (I), chloride, and bromide in acetonitrile for the overall reduction of these inter-

halogens to iodide. However, specific electrode reactions could not be assigned to the individual waves. The limiting current plateau potential in the polarograms produced due to the cathodic reduction of the halogen cation in the present studies is +0.3–+0.1 V. This potential range and the proportionality of the diffusion current with concentration of halogen cyanides provide favorable conditions for performing the amperometric titrations using a rotating platinum indicator electrode.

Amperometric Titrations

In the direct amperometric titrations of vanadium (II), tin (II), and potassium propyl xanthate with iodine cyanide and bromine cyanide at +0.15 V (vs SCE), the current is practically zero in the beginning and remains constant until the end point is reached when it starts increasing gradually and slowly due to the cathodic reduction of the unconsumed halogen cation resulting in a straight line plot (Fig. 2). Since iodide is added to the solution of the metal ions before titrating with bromine cyanide, the current in these cases is due to the cathodic reduction of iodine formed. The titrations of tin (II) and vanadium (II) are possible over a wide range of acid concentration but only in the presence of the iodide. It may be mentioned that the electrometric titrations of vanadium (II) cover the wider range of its concentration whereas it can be estimated visually with iodine cyanide using starch indicator only in very dilute solutions. In concentrated solutions, the end point cannot be accurately noticed due to the violet color of vanadium (II) itself. In such cases, it is possible to add a known excess of iodine cyanide to vanadium (II) and back-titrate the excess with tin (II) when the end point is marked by the disappearance of the violet-blue color. During the electrometric titration, the violet color of vanadium (II) starts fading and becomes yellowish through blue at the equivalence point. The color becomes reddish yellow when excess of iodine cyanide is added. Iron (II) does not affect the accuracy and the course of the reaction and as little as 0.1 mg of vanadium (II) can be electrometrically estimated with iodine cyanide 40 times in excess of iron (II). Vanadium (II), tin (II), and the xanthate react with these oxidants in the molar ratio 2:1, 1:1, and 2:1, respectively. These stoichiometries and the use of starch as an indicator in the visual titrations suggest the reduction of the halogen cation to the coresponding halide.

In the reverse amperometric titrations of bromine cyanide with these reductants, the cathodic current due to the reduction of bromine cyanide or the evolved iodine, as the case may be, decreases linearly as the titration proceeds till the end point is reached after which it remains constant indicating the completion of the reaction (Fig. 3).

The shape of the reverse amperometric titration curves of iodine cyanide with these reductants is, however, neither the one commonly obtained nor similar to that in the corresponding titations of bromine cyanide. The initial current, which is due to the reduction of iodine cation, starts increasing instead of decreasing upon adding reductant solution until half of the theoretically required amount has been added (Fig. 3). In these titrations, iodine cyanide is reduced to iodide, which liberates iodine subsequent upon its reaction with unreacted oxidant imparting its color to the solution. The observed increase in current is because of the formation of iodine which more than compensates the decrease in current intensity due to corresponding consumption of iodine cyanide. However, the plot is not a straight line in more concentrated solutions, but the half equivalence point is accurately located by extrapolation even in such cases. When iodine cyanide has been totally converted into iodine, the cathodic current decreases gradually and linearly assumes zero value at the equivalence point. With further additions of the reductant, the current remains constant.

The above explanation of the increase in current in the reverse titrations of iodine cyanide has been verified by titrating iodine cyanide with iodide in hydrochloric acid medium where they react in 1:1 molar ratio forming iodine (the curve obtained is depicted in Fig. 3). However, bromine cyanide could neither be titrated with bromide under similar conditions electrometrically nor is there any color of bromine in the solution, indicating that they do not react in a way iodine cyanide and iodide do. This justifies the different shape of the reverse amperometric titration curves of bromine cyanide. The amperometric titration curve of bromine cyanide with iodide is similar to that of iodine cyanide. In the direct titrations of iodide with halogen cyanides (Fig. 2), the current, which is zero to start with, increases upon gradual additions of the oxidant due to the cathodic reduction of iodine formed. After the equivalence point is reached, the increase in the current intensity is relatively less and is due to the cathodic reduction of halogen cyanide. The reaction is selective and as little as 2 mg of iodide can be estimated even 100 times in excess of chloride and bromide. Acetate, bicarbonate, sulfate, nitrate, and iron (II) also do not interfere, but the oxalate does.

Biamperometric and Bipotentiometric Titrations

The curves of direct and reverse biamperometric and bipotentiometric titrations of tin (II) chloride and potassium propyl xanthate with these oxidants (Fig. 4) are, however, the expected ones for the titrations of

an irreversible redox system with the reversible one, and vice versa. The titrations are fast, quantitative, and simple.

SUMMARY

Redox potential and polarographic behavior of iodine cyanide and bromine cyanide have been studied and electrometric titrations with potassium propyl xanthate, vanadium (II) chloride, and tin (II) chloride performed to investigate their potentialities as volumetric oxidants and their mode of reduction. Unlike iodine cyanide, bromine cyanide oxidizes the metal ions only in the presence of iodide, but electrometric titration with xanthate is possible even otherwise. Whereas there is no reaction between bromine cyanide and bromide, iodine cyanide can be titrated with iodide. Thus, in the amperometric titrations of iodine cyanide with these reductants at +0.15 V (vs SCE), the current starts increasing instead of decreasing until half the theoretically required amount has been added and then decreases gradually, assuming zero value at the equivalence point.

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Microchemical Tests for Hydantoins and Barbiturate–Hydantoin Mixtures

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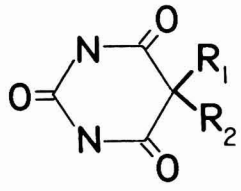
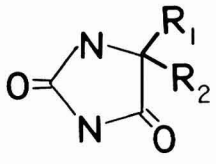
INTRODUCTION

As a result of the high incidence of drug-abuse situations, there is currently a great emphasis placed upon drug identification methods in forensic laboratories. The techniques employed generally involve a combination of color tests, microcrystalline tests, chromatography, and instrumental analysis. For the identification of barbituric acid derivatives, the former two methods of identification are relied upon heavily due to the speed in which the tests may be conducted and the simplicity of procedure and interpretation. In many forensic laboratories, a combination of color tests for purposes of screening followed by one or more microcrystalline tests is considered to represent an adequate procedure for an unequivocal identification of a suspected barbiturate (*1–5*).

The interpretation of commonly used color and microcrystalline tests for barbiturates is, however, complicated by the existence of the hydantoins, a family of materials having structural similarities to the barbiturates. The barbiturates have a six-membered ring while the hydantoins have a five-membered ring lacking a carbonyl group.



BARBITURATE



HYDANTOIN

Certain substituted hydantoins have an anticonvulsant activity and may be prescribed alone or compounded with barbiturates for the control of epilepsy. This may result in interferences or modifications of the microchemical tests normally employed for the identification of the substituted barbituric acid derivatives. The ability of diphenylhydantoin to

give color tests similar to barbiturates has previously been noted (*6*).

The purpose of this study was to establish the nature of interferences and modifications of color and microcrystalline tests for barbiturates when these materials are encountered in the presence of hydantoins. The results may serve to alert the analyst to certain considerations relative to the microchemical identification of hydantoins and hydantoin–barbiturate mixtures. The primary thrust of the study was not the microchemical identification of hydantoins, but rather the influence of hydantoins on microchemical tests for barbiturates.

EXPERIMENTAL

Materials

The color and microcrystalline tests described in this study were performed on pure samples of the following hydantoins:

Hydantoin
Diphenylhydantoin
Dimethylhydantoin
Methylethylhydantoin
Methylisobutylhydantoin
Methylene-*bis*-dimethylhydantoin
Monomethylol–dimethylhydantoin
Dimethylhydantoin–formaldehyde resin

In addition, microcrystalline tests were performed on 1:1 (v/v) mixtures of each hydantoin and phenobarbital, amobarbital, secobarbital, and pentobarbital.

Color Tests

Dille–Koppanyi test (5). Reagent (a) consists of 0.1 g of cobaltous acetate dissolved in 100 ml of absolute methanol to which is added 0.2 ml of glacial acetic acid. Reagent (b) is a 5% (v/v) solution of isopropylamine in absolute methanol. To conduct the test, a few milligrams of sample are placed in a spot plate, and 2 drops of reagent (a) are added. One drop of reagent (b) is then added and the color noted. A stable blue–violet color indicates the presence of barbituric acid or one of its derivatives.

Zwikker test (5). Reagent (a) consists of a 0.5% aqueous solution of cupric sulfate. Reagent (b) is a 5% (v/v) solution of chloroform in pyridine. The test is performed by placing a few milligrams of sample in a small test tube, adding 0.5 ml of the cupric sulfate solution, and mixing gently. An equal volume of the chloroform solution is then added and the tube shaken briskly. The color of the lower (chloroform) layer is noted. If the sample contains a barbituric acid or its salt, the lower

TABLE 1

REACTION OF BARBITURATES AND HYDANTOINS WITH THE DILLE–KOPPANYI TEST

	Color with Dille–Koppanyi test
Phenobarbital	Blue–violet
Amobarbital	Blue–violet
Secobarbital	Blue–violet
Pentobarbital	Blue–violet
Hydantoin	Blue–violet
Diphenylhydantoin	Blue–violet
Dimethylhydantoin	Blue–violet
Methylethylhydantoin	Blue–violet
Methylisobutylhydantoin	Blue–violet
Methylene–*bis*–dimethylhydantoin	Blue–violet
Monomethylol–dimethylhydantoin	Blue–violet
Dimethylhydantoin–formaldehyde resin	Blue–violet

layer will be blue–violet. If the sample contains a thiobarbituric acid or its salt, the lower layer will be bright green. A drop of glacial acetic acid is then added to the mixture. If the sample contains a barbituric acid or its salt, the blue–violet color will fade to a light blue. If the sample contains a thiobarbituric acid or its salt, the bright-green color will fade to a faint green.

Microcrystalline Tests

Waganaar's Test (1). The reagent consists of a 5% cupric sulfate solution with sufficient ethylenediamine added to give a decidedly purple

TABLE 2

REACTION OF BARBITURATES AND HYDANTOINS WITH THE ZWIKKER TEST

	Color with $CHCl_3$—Pyridine	Color with acetic acid
Phenobarbital	Blue–violet	Light blue
Amobarbital	Blue–violet	Light blue
Secobarbital	Blue–violet	Light blue
Pentobarbital	Blue–violet	Light blue
Hydantoin	—	—
Diphenylhydantoin	Blue	Light blue
Dimethylhydantoin	—	—
Methylethylhydantoin	—	—
Methylisobutylhydantoin	Blue	Light blue
Methylene–*bis*–dimethylhydantoin	Blue	Light blue
Monomethylol–dimethylhydantoin	—	—
Dimethylhydantoin–formaldehyde resin	Blue	Light blue

TABLE 3

REACTIONS OF HYDANTOINS AND MICROCRYSTALLINE REAGENTS

	Waganaar's	Davis silver	H_2SO_4–H_2O	KOH–H_3PO_4	I–KI–$2H_3PO_4$	$NiCl_2$
Hydantoin	Coffin-shaped prisms	Irregular scattered needles	No crystals	No crystals	No crystals	No crystals
Diphenylhydantoin	Needles and rods, isolated and in aggregates	Needles and rods, but small rectangular plates if first dissolved in 2% KOH.	Irregular rods, various sizes	Long irregular rods, isolated and in aggregates	Branched aggregates and tufts of needles	No crystals
Dimethylhydantoin	No crystals	No crystals	No crystals	No crystals	No crystals	No crystals
Methylethylhydantoin	No crystals	No crystals	No crystals	No crystals	No crystals	No crystals
Methylisobutylhydantoin	No crystals	No crystals	No crystals	Rectangular plates, serrated edges, isolated and in aggregates	Rosettes of rectangular rods and plates	No crystals
Methylene–*bis*-dimethylhydantoin	Small rectangular plates	Small needles and rectangular rods	Rods, isolated and in aggregates	"Snow-flake"-shaped crystals, with light diagonals	Square plates with light diagonals, and some "snow-flake" shaped crystals	Isolated rods and blades
Monomethylol–dimethylhydantoin	No crystals	No crystals	No crystals	No crystals	No crystals	No crystals
Dimethylhydantoin–formaldehyde resin	Oily droplets, but no crystalline precipitate	No crystals	No crystals	No crystals	Immediate red amorphous precipitate, grainy	No crystals

TABLE 4

MIXED HYDANTOINS AND BARBITURATES (1:1) WITH WAGANAAR'S REAGENT

	Phenobarbital	Amobarbital	Secobarbital	Pentobarbital
Hydantoin	Typical phenobarbital–Waganaar's crystals	Typical amobarbital–Waganaar's crystals	Small isolated needles in addition to rosettes of regular blades, typical of secobarbital–Waganaar's crystals, but smaller	Typical pentobarbital–Waganaar's crystals
Diphenylhydantoin	Grainy amorphous ppt. in addition to a few crystals typical of phenobarbital–Waganaar's	Grainy amorphous ppt. in addition to a few crystals typical of amobarbital–Waganaar's	Grainy amorphous ppt. in addition to rosettes of regular blades typical of secobarbital–Waganaar's	Sheaves of needles in addition to a few crystals typical of pentobarbital–Waganaar's
Dimethylhydantoin	Typical phenobarbital–Waganaar's crystals	Typical amobarbital–Waganaar's crystals	Typical secobarbital–Waganaar's crystals	Typical pentobarbital–Waganaar's crystals
Methylethylhydantoin	Typical phenobarbital–Waganaar's crystals	Typical amobarbital–Waganaar's crystals	Small needles, developing into rods, in addition to typical secobarbital–Waganaar's crystals	Coarse rods, forming barrel-shaped aggregates, otherwise typical of pentobarbital–Waganaar's, but more poorly formed

Methylisobutylhydantoin	Typical phenobarbital–Waganaar's crystals	Coarse needles and rods, isolated and in aggregates, otherwise typical of amobarbital–Waganaar's, but with serrated edges	Fine needles in addition to crystals typical of secobarbital–Waganaar's	Typical pentobaribital–Waganaar's crystals
Methylene-*bis*-dimethylhydantoin	Hexagonal plates developing into tablets in addition to a few crystals typical of phenobarbital–Waganaar's	Small rectangular plates in addition to typical amobarbital–Waganaar's crystals	Small rectangular plates in addition to typical secobarbital–Waganaar's crystals	Small rectangular plates in addition to typical pentobarbital–Waganaar's crystals
Monomethylol–dimethylhydantoin	Typical phenobarbital–Waganaar's crystals	Typical amobarbital–Waganaar's crystals	Fine needles, isolated or in aggregrates, in addition to typical secobarbital–Waganaar's crystals	Typical pentobarbital–Waganaar's crystals
Dimethylhydantoin–formaldehyde resin	Typical phenobarbital–Waganaar's crystals	Typical amobarbital–Waganaar's crystals	Fine needles in addition to rosettes of regular blades, typical of secobarbital–Waganaar's, but smaller	Quick-forming pointed rods, isolated or in aggregates

color to the solution. To conduct the test, a drop of the reagent is added to a small amount of the sample on a microscope slide, mixed gently with a microspatula, and the crystal formation observed at a magnification of 100X.

Davis silver test (1). The reagent is a 10% aqueous silver nitrate solution to which is added 15% ethylenediamine by volume. The test is performed in the same manner as described above in the Waganaar's test. The use of a polarizing microscope is recommended for the examination of the crystals formed with this reagent.

Concentrated sulfuric acid and water (1). A small amount of the sample to be tested is dissolved in a drop of concentrated sulfuric acid, and a drop of water is added to precipitate the crystals, which are observed at a magnification of 100×.

I–KI–2H_3PO_4 test (2). The reagent is prepared by adding 1.5 ml of a 1:16 (w/w) solution of I:KI to 20 ml of water, then adding 10 ml of syrupy phosphoric acid. The reagent is cooled and decanted before using. The test is performed by dissolving a small amount of the sample to be tested in a drop of 2% KOH, and then adding a drop of the reagent to precipitate the crystals, which are observed at a magnification of 100×.

$NiCl_2$ test (2). The reagent is prepared by dissolving 0.2 g of $NiCl_2 \cdot H_2O$ and 0.5 ml triethanolamine in 19.5 ml of distilled water. The test is performed in the same manner as described above in the Waganaar's test.

RESULTS AND DISCUSSION

As shown in Tables 1 and 2, all of the hydantoins tested gave a positive result with the Dille–Koppanyi test, and four of the hydantoins gave a reaction with the Zwikker test. In both tests, the shade of color observed with barbiturates alone was discernibly different from that observed with the hydantoins. The difference is subtle, however, and although readily apparent when the barbiturates and hydantoins are tested at the same time, may possibly be confused when only one family is tested. The prevailing reactivity of barbiturate–hydantoin mixtures was that of the barbiturates. It was noted that the colors formed with the Dille–Koppanyi reagent and two hydantoins, unsubstituted hydantoin and monomethylol–dimethylhydantoin, were transient, while the remaining hydantoins and barbiturates gave colors which were persistent.

The reactivity of the hydantoins and commonly used microcrystalline reagents is indicated in Table 3. It is evident that certain hydantoins do indeed form crystals with these reagents. The crystals which are formed from the pure hydantoins and the various reagents are, however,

TABLE 5

MIXED HYDANTOINS AND BARBITURATES (1:1) WITH DAVIS SILVER REAGENT

	Phenobarbital	Amobarbital	Secobarbital	Pentobarbital
Hydantoin	Irregular tufts of needles	Irregular tufts of needles	Irregular tufts of needles	Irregular tufts of needles
Diphenylhydantoin	Typical phenobarbital–Davis Ag crystals, very slow growing, in a grainy amorphous precipitate [a]	Minute rhombs typical of amobarbital–Davis Ag, but more poorly formed	Grainy amorphous ppt.	No crystal formation
Dimethylhydantoin	Typical phenobarbital–Davis Ag crystals	Typical amobarbital–Davis Ag crystals	Barrel-shaped plates	Typical pentobarbital–Davis Ag crystals
Methylethylhydantoin	Rhombs and blades typical of phenobarbital–Davis Ag, but more poorly formed [a]	Typical amobarbital–Davis Ag crystals, but slowly forming and smaller	Small elliptical plates with squared ends	No crystal formation
Methylisobutylhydantoin	No crystal formation	Typical amobarbital–Davis Ag crystals, but slowly forming and smaller	Small elliptical plates with squared ends, isolated and in aggregates	No crystal formation
Methylene-*bis*-dimethylhydantoin	Typical phenobarbital–Davis Ag crystals	Small square and rectangular plates	Amorphous grainy precipitate slowly forming small elliptical plates	Small square and rectangular plates
Monomethylol–dimethylhydantoin	Typical phenobarbital–Davis Ag crystals, but more poorly formed [a]	Typical amobarbital–Davis Ag crystals	Barrel-shaped plates, in addition to other crystals typical of secobarbital–Davis Ag	Typical pentobarbital–Davis Ag crystals
Dimethylhydantoin–formaldehyde resin	Typical phenobarbital–Davis Ag crystals, but more poorly formed	Typical amobarbital–Davis Ag crystals	Ellipse-shaped plates with squared ends	Typical pentobarbital–Davis Ag crystals

[a] Crystal formation requires scratching the slide and slow growth under a coverslip; technique is important in this test, and experimentation with known materials is essential.

TABLE 6

MIXED HYDANTOINS AND BARBITURATES (1:1) WITH CONC. H_2SO_4–H_2O

	Phenobarbital	Amobarbital	Secobarbital	Pentobarbital
Hydantoin	Typical phenobarbital–H_2SO_4/H_2O crystals	Typical amobarbital–H_2SO_4/H_2O crystals	Typical secobarbital–H_2SO_4/H_2O crystals	Typical pentobarbital–H_2SO_4/H_2O crystals
Diphenylhydantoin	Crystals typical of (a) phenobarbital–H_2SO_4/H_2O and (b) diphenylhydantoin–H_2SO^4/H_2O; no hybrid crystals noted	Coarse dendrites	Irregular tufts of needles	Crystals typical of (a) pentobarbital–H_2SO_4/H_2O and (b) diphenylhydantoin–H_2SO_4/H_2O; no hybrid crystals noted
Dimethylhydantoin	Typical phenobarbital–H_2SO_4/H_2O crystals	Typical amobarbital–H_2SO_4/H_2O crystals	Typical secobarbital–H_2SO_4/H_2O crystals	Typical pentobarbital–H_2SO_4/H_2O crystals
Methylethylhydantoin	Typical phenobarbital–H_2SO_4/H_2O crystals	Typical amobarbital–H_2SO_4/H_2O crystals	Typical secobarbital–H_2SO_4/H_2O crystals	Typical pentobarbital–H_2SO_4/H_2O crystals
Methylisobutylhydantoin	Small fine burrs	Typical amobarbital–H_2SO_4/H_2O crystals	Typical secobarbital–H_2SO_4/H_2O crystals	Typical pentobarbital–H_2SO_4/H_2O crystals
Methylene-*bis*-dimethylhydantoin	Irregular rosettes of needles and blades	Typical amobarbital–H_2SO_4/H_2O crystals	Typical secobarbital–H_2SO_4/H_2O crystals	Typical pentobarbital–H_2SO_4/H_2O crystals
Monomethylol-dimethylhydantoin	Typical phenobarbital–H_2SO_4/H_2O crystals	Typical amobarbital–H_2SO_4/H_2O crystals	Typical secobarbital–H_2SO_4/H_2O crystals	Typical pentobarbital–H_2SO_4/H_2O crystals
Dimethylhydantoin-formaldehyde resin	Small fine burrs	Typical amobarbital–H_2SO_4/H_2O crystals	Small rods and rectangular plates, isolated and in aggregrates	Small fine burrs, in addition to the elliptical plates typical of pentobarbital–H_2SO_4/H_2O

TABLE 7

MIXED HYDANTOINS AND BARBITURATES (1:1) WITH $KOH-H_3PO_4$

	Phenobarbital	Amobarbital	Secobarbital	Pentobarbital
Hydantoin	Crystals typical of phenobarbital–KOH/H_3PO_4	Crystals typical of amobarbital–KOH/H_3PO_4	Crystals typical of secobarbital–KOH/H_3PO_4	Fine needles, isolated and in aggregrates
Diphenylhydantoin	Crystals typical of (a) phenobarbital–KOH/H_3PO_4 and (b) diphenylhydantoin–KOH/H_3PO_4; no hybrid crystals noted	Coarse dendrites	Crystals typical of secobarbital–KOH/H_3PO_4 in addition to grainy amorphous precipitate	Crystals typical of pentobarbital–KOH/H_3PO_4 in addition to grainy amorphous precipitate
Dimethylhydantoin	Crystals typical of phenobarbital–KOH/H_3PO_4	Crystals typical of amobarbital–KOH/H_3PO_4	Crystals typical of secobarbital–KOH/H_3PO_4	Crystals typical of pentobarbital–KOH/H_3PO_4
Methylethylhydantoin	Crystals typical of phenobarbital–KOH/H_3PO_4	Crystals typical of amobarbital–KOH/H_3PO_4	Crystals typical of secobarbital–KOH/H_3PO_4	Crystals typical of pentobarbital–KOH/H_3PO_4
Methylisobutylhydantoin	Crystals typical of phenobarbital–KOH/H_3PO_4	Crystals typical of amobarbital–KOH/H_3PO_4	Flat rods in aggregrates	Crystals typical of pentobarbital–KOH/H_3PO_4, although smaller in size
Methylene-*bis*-dimethylhydantoin	Isolated rods in addition to crystals typical of phenobarbital–KOH/H_3PO_4	Crystals typical of amobarbital–KOH/H_3PO_4	Serrated blades, generally twinned	Serrated irregular blades, in addition to crystals typical of pentobarbital–KOH/H_3PO_4
Monomethylol–dimethylhydantoin	Crystals typical of phenobarbital–KOH/H_3PO_4	Crystals typical of amobarbital–KOH/H_3PO_4	Crystals typical of secobarbital–KOH/H_3PO_4	Crystals typical of pentobarbital–KOH/H_3PO_4
Dimethylhydantoin–formaldehyde resin	Crystals typical of phenobarbital–KOH/H_3PO_4, forming slowly from an amorphous oily ppt.	Crystals typical of amobarbital–KOH/H_3PO_4	Crystals typical of secobarbital–KOH/H_3PO_4	Crystals typical of pentobarbital–KOH/H_3PO_4

sufficiently characteristic to be distinguished from the barbiturates tested.

The descriptions of the crystals in Tables 3–7 conform to the nomenclature of Farmilo and Genest (*7*).

The results of testing mixtures of the hydantoins and barbiturates with commonly used microcrystalline reagents were much more profound. These results are tabulated in Tables 4–7. Many of the mixtures tested showed significant modification in crystal formation relative to the barbiturate component as tested alone. The implications of this to the identification of the barbiturate are patent. A mixture of barbiturate and hydantoin in which the amount of hydantoin is equal to or greater than the amount of barbiturate may in certain instances prevent the analyst from being able to readily identify the barbiturate component. Hybrid crystals, however, do appear to be characteristic of a particular mix.

A number of other modifications were observed during the microcrystalline testing of barbiturate–hydantoin mixtures. These modifications were not tabulated because they were subtle changes which are unlikely to prevent the analyst from identifying a particular barbiturate. These modifications of crystal form appear to be a function of the ratio of hydantoin to barbiturate and the competition of the two for the reagent.

SUMMARY

Substituted hydantoins, a number of which possess anticonvulsant activity and are prescribed alone or compounded with barbiturates, were observed to enter into a number of reactions commonly utilized to identify barbituric acid derivatives. The reactivity of the hydantoins with the Dille–Koppanyi and the Zwikker tests is sufficiently similar to that of the barbiturates to present the possibility of confusion. Mixtures of the hydantoins and barbiturates may complicate the type of microcrystalline testing utilized for identification of particular barbiturates. In certain instances, microcrystalline tests fail to either yield the crystal form which would normally be expected of the barbiturate component alone, or significantly alter the expected crystal form to such an extent that identification of the barbiturate component is not possible. Hybrid crystals formed from a mixture of barbiturate and hydantoin appear to be characteristic of the particular ratio of the respective barbiturate and hydantoin. Identifications of barbiturate–hydantoin mixtures may be effected by means of a judicious selection of appropriate microcrystalline tests.

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MICROCHEMICAL JOURNAL 17, 540–545 (1972)

Benzoylacetanilide as a Spectrophotometric Reagent for Iron(III)

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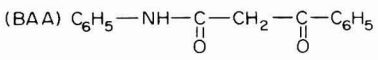
INTRODUCTION

During the last few years, studies on the complex compounds of benzoylacetanilide:



and other β-diketones with metal ions have received major attention (*1–4*). The ability of these compounds to form stable metal chelates is due to the presence of keto–enol tautomerism:

which results in the formation of six-membered chelate ring. Benzoylacetanilide (BAA) reacts with Fe(III) to form a violet colored complex soluble in 60% water–ethanol mixture. The present paper deals with the spectrophotometric studies of $Fe(BAA)_3$ complex regarding composition, stability constant, free energy of formation. Spectrophotometric estimation of nanograms of Fe(III) and the effect of diverse ions in the estimation were also investigated.

EXPERIMENTAL METHODS

Benzoylacetanilide was obtained from Aldrich Chemicals Company, Wisconsin. A standard solution of iron was prepared by dissolving $FeCl_3$ (BDH) in dilute HCl and the iron content was estimated. All other chemicals used were of BDH, A.R. quality. The pH of the solution was adjusted by adding dilute solutions of Na_2CO_3 and glacial acetic acid.

Absorbance measurement was made on 139-Uv-Vis Hitachi-Perkin-

Elmer spectrophotometer using 1-cm effective light-path. The pH measurement was made using Beckman pH meter (Model H2). All the experiments were performed in 60% ethanolic medium. Stock solutions of diverse ions were prepared from the nitrate, chloride, or sulfate of the corresponding metals.

Spectrophotometric Investigations

The nature of the complex was studied by the method suggested by Vosburgh and Cooper (*5*) and found that only one complex is formed with λ_{max} at 540 nm under the conditions of study (Fig. 1). The pH range for the stable existence of the complex was found to be 2 to 4. For subsequent studies, pH 3.0 ± 0.1 was selected since the complex showed maximum extinction at this pH. The empirical formula of the complex in solution was established by three independent methods as continuous variation (*6*), slope-ratio (*7*) and mole-ratio (*8*) and found that a stable complex is formed between 1 mole of Iron and 3 moles of BAA. The results have been given in Fig. 2, 3, and 4, respectively. In Fig. 4, the curve is found to be continuous without a sharp break since the complex is very weak (dissociated).

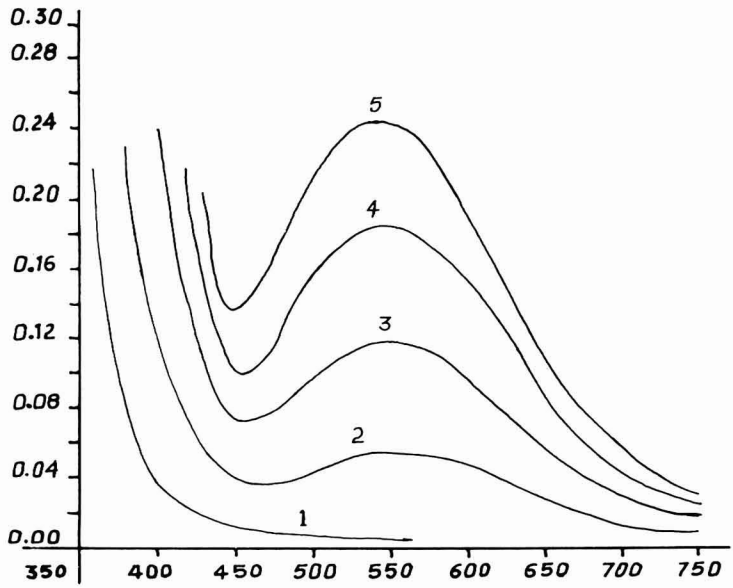


FIG. 1. Absorption spectra of the mixtures of ferric chloride and benzoylacetanilide (BAA): (1) $FeCl_3$, 4 × 10^{-4} *M*. (2) $FeCl_3$, 4 × 10^{-4} *M*; BAA, 4 × 10^{-4} *M*. (3) $FeCl_3$, 4 × 10^{-4} *M*; BAA, 8 × 10^{-4} *M*. (4) $FeCl_3$, 4 × 10^{-4} *M*; BAA, 1.2 × 10^{-3} *M*. (5) $FeCl_3$, 4 × 10^{-4} *M*; BAA, 1.6 × 10^{-3} *M*.

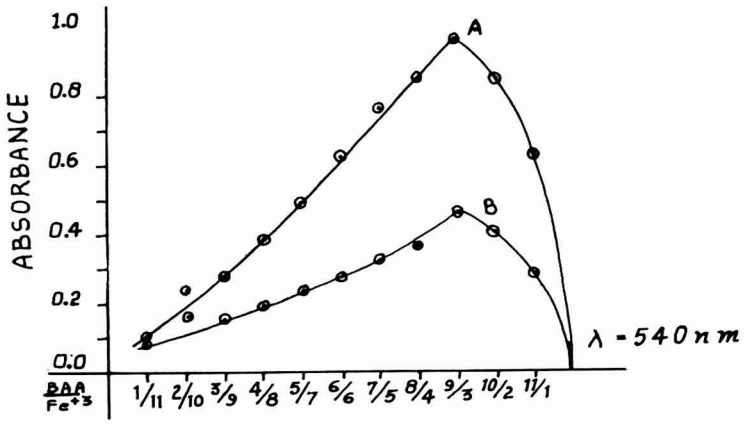


FIG. 2. Job's curves for $Fe(BAA)_3$ complex: (1) Fe^{3+} = BAA, 4×10^{-4} *M*. (2) Fe^{+3} = BAA, 2×10^{-2} *M*.

Evaluation of Stability Constant

The molecular extinction coefficient (ε) of the complex was calculated and was found to be 1.75×10^3. Stability constant of the complex has been calculated by the formula:

$$K = \frac{[\text{complex}]}{[(Fe^{3+})_{\text{total}} - \text{complex}]\,[(\text{ligand})_{\text{total}} - 3(\text{complex})]^3}.$$

Concentration of the complex was calculated from the observed extinction of the complex. Stability constant calculated by using above formula

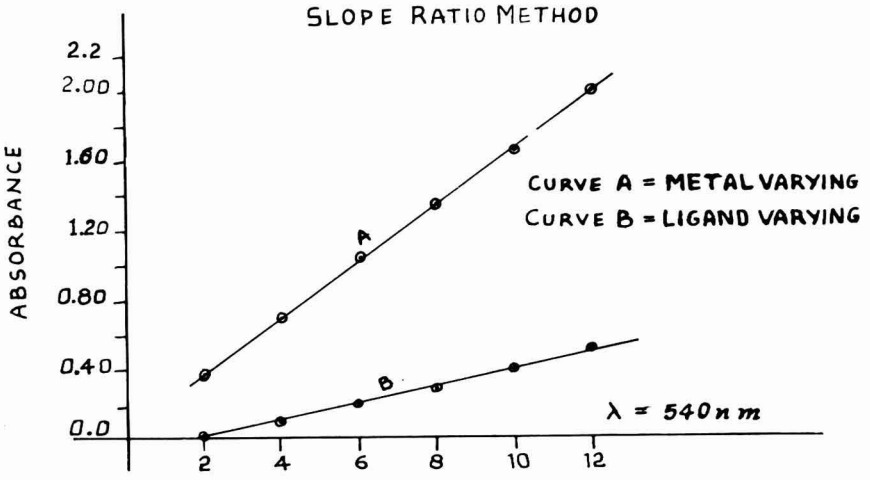


FIG. 3. Slope-ratio method: (A) metal varying (B) ligand varying.

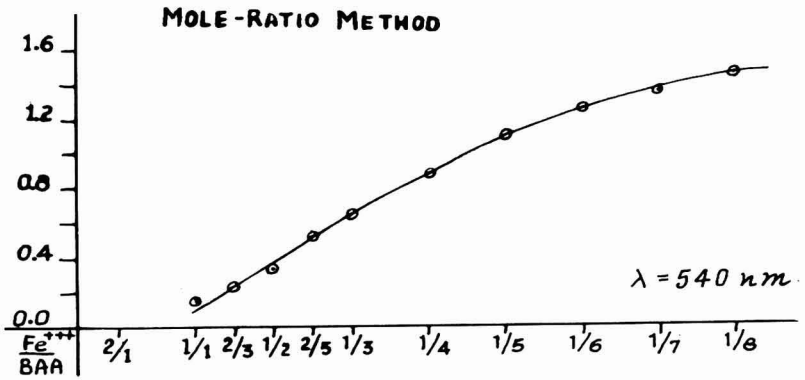


FIG. 4. Mole-ratio curve or spectrophotometric titration of a constant amount of Fe(III) and varying amount of BAA. Concentration of Fe^{+3}, 1×10^{-3} *M*.

was found to be log $K = 7.8 \pm 0.1$ at 20°C. The free energy of formation of the chelate at 20°C is $\Delta F = -10.53$ kcal/mole.

Conformity to Beer's law and sensitivity. The color system obeys Beer's law at 540 nm for iron concentration between 5 to 60 ppm. The optimum range being 10 to 40 ppm. The results have been given in Fig. 5. The spectrophotometric sensitivity of the reagent, defined by Sandell (*9*) was found to be 0.032 μg of Fe/cm^2.

Effect of reagent concentration. It was found that at least a 20-fold concentration of reagent is essential for the effective spectrophotometric determination of Fe(III) with benzoylacetanilide at pH 3.0 ± 0.1.

Reproducibility. The reproducibility and accuracy of the method was

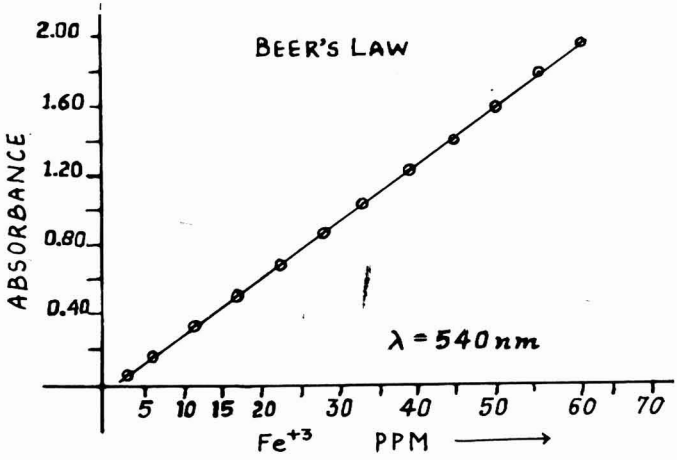


FIG. 5. Beer's Law curve at 540. nm.

TABLE 1

Fe(III) soln taken (ppm)	Absorbance at 540 nm	Error (%)
5.00	0.158	−1.25
10.00	0.316	−1.25
15.00	0.485	+1.04
20.00	0.635	−0.80
25.00	0.805	+0.62
30.00	0.970	+1.04
35.00	1.105	−1.30
40.00	1.270	−0.80

studied by analyzing solutions containing known amounts of iron. To aliquot quantities of Fe(III) solutions taken in a 25-ml flask, 10 ml of 0.1 *M* BAA solution was added and the volume in each flask was kept at 25 ml after adjusting the pH 3.0 ± 0.1. The results are recorded in Table 1.

Effects of Diverse Ions

The diverse ions were added individually to solutions containing 22.4 ppm of Fe(III) and the color was developed. The results are shown in Table 2. The tolerance limits are taken in such amount that the error is less than ± 2% of Fe(III) concentration. In every case large excess of ligand solution was added.

It was found that $C_2O_4^{2-}$, F^-, SCN^- and organic acids interfered even

TABLE 2

EFFECT OF DIVERSE IONS

All solutions contained 22.4 ppm of iron(III); pH = 3.0 ± 0.1.

Ion added	Conc tolerated (ppm)
Cu^{2+}	75
Ni^{2+}	100
Co^{2+}	50
UO_2^{2+}	20
Cd^{2+}	250
Al^{3+}	44
Hg^{2+}	200
Mn^{2+}	25
Ba^{2+}	40
Cl^-	350
I^-	50
SO_4^{2-}	150

when present in very small quantities and hence they have to be removed before carrying out these measurements.

SUMMARY

Benzoylacetanilide (BAA) forms violet colored complex $Fe(BAA)_3$ with Fe(III) in 60% alcohol with λmax at 540 nm. The stability constant (log K) and free energy of formation of the complex at 20°C are log $K = 7.8 \pm 0.1$ and $\triangle F = -10.53$ kcal/mole, respectively. The molar adsorptivity, sensitivity, effect of diverse ions in spectrophotometric determination of iron have also been investigated.

ACKNOWLEDGMENT

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MICROCHEMICAL JOURNAL 17, 546–555 (1972)

Determination of Traces of Free Acid by the Polarographic Reduction of Quinones [1]

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INTRODUCTION

A major problem in organic polarography is obtaining information of the role of protonation in electrochemical processes of organic compounds in relation to the properties of solvent. The presence of proton donors often leads to pronounced changes in polarographic characteristics of those compounds (*2, 6, 12*). Despite the many works developed recently, very few dealt with the explanation of the results in terms of correlation with the proton-donating strength of the acid in the given solvent.

During the course of an investigation on the polarographic reduction of quinones (*8–11*), the contribution of protonation to the electrode processes became evident in relation to basicity of solvent. In aprotic solvents, quinones are reduced first to semiquinone anions and then to dianions (*6*), whereas in amphiprotic solvents, two waves merge together and give a single wave due to two-electron reduction because of the sufficiently high rates of proton addition from the solvents. By the addition of acid to the solution, a new reduction wave was produced which preceded the original waves of quinones. Detailed investigation showed that the feature of the new wave is related to the acid strength and the rate of supply of proton-donating species from the bulk solution to the electrode surface. Analytical application of the experimental results to the determination of minute quantity of acid was examined.

The present paper is concerned with the extension of our earlier studies (*8–11*) to the analysis of traces of acid component in commercial reagents.

EXPERIMENTAL

2-Methoxyethanol (methyl cellosolve) was chosen in the present experiment for comparison of the polarographic characteristics of quinone

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in nonaqueous solvents with those in aqueous solutions. 2-Methoxyethanol is a satisfactory nonaqueous solvent for the polarographic study of organic compounds (*5, 7*).

Guaranteed reagent-grade 2-methoxyethanol was refluxed with anhydrous iron (II) sulfate for 1 hr at atmospheric pressure, the distillate at 125 ± 0.5°C being collected (*5*). Methyl-*p*-benzoquinone (MQ) was purified by repeated sublimation of the chemical reagent (mp 67.7°C). The supporting electrolyte used was 0.25 *M* sodium perchlorate unless otherwise stated, which was purified by crystallizing the guaranteed reagent from ethanol and ether.

DC polarograms were recorded on a Yanagimoto pen-recording polarograph model PA-102. The mercury flow rate and the drop time of the dropping mercury electrode used were 1.16 mg/sec and 6.04 sec in aqueous solution, and 1.24 mg/sec and 5.84 sec in 2-methoxyethanol solution, respectively. These values were measured in air-free supporting electrolyte solutions at open circuit and at 25°C. The potentials were referred to a saturated calomel electrode (SCE) in aqueous solution connected with a $NaNO_3$–agar salt bridge, while a mercurous sulfate electrode was used as a reference electrode in 2-methoxyethanol solution. The ohmic resistances between cathode and anode in both solutions were about 1.2 kΩ and 300 Ω, respectively. All measurements were carried out at 25°C.

RESULTS AND DISCUSSION

Effects of Acids on DC Polarogram of MQ

DC polarograms of MQ in aqueous solution in the absence and presence of acetic acid are shown in Fig. 1. A single wave due to the two-electron reduction is seen as curve a in Fig. 1. The addition of a small amount of acetic acid to the solution caused a new wave at a more positive potential than the original reduction wave of MQ (curves b and c in Fig. 1). The new waves were also produced by the addition of other acids, such as perchloric acid, nitric acid, benzoic acid, etc. The total limiting current (i_t) is proportional to the concentration of MQ, whereas the current of the new wave (i_H) at constant acid concentration remains unchanged irrespective of MQ concentration provided that MQ is present in excess. As shown in Fig. 2, i_H is proportional to the concentration of added acid (in the range of $i_H \leqq i_t$). The similar results were also obtained in 2-methoxyethanol solution.

The half-wave potential of the new wave changes in relation to the strength of the added acid. A decrease in acid strength leads to a shift of the half-wave potential to negative potential side. The linear relation is clearly obtained in 2-methoxyethanol solution under the condition

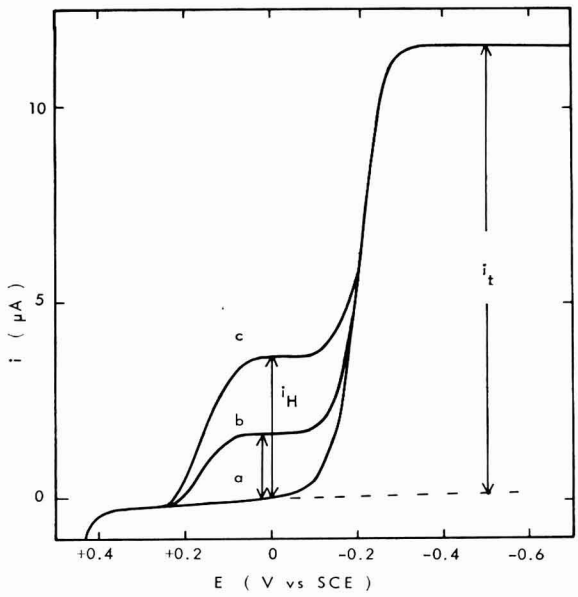

FIG. 1. Polarograms of 2.20×10^{-3} *M* MQ in aqueous solutions. Concentration of acetic acid: (a) 0; (b) 4.89×10^{-4}; (c) 1.02×10^{-3} *M*.

that the total acid concentration keeps constant, and the result is shown in Fig. 3, in which the half-wave potentials are plotted against pK_a of the added acid.[2] In aqueous solution, the half-wave potential remains nearly constant at lower pK_a, but shifts linearly to the negative potential side at higher pK_a. The leveling effect of the solvent is evident in the $E_{1/2}$ vs pK_a curve in aqueous solution (curve A in Fig. 3). Further confirmation of the constancy of the half-wave potential at lower pK_a was attained by the addition of perchloric or nitric acid, both of which gave new waves having the same half-wave potential at +0.21 V vs SCE.

These phenomena are elucidated by the following discussion. The new wave of quinone in the presence of acid can be explained from the standpoint of protonation prior to the reduction of quinone (*4, 11*). The availability of protons has resulted in a lowering of the free energy for the reduction of quinone. In our previous work (*11*), the reaction mechanism has been shown as:

$$2HA \rightleftarrows 2H^+ + 2A^-; \quad (1)$$

$$Q + H^+ \rightleftarrows QH^+ \quad ; \quad (2)$$

$$QH^+ + H^+ + 2e \rightleftarrows QH_2 \quad ; \quad (3)$$

[2] K_a is the dissociation constant of acid in aqueous solution. The use of K_a values obtained in aqueous solutiton instead of those in 2-methoxyethanol does not affect the relation between the half-wave potential and pK_a (*3*).

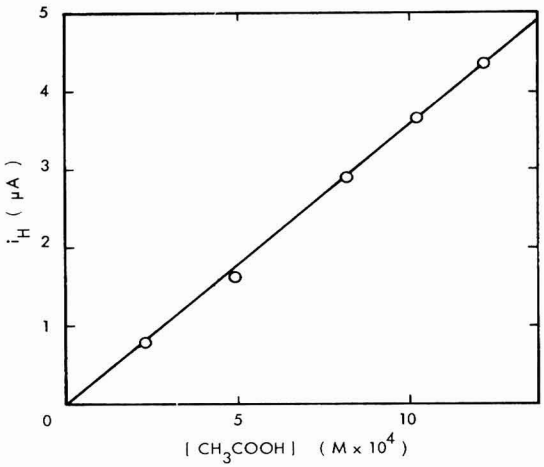

FIG. 2. Relation between i_H and concentration of acetic acid obtained in aqueous solution containing 2.20×10^{-3} *M* MQ.

where HA and Q denote acid and quinone, respectively. On the condition that protonated quinone QH^+ is produced rapidly at the electrode surface through Eq. (2), the electrode potentials of reduction of quinone in the presence of HA are determined by the reaction (3). Then the half-wave potential is expressed by

$$E_{1/2} = (RT/F) \ln [H^+] + \text{const.} \tag{4}$$

The total concentration of acid, i.e., the amount of acid added, is given by:

$$[HA]_t = [HA] + [H^+] + [QH^+] \tag{5}$$

In Eq. (5), the concentration of QH^+ can be neglected (*4*). On substituting K_a into Eq. (5), we have

$$[HA]_t = [H^+]^2/K_a + [H^+]. \tag{6}$$

From Eqs. (4)–(6), we can expect how the acid strength affects the half-wave potential of the new wave under the condition that $[HA]_t$ keeps constant. For very strong acid,[3] $[HA]_t$ becomes practically equal to $[H]^+$, and consequently, the half-wave potential of the new wave becomes independent of K_a. Contrary to this, $[HA]_t$ is approximately equal to $[H^+]^2/K_a$ for very weak acid,[3] and we can rewrite Eq. (4) as,

$$E_{1/2} = (RT/2F) \ln K_a + \text{const.}, \tag{7}$$

then a linear relation is expected between $E_{1/2}$ and pK_a. The relation between $E_{1/2}$ and pK_a in 2-methoxyethanol solution corresponds to the

[3] For the validity of these approximations, see ref. (*4*).

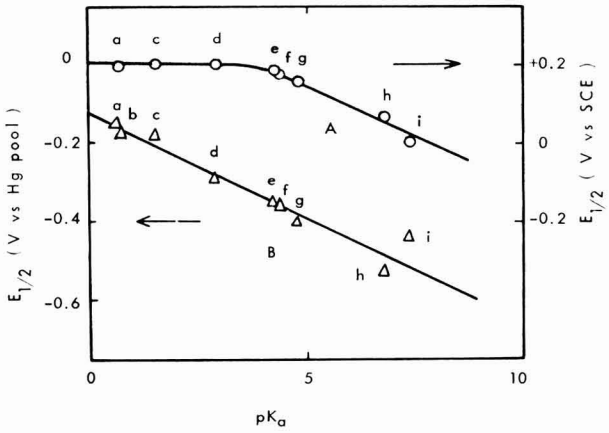

FIG. 3. Relation between half-wave potential of new wave and pK_a obtained in aqueous (A) and 2-methoxyethanol (B) solutions keeping $[HA]_t = 7 \times 10^{-4}$ *M*. (a) CCl_3COOH; (b) $C_6H_5SO_3H$; (c) $Cl_2CHCOOH$; (d) $ClCH_2COOH$; (e) C_6H_5COOH; (f) $C_6H_5CH_2COOH$; (g) CH_3COOH; (h) 2,6-$Cl_2C_6H_3OH$; (i) 2,5-$Cl_2C_6H_3OH$.

later case (curve B in Fig. 3). The former case is realized in the data obtained at lower pK_a in aqueous solution, but even in this solution, the relation tends to exhibit the latter case with the increasing pK_a (curve A in Fig. 3.

The currents of the new wave for a given concentration of acid, $i_H/[HA]_t$, are plotted against pK_a in Fig. 4. The results in Fig. 4 suggest that the current of the new wave is controlled by the diffusion of proton-donating species. In aqueous solution, an increase in pK_a leads to a significant decrease in i_H. The greater values of i_H obtained in the presence of strong acids indicate that the current of the new wave is controlled by the diffusion of hydronium ion. (Agreement between i_H value for strong acid and that calculated by the diffusion coefficient of hydronium ion is reasonable.) The decrease in i_H with increasing pK_a means that the contribution of the undissociated acid species to the supply of proton to the electrode surface becomes more predominant than that of hydronium ion. In 2-methoxyethanol, the currents are smaller and independent of pK_a but dependent on radius of undissociated acid molecule [see ref. (*11*)]. This fact clearly indicates the lower degree of dissociation of acid in this solvent than in aqueous solution.

Analytical Application

The experimental results mentioned above can be applied to the determination of minute quantities of free acids in commercial reagents

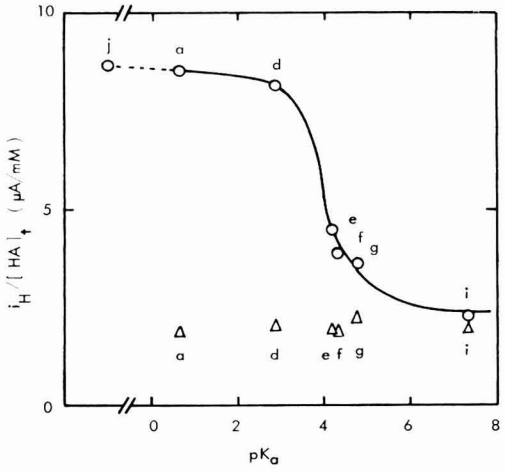

FIG. 4. pK_a dependence of $i_H/[HA]_t$ obtained in aqueous (○) and 2-methoxyethanol (△) solutions. a ~ *i* are the same as in Fig. 3. *j* is obtained with $HClO_4$ or HNO_3.

as an impurity. Water is found to be a satisfactory solvent for the present method because of a higher i_H value. Contrary to this, even if the wave-height obtained in 2-methoxyethanol is lower than that obtained in aqueous solution, it can still be utilized to the determination of acids more than 10^{-4} *M* in concentration. Furthermore, this solvent can be conveniently used for some organic acids, especially those insoluble in water. The fact that the use of 2-methoxyethanol gives rise to a clear shift of half-wave potential of the new wave with acidity allows of the simultaneous determination of mixed acids [see ref. (*9*)]. Some examples of application are shown below.

Determination of Free Acid in Salts Which Can be Used as a Supporting Electrolyte

Figure 5 shows the polarogram of MQ in aqueous solution containing 0.350 *M* sodium benzoate as a supporting electrolyte. Sodium benzoate used was reagent-grade chemicals without further purification. A small wave preceded the main reduction wave of MQ as clearly seen. The small wave disappeared in the solution of purified sodium benzoate, and reappeared at the same potential as in Fig. 5 by the addition of benzoic acid. Then it was confirmed that the small wave was caused by the presence of free benzoic acid in supporting electrolyte reagent, and the content of the acid was determined as 0.077 wt.%. By the same method, the contents of free perchloric acid in perchlorates, free sulfuric acid in sulfates, and free salicylic acid in sodium salicylate

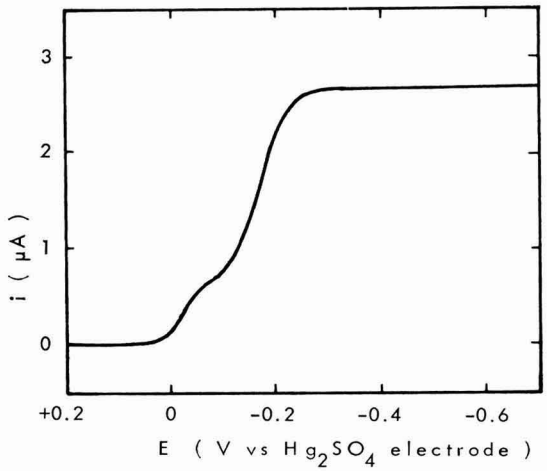

FIG. 5. Polarogram of 2.0×10^{-4} *M* MQ in aqueous solution containing 0.350 *M* sodium benzoate (not purified) as a supporting electrolyte.

were also determined. The method can be utilized to determine the traces of acid in salts as low as 5 ppm.

Determination of Free Acid in Solvents

The polarogram of MQ obtained in the mixed solvent of acetic acid butyl ester and 2-methoxyethanol (1:1) containing 0.25 *M* of sodium

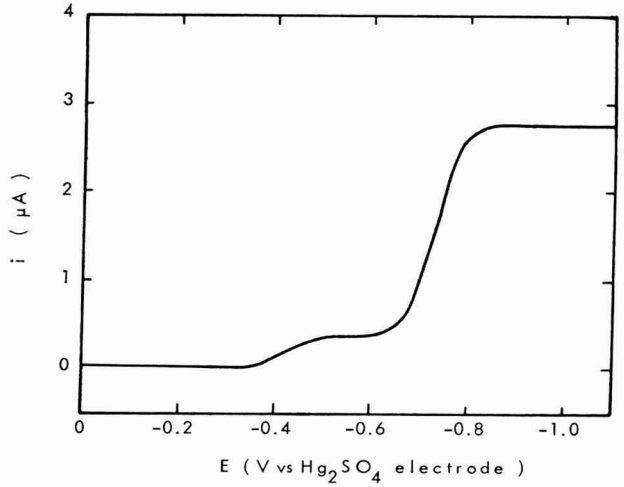

FIG. 6. Polarogram of 3.0×10^{-4} *M* MQ obtained in mixed solvent of acetic acid butyl ester (not purified) and 2-methoxyethanol (1:1).

perchlorate is shown in Fig. 6,[4] in which a small wave was also found on the polarogram. Acetic acid butyl ester obtained commercially (GR-grade reagent) was used without purification. Such an ester often contains a small amount of free acid caused by hydrolysis. By the addition of acetic acid to the solution, the height of the wave was increased. The small wave was not found in the purified acetic acid butyl ester-2-methoxyethanol. (Acetic acid butyl ester was distilled after treating with sodium carbonate). These facts indicate that the small wave in Fig. 6 is attributed to the presence of free acetic acid in the ester. The content of acetic acid in acetic acid butyl ester was determined from the polarogram to be 0.0016 wt%. The present method will be applicable to determine trace acid in solvents as low as 1 ppm providing that the solvent is available for polarographic analysis.

ADVANTAGEOUS POINTS OF THE PRESENT METHOD

The possibility of the determination of acid by means of polarographic reduction of quinone was pointed out first by Abbott *et al.* (*1*). The method was extended to the determination of various kinds of acids in salts and solvents during the course of our investigation (*8–11*). The use of nonaqueous solvent made possible the determination of organic acids which are insoluble in water. Further extension of the method was a simultaneous determination of mixed acids using nonaqueous solvents (*9*).

Since acid is reduced on a dropping mercury electrode at highly negative potential, the direct polarographic analysis of acid often encounters some difficulties, such as an interference of discharge of supporting electrolyte or decomposition of solvent. Sometimes an irregularity of drop time of mercury flow is found at a highly negative potential region, especially in organic solvents. In the present method, acid is determined by means of the polarographic reduction of quinone, instead of a reduction wave of acid itself. The presence of acid gives rise to the new wave on polarogram of quinone in positive potential region without any interference as stated above. This is also one of the favorable points of the present method.

On the other hand, the present method has the limitation of the applicability to the determination of very weak acids. A weaker acid produces the new wave at more negative potential, and, consequently, the new wave becomes closer to the main reduction wave of quinone. In

[4] Mixing 2-methoxyethanol in other solvents is not always necessary for the present purpose. In this case, 2-methoxyethanol was used to increase the solubility of sodium perchlorate. Free acetic acid in acetic acid ethyl ester was determined without the addition of 2-methoxyethanol [see ref. (*10*)].

the present experiment, well-defined new waves cannot be obtained by weak acids whose pK_a is more than 8.

Stability of Quinone in Solution

Quinone is rather unstable in solution and decomposes through photochemical processes. Reproducible polarograms are scarcely obtained with *p*-benzoquinone in organic solvents. The changes, in the color of the solution and in the shape of the polarogram of *p*-benzoquinone, become significant with a lapse of time. On the other hand, *p*-benzoquinone is rather stable in aqueous solution and reproducible polarograms can be obtained within 1 hr after preparation of the solution. Alkyl-substituted quinones are more stable, even in organic solvents.

Besides MQ, similar results to those described above were also obtained with other quinones, such as *p*-benzoquinone, 1,4-naphthoquinone, anthraquinone, and their derivatives. In the present method, the use of MQ is recommended, especially for organic solvents. Anyway, it is necessary to use the freshly prepared solution of quinone in experiment.

SUMMARY

The effects of proton donors on the polarographic reduction of methyl-*p*-benzoquinone (MQ) and some examples of analytical application of the experimental results have been presented in this report. The presence of a small amount of acid gives rise to a new reduction wave which preceded the original reduction wave of MQ. The new wave is affected by the strength of added acid and the basicity of solvent. The comparison between the results obtained in aqueous and 2-methoxyethanol solutions has been made to demonstrate that the new wave is diffusion-controlled and attributed to the protonation of MQ at the electrode surface prior to the electron transfer.

The current of the new wave is proportional to the concentration of acid present. This fact has been applied to the determination of minute quantities of free acid contained in commercial reagents. The contents of free benzoic acid in sodium benzoate and free acetic acid in acetic acid butyl ester have been determined by this method. The method is applicable to the determination and the detection of small amounts of free acid contained in salts and solvents as low as the ppm range.

ACKNOWLEDGMENT

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Automation in Organic Analysis
II. Simultaneous Microdetermination of Oxygen and Nitrogen

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INTRODUCTION

Up to now, the only method of simultaneous determination of oxygen and nitrogen in organic compounds was that of Terent'yev and coworkers (*1*). They decomposed the sample in a closed evacuated tube containing nickelized carbon at 900°C, and the gaseous products formed were rinsed with helium into a chromatographic column filled with a molecular sieve 5A. On the molecular sieve carbon monoxide was separated from nitrogen and their quantity was determined using a catharometer. The mentioned procedure was used for the analysis of nonvolatile substances only, containing C, H, O, and N. Absolute error of the determination of oxygen was ±0.40%, and in the case of nitrogen it was always positive (up to +0.80%). For the sake of completeness let us mention the determination of oxygen according to Walisch and Marks (*2*), as these authors also indicate the amount of nitrogen, when analyzing nitrogen-containing substances, in addition to the amount of the determined oxygen. In the case of their method the accuracy of nitrogen determinaton depends upon the type of the analyzed substance and the error amounts up to 3% (absolute).

EXPERIMENTAL

Investigation of the Conditions of the Determination

After thermal decomposition of the analyzed compound each of its elements is included in various pyrolytic fragments. The condition for a simultaneous determination of the two elements is the transformation of the various products of decomposition to uniform compounds of each of both the determined elements, followed by the separation of these two compounds from other products and from each other, and their quantitative determination. Unless the determined element is not transformed to a single compound, its amount is usually determined

from quantitative analysis of several compounds, and the result of the analysis is then naturally inaccurate.

For the transformation of the oxygen of an organic substance to carbon monoxide as a uniform derivative, we intended to make use of previously published methods of oxygen determination (*3*). Under these conditions, however, the nitrogen of an organic compound is transformed to a mixture of NH_3, CHN, NO, and N_2, which must be transformed to a uniform compound if the determination of nitrogen is to be carried out. Therefore, we first determined experimentally how these compounds behave during the passage through a layer of heated platinized carbon, and whether by this alone a simultaneous determination of oxygen and nitrogen would not be possible. The reactions were first investigated qualitatively, because ammonia and hydrogen cyanide are partly adsorbed on the carbon from which they can be eluted only slowly (requiring more than 1 hr). For our orientation we used the following scale for the amount of the compounds going out from the reaction tube:

0, traces, +, ++, +++.

Ammonia was detected using a universal indicator paper, hydrogen cyanide with a paper wetted with a cupric acetate and benzidine acetate solution, nitrogen-II-oxide with an iodine–starch paper kept close to the mouth of the reaction tube (in air NO is immediately oxidized to NO_2, which reacts with the iodine–starch reagent). Elemental nitrogen was determined with a catharometer after ammonia was absorbed on oxalic acid, hydrogen cyanide on soda asbestos, and carbon monoxide was separated by column chromatography (Porapak Q, 80–100 mesh; i.d. 3 mm; length 3 m; temperature −80°C).

In order to elucidate the effect of platinized carbon we first found out whether NO, HCN, NH_3, and N_2 react with hydrogen in an "empty tube." Into a stream of H_2 (30 ml/min) passing through a quartz tube (i.d. 10 mm, a 4-cm section of which was heated at 1100°C), we consecutively injected (inj.) 0.3-ml doses of the investigated gases:

Inj.	Detected	Inj.	Detected	Inj.	Detected	Inj.	Detected
NO	NO +++	HCN	HCN +++	NH_3	NH_3 +++	N_2	N_2 +++
	NH_3 traces		NH_3 traces		N_2 traces		

When studying these reactions on platinized carbon we proceeded in a similar manner (as in the case of "empty tube"), with the difference that the quartz tube contained a 4-cm-long layer of platinized carbon (50% Pt). The carbon was heated at 1050°C, which is necessary for the determination of oxygen (*3*), and which, therefore, guarantees the

transformation of oxygen-containing products of the hydropyrolysis to carbon monoxide.

Inj.	Detected	Inj.	Detected		Inj.	Detected
N_2	N_2 +++	NH_3	NH_3 +++	(75%)	HCN	HCN +++
			N_2 +	(25%)		NH_3 +
						N_2 traces

The reactions of nitrogen-II-oxide on platinized carbon were investigated in dependence on temperature. The procedure was the same as in the preceding case, but the temperature of the platinized carbon layer was gradually increased. The injected amount was always 0.3 ml of nitrogen-II-oxide.

25°C	NO	0	750°C	NO	0	1050°C	NO	0
	NH_3	traces(?)		NH_3	+++		NH_3	++
	HCN	0		HCN	traces		HCN	+++
	N_2	0		N_2	0		N_2	traces
500°C	NO	0	950°C	NO	0	1100°C	NO	0
	NH_3	++		NH_3	+++		NH_3	traces
	HCN	0		HCN	+++		HCN	+++
	N_2	0		N_2	traces		N_2	traces

On the basis of these results we came to the conclusion that nitrogen monoxide reacts with hydrogen during the passage through platinized carbon under formation of NH_3 and HCN (if taking into consideration only nitrogen-containing compounds); their ratio depends on the temperature of the carbon. The detected small amount of elemental nitrogen originates from partly decomposed ammonia. It is interesting to note that nitrogen monoxide could not be detected at room temperature (25°C) for the platinized carbon, either. It was observed, however, that a short time after the furnace was switched on and began to heat the platinized carbon, on which nitrogen oxide was previously dosed, ammonia started to go out of the reaction tube. This indicates that at room temperature nitrogen monoxide is sorbed on the carbon and on heating it is reduced to ammonia.

Summarizing, we may state that of nitrogenous pyrolytic products passing through a layer of heated platinized carbon, only nitrogen, ammonia, and hydrogen cyanide remain in the hydrogen flow. Hence, it is evident that a layer of platinized carbon (as the only filling of the reaction tube) does not suffice for simultaneous determination of oxygen and nitrogen.

As we used hydrogen as carrier gas, the possibility arose of decreasing

the number of nitrogen-containing hydrogenation products. In preliminary experiments we found that on a common hydrogenaton catalyst, nickel (a 3-cm-long layer in a tube of i.d. 10 mm), nitrogen oxide is quantitatively hydrogenated to ammonia and water from 200°C up, while a quantitative reduction of hydrogen cyanide to ammonia and methane occurs from 400°C up (at 350°C HCN came out only in traces). However, this fact cannot be utilized directly for the determination of nitrogen, because (as was already said) ammonia is partly sorbed on the carbon. Therefore, it is necessary to let the nitrogen pass through the carbon only in the form of elemental nitrogen.

Hence, the main problem consisted in the decomposition of ammonia in a stream of hydrogen to nitrogen and water. For this purpose a catalyst had to be found. We started from the known fact, i.e., that catalysts which cause hydrogenation at lower temperatures dehydrogenate at elevated temperatures. Therefore, we chose the following metals for our experiments: Ag, Co, Cu, Fe, Ni, and Pt.

The decomposition of ammonia on these metals was investigated in the following manner: ammonia (1 ml each time) was injected into a stream of hydrogen (30 ml/min) which was led into a quartz tube (i.d. 10 mm) with a 3-cm-long layer of the catalyst which was gradually heated up to 1000°C. The undecomposed ammonia was captured in a trap with oxalic acid and the amount of the passed elemental nitrogen was determined quantitatively using a catharometer.

Of the above-mentioned catalysts, only iron, cobalt, and nickel decomposed ammonia quantitatively at a temperature above 900°C. A curve of ammonia decomposition on nickel is shown in Fig. 1.

In the proposed method of simultaneous determination of oxygen and nitrogen we used nickel because it was most easily accessible in pure form, and we placed it together with platinized carbon (50% Pt) into the reaction tube. Experimentally we checked that the filling should be placed in the reaction tube in the following order: nickel and then platinized carbon. If the sequence was reversed, ammonia was partly adsorbed on the carbon and its slow elution prevented the determination of nitrogen (see Fig. 2).

Originally metallic nickel was prepared from nickel nitrate of analytical grade. This nickel gave good results for nitrogen, but in the case of its location before the platinized carbon low results were obtained for oxygen. After a series of experimental tests we found that the retention of oxygen is caused by the alkalies present in nickel. We did not determine the permitted amount of alkalies, but made a practical experiment instead. A part of the prepared catalyst was placed into a quartz test tube with redistilled water and the mixture boiled

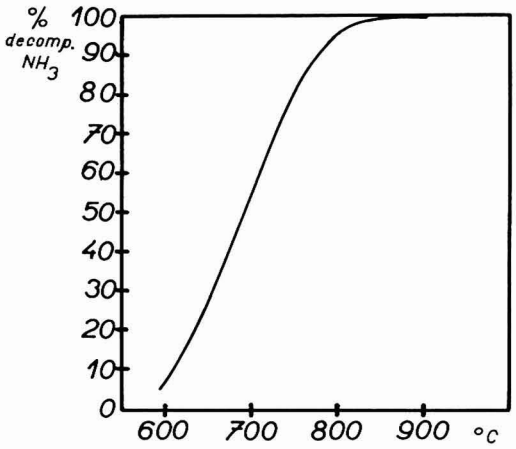

FIG. 1. Temperature dependence of ammonia decomposition on a 3-cm-long layer of nickel.

for 2 min. If, after this treatment the water was not alkaline (on phenolphthalein), the catalyst was a good one. On the basis of these experiences we prepared the catalyst from a nickel sheet by the procedure described in the section on Reagents.

The fact that alkalies indeed retain oxygen was ascertained in the following manner. A 4-cm-long layer of platinized carbon was placed into the reaction tube which was then heated to 1050°C. Carbon dioxide (0.1 ml) was then injected in a flow of hydrogen. The CO formed was determined with a catharometer, which gave the peak of a given area. Then a 3-cm-long zone of nickel was inserted before the platinized carbon. Nickel was prepared by the procedure described in the section on Reagents; when boiled in water, it did not make the solution alkaline. Carbon dioxide (0.1 ml) was again injected and the area of the CO peak was the same as in the absence of nickel. This pure nickel was then boiled in 0.5% sodium carbonate solution, washed with 50 ml of water, dried, annealed under hydrogen at 1100°C, and again placed into the reaction tube before the platinized carbon. Injection of 0.1 ml of CO_2 again gave a peak of CO, which had an area 28% smaller than that obtained without the presence of alkali on nickel, i.e., 28% of oxygen dosed in the form of CO_2 was retained.

Nitrogenous pyrolytic products were thus transformed to elemental nitrogen, while oxygen-containing products appeared as carbon monoxide. They were separated on a chromatographic column filled with Porapak Q and cooled at −80°C (ethanol and solid carbon dioxide).

Hydrogen halides formed on pyrolysis of halogenated compounds are

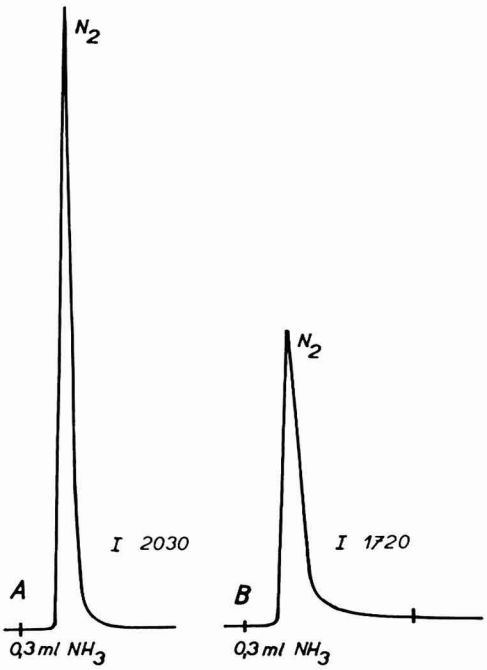

FIG. 2. Effect of the location of the fillings of nickel and platinized carbon on ammonia decomposition. A—3 cm of Ni + 8 cm of GTG carbon (50% Pt); B—8 cm of GTG carbon (50% Pt) + 3 cm of Ni; I—area of peak.

removed by soda asbestos inserted between the reaction tube and the chromatographic column.

Procedure for Simultaneous Determination of Oxygen and Nitrogen

REAGENTS

Activated copper, molecular sieve 5A, platinized carbon (50% Pt); for preparation see the previous paper (*3*).

Porapak Q—a porous copolymer vinylbenzene–divinylbenzene (Waters Associates, Framingham, MA).

Copolymer of 80- to 100-mesh granulation was activated before the first use by 2-hr heating in a hydrogen current at 230°C.

Nickel—A nickel sheet was dissolved in a quartz dish in excess nitric acid which was redistilled in a quartz apparatus and diluted with water (1:1). When heating the quartz dish with a burner, water was first evaporated with the excess of nitric acid and then was decomposed under evolution of NO_2 and O_2. Nickel oxide remained on the dish,

which was reduced with hydrogen in a quartz tube at 500°C to nickel. The metal obtained in this manner was ground, sieved, and the 0.3- to 1.0-mm fraction was annealed for 6 hr at 1050°C in a stream of hydrogen.

Anhydrone

Soda asbestos

Apparatus

The apparatus is shown in Fig. 3. A detail of the filling of the reaction tube and the location of the furnace is shown in Fig. 4.

All ground glass joints were lubricated with Apiezon L (Edwards High Vacuum), and secured with springs.

For the weighing of solid samples a boat was used made of platinum sheet of 0.05-mm thickness, 2.5 × 2.5 × 10 mm large. The liquids were weighed in quartz capillaries provided with handles. For the insertion of the boat (capillary) into the reaction tube a small quartz tube was used; this is described in detail in the section on working procedure.

Working procedure and results

The boat (capillary) with the sample is inserted into a thin-walled quartz tube of 3.5 mm i.d. and 25 cm long. The valve *5* is slowly opened, and after equalization of the overpressure in the apparatus with outer atmospheric pressure, the quartz tube containing the boat (capillary) is inserted through the open valve into the reaction tube *6*. Using

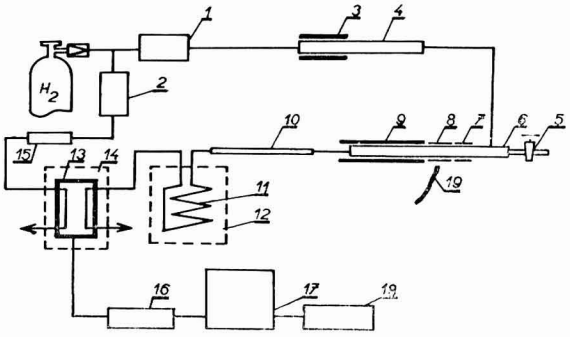

Fig. 3. Scheme of the apparatus. *1, 2*—manostats; *3*—furnace 500°C; *4*—purifying tube filled with active copper and molecular sieve 5A; *5*—valve; *6*—reaction tube; *7*—safety furnace; *8*—decomposition furnace; *9*—reaction furnace 1050°C, *10*—absorption tube with soda asbestos and molecular sieve 5A; *11*—chromatographic column with Porapak Q; *12*—thermostat (ethanol and solid carbon dioxide); *13*—catharometer; *14*-thermostat; *15*—trap with molecular sieve 5A; *16*—Wheatstone bridge; *17*—recorder; *18*—integrator; *19*—inlet of cooling air.

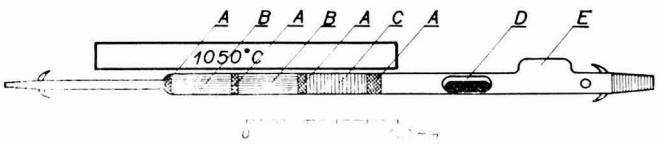

FIG. 4. Filling of the reaction tube and location of the furnace. A—quartz wool; B—platinized carbon (50% Pt); C—nickel; D—insert with a sealed-in iron nucleus; E—chamber.

a stainless-steel wire the boat (capillary) is pushed out into the reaction tube. It should be 10 mm from the side inlet of hydrogen, in the direction of the valve. The small quartz tube is then pulled out. Simultaneously, the decomposition oven *8* is cooled with a current of air (from tubing *19*), so that the sample is inserted into a cold tube.

The air is displaced by hydrogen from the boat and the apparatus within 1 min. The insert *D* (Fig. 4) is then held by a magnet in the chamber *E* and the boat (capillary) is pushed by the stainless-steel wire into the decomposition oven *8*. The valve is closed and 3 min are allowed to pass until the pressure and the flow of the carrier gas are constant. During this time the shifting of the recording paper of the recorder EZ 4 (Laboratory apparatus, Prague) is switched on and integrator AIK (*4*) (Research Institute for Macromolecular Chemistry, Brno) is adjusted.

After a 3-min interval the insert *D* is pushed with the magnet into the safety furnace *7* which is switched on. After 5 sec, when the temperature of the small furnace has attained 1000–1100°C, the decomposition furnace is switched on and its air cooling is interrupted. The decomposition furnace is hot (1000–1100°C) within 5 sec, and the analyzed substance is thus practically immediately decomposed. The safety furnace and the decomposition furnace are switched off after 20 sec. The products of hydropyrolysis in the meanwhile pass through the fillings of nickel and platinized carbon where the nitrogenous products are transformed to elemental nitrogen, the carbonaceous ones to carbon monoxide, halogens to hydrogen halides, and methane is decomposed (*3*). Behind the reaction tube hydrogen halides are removed by the soda asbestos. Only carbon monoxide and nitrogen are swept with the carrier gas. These gases are separated on a chromatographic column and enter the measuring cell of the catharometer (first nitrogen, then carbon monoxide), where they cause reactions proportional to their concentrations in the hydrogen. The signal from the Wheatstone bridge is taken down by the recorder which first draws the chromatographic peak of the nitrogen and then that of carbon monoxide. The

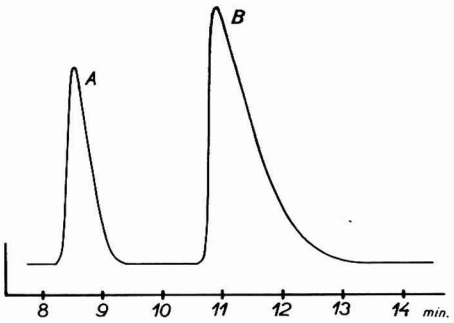

FIG. 5. Chromatographic record of a simultaneous determination of oxygen and nitrogen. A—nitrogen; B—carbon monoxide.

integrator gradually adds the areas of both peaks and expresses them by the number of impulses. The amount of oxygen and nitrogen present in the analyzed sample is then read from the calibration straight lines, which were constructed on the basis of the results of the analyses of the test substance (acetanilide). This is done by plotting the number of integrator impulses against the micrograms of oxygen or nitrogen.

The chromatographic record obtained during one determination is shown in Fig. 5 and the results of the analyses are in Table 1.

DISCUSSION

Quantitative transformation of nitrogen and oxygen of an analyzed organic compound to uniform compounds, elemental nitrogen and carbon monoxide, was achieved by using two independent contact layers—nickel and platinized carbon. A mixed catalyst—nickelized carbon—was also tested (20, 50, 70, and 90% Ni). However, ammonia did not decompose quantitatively on this catalyst in a hydrogen current, even at 1200°C.

The disadvantage of this method is the fact that this procedure cannot be used for serial analyses of sulfur-containing compounds. The errors of determination of both elements are within the required limits for the first analysis of the sulfur-containing compound ($\pm 0.30\%$). Nickel (also cobalt) is poisoned by the sulfur present in the analyzed compound, and, therefore, in the next analysis ammonia is not decomposed quantitatively and nitrogen cannot be determined. In the case of iron the situation is somewhat different. The first traces of sulfur catalyze the binding of nitrogen by iron, so that no undecomposed ammonia comes out from the tube, but almost no nitrogen either.

In spite of the difficulties encountered, we believe that the problem of the determination of oxygen and nitrogen in a single sample of a sul-

TABLE 1

RESULTS OF ANALYSES

Sample	Weight (mg)	Oxygen (%)			Nitrogen (%)		
		Calcd	Found	Δ %	Calcd	Found	Δ %
Acetanilide	1.882	11.83	11.95	+0.12	10.37	10.41	+0.04
C_8H_9NO	1.972		11.83	±0.00		10.39	+0.02
	1.857		11.68	−0.15		10.37	±0.00
	1.847		11.58	−0.25		10.44	+0.07
2-Naphtholazobenzenecarboxylic acid	1.900	16.44	16.47	+0.03	9.58	9.31	−0.27
$C_{17}H_{12}N_2O_3$	1.840		16.30	−0.14		9.83	+0.25
Phthalic acid imide	1.780	21.76	21.76	±0.00	9.52	9.30	−0.22
$C_8H_5NO_2$	1.801		21.82	+0.06		9.66	+0.14
	1.733		21.87	+0.11		9.52	±0.00
Benzoylaminoacetic acid	1.933	26.80	27.00	+0.20	7.82	7.91	+0.09
$C_9H_9NO_3$	1.794		26.88	+0.08		7.91	+0.09
	1.967		26.89	+0.09		7.68	−0.14
Dinitrochlorobenzene	1.719	31.59	31.41	−0.18	13.83	13.73	−0.10
$C_6H_3N_2O_4Cl$	1.820		31.44	−0.15		13.74	−0.11
	1.695		31.35	−0.24		13.63	−0.20

furous organic substance will be resolvable in principle by two methods which we intend to investigate in the future. Either a substance will be used which, when put between the decomposition space and the reactive filling will be able to retain sulfur quantitatively, or else a catalyst will be used which is inert to sulfur. The first way seems more difficult, because only such a substance may be used as an adsorbent, which will bind sulfur quantitatively at elevated temperature from the hydrogen current, but otherwise must be inert to oxygen and nitrogen. However, the majority of substances capable of fixing sulfur in other atmospheres in the form of sulfides or sulfates liberate sulfur in a hydrogen atmosphere in the form of hydrogen sulfide. Therefore, the second method seems more viable, i.e., the finding of a catalyst which would be inert to sulfur. We consider that the work by Goodloe and Frazer (*5*) might serve for the solution of this problem. These authors, during their hydrogenation method of oxygen determination, used nickel chromite $Ni(CrO_2)_2$ (or more recently a better one: $NiO.Cr_2O_3$) both as cracking and hydrogenation catalyst at 750 and 400°; they stated that sulfur does not lower the effect of the catalyst. When analyzing nitrogen-containing substances, they did not find ammonia among the gaseous products coming out of the reaction tube. Therefore, they stated that on nickel chromite, hydrogenation of nitrogen to ammonia does not take place. However, we believe that nickel chromite also catalyzes the decomposition of ammonia. This hypothesis is supported by the fact that, during the pyrolytic decomposition of the organic compound in hydrogen gas, ammonia is always formed to a greater or lesser extent(*6*). If the above-mentioned authors did not find it in the gaseous products, ammonia must be decomposed on nickel chromite. We checked this statement experimentally by using a 3-cm-long layer of nickel chromite in a quartz tube connected to the source of hydrogen current into which ammonia was injected. We found that ammonia decomposed quantitatively from 650°C up. However, the catalyst prepared by us also retained a small amount of oxygen dosed as carbon dioxide. As there is no reason for this adsorption, we consider that the problem consists in the purity of the catalyst, which may be solved by a suitable preparative procedure. If this supposition is correct, the substitution of nickel chromite for nickel will enable a simultaneous determination of oxygen and nitrogen in sulfur-containing compounds. It will also be possible to elaborate a new variant of a simultaneous determination of oxygen and nitrogen using nickel chromite as the sole filling in the reaction tube. In this case, the oxygen-containing products of hydropyrolysis would be transformed to water, nitrogenous compounds to elemental nitrogen, and after their separation on a gas-chromatographic column they would be determined with a catharometer.

We consider these enumerated possibilities of the utilization of nickel chromite for simultaneous determination of oxygen and nitrogen as a preliminary communication, and we shall continue their investigation.

SUMMARY

A simple, rapid, and accurate method of simultaneous determination of oxygen and nitrogen in organic compounds, containing (in addition to C, H, and O) Cl, Br, and I, is described. The principle of the method consists in the pyrolytic decomposition of the analyzed compound in a current of hydrogen at 1000–1100°C and the conducting of the hydropyrolytic product through layers of nickel and platinized carbon (50% Pt) heated at 1050°C. On this filling the nitrogen-containing products are transformed to elementary nitrogen, the oxygen-containing products are transformed to carbon monoxide, the halogens to hydrohalogenides, and methane is decomposed. After removing hydrohalogenides by soda asbestos, carbon monoxide and nitrogen are separated on a chromatographic column filled with Porapak Q, and determined with a catharometer. The whole analysis, including the weighing of the sample, requires 25 min. Sulfur interferes with the determination.

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The Microdetermination of Soluble Iodides in Biological Fluids

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INTRODUCTION

Actually the iodine concentration of the milk produced in a particular region may be considered as an index of endemic goiter incidence in that locality. The ingestion of iodine rich diets, due to the consumption of large amounts of sea foods or seaweeds, leads to notable rise in blood iodine level. A similar increase results when iodides are being given. Therapeutic doses of iodide, e.g., 1 g of sodium iodide, may raise the level temporarily by several hundred per cent. Soluble iodides when orally administered are almost completely absorbed, with no and if any very little of them appear in the feces. Thus the excretion of soluble iodides is almost entirely in the urine (*4*). Hence the determination of soluble iodides in biological fluids is of diagnostic interest.

The present work is an extension of a previous report on the microdetermination of soluble iodides with *N*-bromosuccinimide (*2*), but involves a modification of the procedure.

MATERIALS AND METHODS

Equipment and Reagents

1. Microburette of 5-ml capacity graduated to 0.01 ml.
2. Graduated pipettes of 1-, 2-, 5-, and 10-ml capacity.
3. Volumetric flasks of 100-ml capacity.
4. Standard *N*-bromosuccinimide solution, e.g., 0.1 and 0.01% (w/v) aqueous.
5. Soluble starch solution 1%, aqueous.
6. Trichloroacetic acid 10% (w/v), aqueous.
7. Methyl red solution, 0.04% (w/v), alcoholic.

Reaction Between N-Bromosuccinimide and Potassium Iodide in Neutral Medium

A 1.66-g portion of potassium iodide (0.01 mole) was dissolved in 20 ml of distilled water. Then 1.78 g of *N*-bromosuccinimide (0.01

mole) was suspended in 10 ml of distilled water and gradually added with shaking to the iodide solution.

The solution acquired a yellowish brown color. The presence of iodine monobromide was confirmed by extracting the solution with 5 ml of chloroform in a separating funnel when the lower chloroform layer became brown colored.

The upper colorless aqueous layer was separated and treated with 5% hydrochloric acid (v/v), added dropwise with shaking, until the solution was just acid to litmus paper. The solution was then distilled *in vacuo*. The solid residue was crystallized from benzene and proved to be succinimide by melting point and mixed melting point determinations with an authentic sample. Yield 0.7 g.

Validity of the Reaction for Quantitative Determination

The reaction between *N*-bromosuccinimide and soluble iodides, e.g., potassium iodide or sodium iodide in aqueous medium was shown to be valid for quantitative determination.

An accurately measured volume, e.g., 5 ml of solution containing 0.166 g (1 mmole) of potassium iodide or 0.1499 g (1 mmole) of sodium iodide per 100 ml was placed in a 100-ml conical flask and 1 ml of starch solution was added. The mixture was titrated with 0.178% (1 mmole) or 0.356% (2 mmole) *N*-bromosuccinimide solution (w/v), respectively, that was added drop by drop from a microburette, with vigorous shaking. The first drop of the *N*-bromosuccinimide solution added liberated iodine from the soluble iodide which gave a blue color with starch indicator. The *N*-bromosuccinimide was run in gradually with shaking until the blue color was just discharged, and the volume of the titer was noted. It was found that the reaction was stoichiometric in aqueous medium at room temperature; the results were:

Potassium Iodide

Volume of potassium iodide solution (1 mmole/100 ml) taken (ml):					
10	5	4	3	2	1
Titer of *N*-bromosuccinimide solution (1 mmole/100 ml) used (ml):					
10.03	5.03	3.99	3.02	2.01	1.01
Titer of *N*-bromosuccinimide solution (2 mmole/100 ml) used (ml):					
5.01	2.51	2.01	1.51	1.01	0.51
Titer of *N*-bromosuccinimide solution (1 mmole/100 ml) used (ml):					
10.05	5	4.02	2.99	2.01	1.01

Sodium Iodide

Volume of sodium iodide solution (1 mmole/100 ml) taken (ml):					
10	5	4	3	2	1

Titer of *N*-bromosuccinimide solution (2 mmole/100 ml) used (ml):					
5.01	2.52	2	1.50	1.02	0.52

Procedure

To an accurately measured volume, e.g., 5 ml of potassium iodide or sodium iodide solution in a 100-ml conical flask, add 1 ml of starch solution. Titrate the mixture with 0.1 or 0.01% (w/v) *N*-bromosuccinimide solution added dropwise from a microburette, with shaking. A blue color appears after adding the first drop or few drops of *N*-bromosuccinimide solution, respectively. Continue the addition of the *N*-bromosuccinimide solution until the blue color just disappears. This is the end point, and record the volume of the titer. Calculate the potassium iodide or sodium iodide content of the sample solution as follows:

$$\text{Potassium iodide present (mg or } \mu\text{g)} = V \times C\,(166/178),$$

$$\text{Sodium iodide present (mg or } \mu\text{g)} = \text{V} \times C \times (149.9/178),$$

where V is the titer of *N*-bromosuccinimide solution in milliliters, and C is the concentration of the *N*-bromosuccinimide solution in milligrams or micrograms per milliliter.

Determination of Potassium Iodide

Various known volumes of 0.1 and 0.01% potassium iodide solutions were determined by the proposed method as if they were unknowns, with 0.1 or 0.01% (w/v) *N*-bromosuccinimide solution, respectively. The results are shown in Table 1.

Determination of Sodium Iodide

Sodium iodide solutions of different concentrations (from 0.1 to 0.01%) were determined by the proposed method as if they were unknowns, with 0.1 or 0.01% (w/v) *N*-bromosuccinimide solution, respectively, giving analogous and satisfactory results.

Comparative Analysis of Potassium Iodide by the Proposed Method and the Potassium Iodate Method

Comparative analysis of potassium iodide was simultaneously done by the proposed method and the potassium iodate method described in the Dispensatory of the United States (*3*) by determining amounts of potassium iodide ranging from 500 to 100 mg as if they were unknowns.

In the proposed method each accurately weighed amount of potassium iodide was dissolved in distilled water and the volume was completed with the water up to the mark in a 100-ml volumetric flask. The determination was done on 5 or 10 ml of each solution as if it was an

TABLE 1

RECOVERY OF POTASSIUM IODIDE BY THE PROPOSED METHOD

Conc. of KI taken (%)	Vol. of soln taken (ml)	Potassium iodide content (mg)	Titer of 0.1% (w/v) *N*-bromosuccinimide soln [a] (ml)	Potassium iodide found (mg)	Error (%)
0.1	10	10	10.63	9.91	0.90
	9	9	9.50	8.86	1.56
	8	8	8.45	7.88	1.50
	7	7	7.38	6.88	1.71
	6	6	6.40	5.97	0.50
	5	5	5.30	4.94	1.20
	4	4	4.35	4.06	1.50
	3	3	3.28	3.06	2.00
	2	2	2.16	2.01	0.50
	1	1	1.08	1.01	1.00
		(μg)	0.01% [b] (ml)	(μg)	
0.01	10	1000	10.68	996	0.40
	9	900	9.58	893	0.78
	8	800	8.62	804	0.50
	7	700	7.39	689	1.57
	6	600	6.46	602	0.33
	5	500	5.35	499	0.20
	4	400	4.35	406	1.50
	3	300	3.23	301	0.33
	2	200	2.18	203	1.50
	1	100	1.08	101	1.00
	0.5	50	0.55	51	2.00

[a] One ml of 0.1% *N*-bromosuccinimide $\equiv$ 0.9326 mg of potassium iodide.
[b] One ml of 0.01% *N*-bromosuccinimide $\equiv$ 93.26 μg of potassium iodide.

unknown using 0.1% *N*-bromosuccinimide solution. The results are shown in Table 2.

Interfering Substances

In neutral medium the presence of fluoride, chloride, bromide, nitrite, nitrate, sulfate, and acetate does not interfere. On the other hand, there is interference with sulfite, hydrosulfite, and thiosulfate.

Experimental Error

The experimental error of the proposed method does not exceed $\pm 2\%$ when determining concentrations varying from 50 mg to 50 μg of potassium iodide (Table 1, 2).

TABLE 2

COMPARATIVE ANALYSIS OF POTASSIUM IODIDE BY THE PROPOSED METHOD AND THE POTASSIUM IODATE METHOD

Content (mg)	Found by the proposed method (mg)	Error (%)	Content (mg)	Found by the USP method (mg)	Error (%)
50	49.63	0.74	500	499.83	0.03
40	40.24	0.60	400	401.72	0.43
30	30.06	0.20	300	307.10	2.37
20	20.09	0.45	200	206.84	3.42
10	10.00	—	100	103.75	3.75

Application of the Proposed Method

The proposed method was applied for the determination of potassium iodide in amounts ranging from 10 mg to 500 μg in biological fluids, e.g., milk, blood, and urine as if they were unknowns. The procedure for each biological fluid is described below.

A. Milk. A 0.1-g portion of potassium iodide was dissolved in few drops of distilled water and mixed well with buffalo milk up to the mark in a 100-ml volumetric flask. An aliquot of 50 ml of the milk sample was shaken well with 50 ml of 10% trichloroacetic acid and filtered. Various known volumes of the clear filtrate ranging from 10 to 1 ml and containing from 5 to 0.5 mg of potassium iodide, respectively, were titrated as if they were unknowns with 0.1% (w/v) *N*-bromosuccinimide solution using starch indicator. The results are shown in Table 3.

TABLE 3

RECOVERY OF POTASSIUM IODIDE IN MILK BY THE PROPOSED METHOD

Vol of filtrate taken (ml)	Potassium iodide content (mg)	Titer of 0.1% (w/v) *N*-bromosuccinimide soln (ml)	Potassium iodide found (mg)	Error (%)
10	5.0	5.38	5.02	0.40
9	4.5	4.83	4.50	—
8	4.0	4.29	4.00	—
7	3.5	3.76	3.51	0.29
6	3.0	3.23	3.01	0.33
5	2.5	2.68	2.50	—
4	2.0	2.15	2.01	0.50
3	1.5	1.62	1.51	0.67
2	1.0	1.08	1.01	1.00
1	0.5	0.54	0.50	—

B. Blood. A 0.1-g portion of potassium iodide was dissolved in few drops of distilled water in a 100-ml standard flask and mixed well with buffalo blood up to the mark. An aliquot of 50 ml of the blood sample was shaken well with 50 ml of 10% trichloroacetic acid and filtered. Various known volumes of the protein-free filtrate were determined by the proposed method as if they were unknowns using 0.1% (w/v) *N*-bromosuccinimide solution and starch indicator. The results are recorded in Table 4.

C. Urine. A 0.1-g portion of potassium iodide was dissolved in human urine and the volume was completed with the same urine to 100 ml in a volumetric flask. Various known volumes were titrated as if they were unknowns with 0.1% (w/v) *N*-bromosuccinimide solution using starch indicator and the volume of the titer was noted. A blank experiment was simultaneously done by treating an exact volume of the urine sample as that previously titrated with an equal volume of 5% silver nitrate or lead acetate solution to precipitate the soluble iodide in the form of insoluble silver iodide or lead iodide, respectively, and then filtered. A known volume of the clear filtrate equal to the original volume of the previously determined urine sample was titrated with 0.1% (w/v) *N*-bromosuccinimide solution using 2 drops of methyl red indicator and the reading was multiplied by 2 and then subtracted from the titer before calculation. The results are listed in Table 5.

DISCUSSION

It is obvious (Table 2) that the experimental error of the generally accepted potassium iodate method rises when the concentration of

TABLE 4

RECOVERY OF POTASSIUM IODIDE IN BLOOD BY THE PROPOSED METHOD

Vol of protein-free filtrate (ml)	Potassium iodide content (mg)	Titer of 0.1% (w/v) *N*-bromosuccinimide soln (ml)	Potassium iodide found (mg)	Error (%)
10	5.0	5.37	5.01	0.20
9	4.5	4.81	4.49	0.22
8	4.0	4.27	3.98	0.50
7	3.5	3.75	3.50	—
6	3.0	3.23	3.01	0.33
5	2.5	2.71	2.53	1.20
4	2.0	2.18	2.03	1.50
3	1.5	1.64	1.53	2.00
2	1.0	1.08	1.01	1.00
1	0.5	0.53	0.49	2.00

TABLE 5

RECOVERY OF POTASSIUM IODIDE IN URINE BY THE PROPOSED METHOD

Vol of urine taken (ml)	Potassium iodide content (mg)	Titer of 0.1% (w/v) *N*-bromosuccinimide solution − (blank reading) (ml)	Potassium iodide found (mg)	Error (%)
10	10	10.67	9.95	0.50
9	9	9.64	8.99	0.11
8	8	8.54	7.96	0.50
7	7	7.66	7.14	2.00
6	6	6.49	6.05	0.83
5	5	5.38	5.02	0.40
4	4	4.30	4.01	0.25
3	3	3.23	3.01	0.33
2	2	2.16	2.01	0.50
1	1	1.07	1.00	—

potassium iodide diminishes. This finding is supported by a previous report on the microdetermination of soluble iodides with *N*-bromosuccinimide (*2*).

N-Bromosuccinimide is an oxidizing agent and thus can liberate iodine from potassium iodide in aqueous solution (*1*). In the presence of starch solution a blue color develops which serves to detect the end point.

The basis of the proposed method is that *N*-bromosuccinimide reacts readily and quantitatively in aqueous solution with soluble iodides, e.g., potassium iodide or sodium iodide when starch is used as the indicator. The end point is easily detected when the blue color of the indicator just disappears.

The mechanism of the interaction between *N*-bromosuccinimide and potassium iodide in neutral medium has been formulated on the ground of the data gained and is shown by the equations listed below.

Iodine monobromide and potassium succinimide are the products of the reaction. Neutralization of potassium succinimide with 5% hydrochloric acid (v/v) yields succinimide.

The proposed method is simple, rapid, and sensitive to determine concentrations as low as 50 μg of potassium iodide. The experimental error does not exceed $\pm 2\%$ (Tables 1, 2).

Comparison of the proposed method with the official potassium iodate method has shown that the previously accepted method is not highly sensitive to determine low concentration of potassium iodide because the experimental error increases with a decrease in concentration of potassium iodide to as low as 100 mg (Table 2).

Furthermore, the proposed method has been applied successfully to determine potassium iodide in biological fluids such as milk, blood, and urine. The experimental error is within the limits of $\pm 2\%$ (Tables 3, 4, 5) when the amounts of potassium iodide range from 10 to 1 mg in the biological fluid.

SUMMARY

A modified titrimetric method for the microdetermination of soluble iodides, e.g., potassium iodide or sodium iodide is described. The mechanism of the reaction between *N*-bromosuccinimide and potassium iodide in aqueous solution is discussed. The determination is done within the range from 10 mg to 50 μg for soluble iodides. The experimental error does not exceed $\pm 2\%$. Comparative analysis of potassium iodide by the proposed method and the official potassium iodate method is reported. The method has been applied to determine potassium iodide in biological fluids within the limits of 10 to 1 mg giving satisfactory results.

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Chromatographic and Spectroscopic Investigations of the Oxidation Process in Methyl Oleate and Methyl Elaidate

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INTRODUCTION

The study of the autoxidation of aliphatic compounds, especially of long-chained unsaturated acid derivatives is important because of its practical and theoretical interest.

The works dealing with these problems were performed by numerous workers (*1, 4, 15*). Some classes of products formed during autoxidation in fatty substances were thoroughly examined (*2, 3, 7*). Methyl ester of *cis*-9-octadecenoic acid (methyl oleate) was willingly applied as the model compound in the elucidation of the autoxidation process (*6, 11, 12, 17*). This fact may be connected with the abundance of *cis*-9-octadecenoic acid derivatives present in the natural fatty products.

In the prevailing investigations no attention was paid to the influence of the *cis–trans* isomerism on the course of autoxidation of the higher monounsaturated carboxylic acid derivatives.

The purpose of our work was to compare the initial stage of autoxidation in methyl oleate and methyl elaidate (i.e., methyl esters of *cis*- and *trans*-9-octadecenoic acid). The comparison was realized with the help of the following analytical techniques: high-resolution nuclear magnetic resonance spectroscopy and infrared absorption spectroscopy. The additional methods used were measurements of peroxide number, acid number and iodine number, taking into account the chromatographic separation of the essential products of oxidation by adsorption thin-layer chromatography.

REAGENTS AND METHODS

The substrates to our experiments were: oleic acid (B.D.H., England) and elaidic acid (Koch & Light, England). These acids were submitted

to estrification in the 14% solution of boron trifluoride in anhydrous methanol (*16*). The characteristics of the obtained and purified methyl esters are shown in Table 1.

Additionally the purity of the above mentioned esters was examined by gas chromatography and in both cases more than 99.5% of pure compound was stated. These analyses were performed using a Chrom-31 apparatus, produced by Laboratorni Pristroje, Czechoslovakia. The conditions of analyses were as follows: 1.2 m column packed with 3% HIFF 8 BP on Chromosorb P, argon as a carrier gas (flow rate 45 ml/min), column temperature 180°C and flame ionization detector (FID).

The 30-g samples of methyl esters were placed in Petri scales (diameter of a scale 8.5 cm) and submitted to the thermal autoxidation process for 15 days at three temperatures: 40, 50 and 60°C.

The measurements of peroxide number were performed daily, according to Polish standards (*10*).

The acid number and iodine number values were determined on the first and the last day of our experiment (*8, 9*). In order to compare the obtained data, two identical samples of every ester were oxidized simultaneously and the *a/m* measurements of characteristic numbers were performed for both of them. The values presented in Tables 2, 3 and 4 are the arithmetical mean values calculated from two measurements.

For the inquiry with the help of thin-layer chromatography (TLC), unoxidized samples and those after 15 days of oxidation were used. In order to separate the autoxidation products, the adsorption TLC was applied and the conditions were as follows: The chromatographic glass-plates (20 × 20 cm) were covered with Kieselgel H (E. Merck, Germany), the thickness of a layer being 0.3 mm; then they were dried at room temperature for 24 hr and activated at 110°C for 1 hr. On every chromatographic plate, unoxidized and oxidized samples were developed, with every sample in 20 μl of 2.5% (by wt) solutions. The solvent was composed of *n*-hexane and ethyl ether in a volume ratio of 1:1. The same mixture, i.e., *n*-hexane and ethyl ether in a volume ratio of 1:1 was used as a mobile phase.

TABLE 1

THE CHARACTERISTICS OF UNOXIDIZED ESTERS

Ester	Acid no.	Iodine no.	Peroxide no.	Refraction index n_D^{20}
Methyl oleate	1.25	89.7	0.6	1.4538
Methyl elaidate	1.30	90.6	0.4	1.4561

The chromatograms were evoked by means of following substances:

A. 5% solution of ammonium molybdate in 20% sulfuric acid;

B. 2.5% solution of kalium iodide in acetic acid, followed by 1% aqueous solution of starch (*14*);

C. 2.5% chloroformic solution of iodine, followed by 1% aqueous solution of starch (*14*);

D. 1% Dinitrophenylhydrazine solution in 2 *N* HCl (*14*);

E. 0.005% solution of new fuchsine in water (*13*).

The above presented set of evoking agents permitted to identify the following classes of compounds:

A. Compound which could be carbonized;

B. Compounds reducing iodine ions to free iodine;

C. Unsaturated compounds forming iodine adducts;

D. Compounds with free carbonyl groups;

E. This reagent unabled to determine hydrophobic and hydrophilic properties of separated compounds.

The quantitative evaluation of the chromatograms evoked by means of the solution A was performed by the help of a Photovolt model 52-C densitometer, operating in the transmitted light. To registration one used the slotlike light source (5 × 0.5 mm).

The NMR measurements were performed using a Varian, type XL-100 apparatus (Varian, Analytical Instruments Division, USA). In order to register the NMR spectra, one prepared the 28% (% by wt) carbon tetrachloride solutions of unoxidized samples and those after 15 days of oxidation. The carbon tetrachloride used was previously distilled over P_2O_5 and stored over the molecular sieves. The working conditions were as follows: frequency, 100.1 MHz; temp, 20°C; sweep width, 1000 Hz; time, 500 sec; tube o.d., 5 mm; spin rate, 28 rps; filter, 2; spectrum amplification, 16, 20; number of scans, 4.

The iR spectra were performed with the help of an UR-20 spectrophotometer (VEB Carl Zeiss, G. D. R.), under the following operating conditions: solutions, 0.1 mol/l of unoxidized esters and those after 15 days of oxidation in carbon tetrachloride for iR spectroscopy; registration speed, 64 cm^{-1}/min; paper speed, 10 mm/100 cm^{-1}; energy program $s = 4$; time constant $z = 1$; amplification, 8.8 correction filter, 2; attenuator on.

DISCUSSION

Figures 1 and 2 illustrate the changes of peroxide number values in the course of oxidation of methyl oleate (Fig. 1) and methyl elaidate (Fig. 2) at three temperatures: 40, 50 and 60°C for the initial 15 days of this process. The changes of acid number and iodine number values are shown in Table 2.

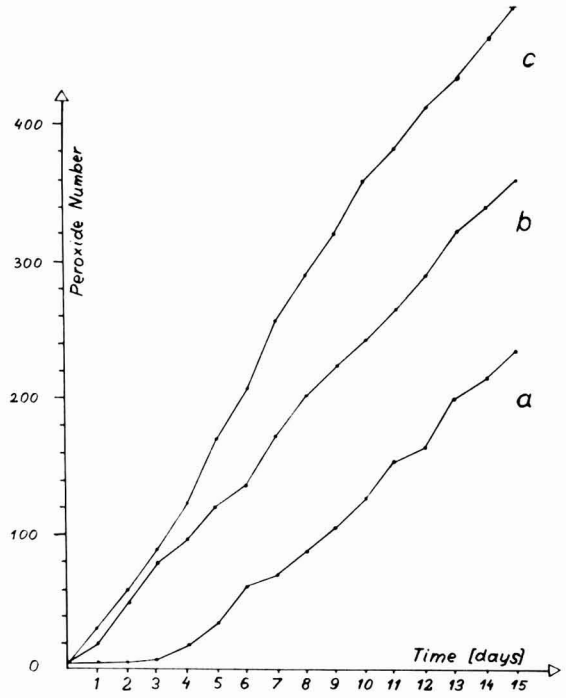


FIG. 1. The values of peroxide number (PN) against the time of oxidation of methyl oleate oxidized at (a) 40, (b) 50, and (c) 60°C.

In the case of methyl oleate, the amounts of peroxide products formed at 40, 50 and 60°C are nearly proportional to the time of oxidation and the process temperature. In the case of methyl elaidate, the peroxide number values got the lower level than those of methyl oleate. For both isomers the peroxide number values increase with the growth of temperature of oxidation. The growth of acid number for the initial 15 days of oxidation of *cis*- and *trans*-isomer confirms formation of low quantities of acidic products. It must be stated, that this process is slightly more efficient in the case of *cis*-isomer. The decrease of iodine number values for both isomers suggests the partial decay of double bonds induced with the oxidation. For *cis*-isomer, the change of iodine number value is slightly more significant, too. With the help of adsorption TLC, the oxidized samples were separated into 9 chromatographic bands. The results of chromatographic inquiries are shown in Fig. 3. Owing to the selective evoking agents, the following classes of products were determined on chromatograms (excluding unoxidized substrates of reaction):

a. Compound having free carbonylic groups;
b. Compounds of peroxidic character;
c. Compound having hydroxylic and carboxylic groups;

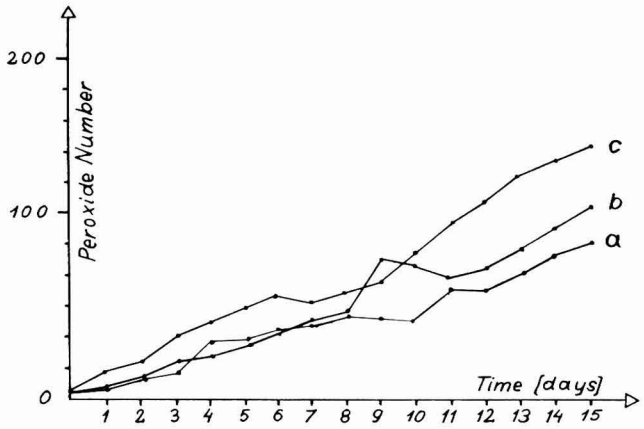


FIG. 2. The values of peroxide number (PN) against the time of oxidation of methyl elaidate oxidized at (a) 40, (b) 50, and (c) 60°C.

d. Oligomers—resting near the starting points.

In order to determine the differences in the course of autoxidation of *cis*- and *trans*-isomer, the light densities of the separated, chromatographic bands were compared. The evaluated densitograms regarded to the samples of esters oxidized at 40 and 60°C.

From the densitograms shown in Fig. 4 one may conclude, that in the case of oleic ester higher amounts of autoxidation products were obtained than in the case of another one. Carrying out the autoxidation at these temperatures one may notice, that with methyl oleate the amounts of unoxidized substrate are lower and those of peroxides are

TABLE 2

THE CHANGES OF THE ACID NUMBER AND IODINE NUMBER VALUES AFTER 15 DAYS OF OXIDATION

Ester	Unoxidized ester	Ester oxidized at (°C): 40	50	60
Acid no.				
methyl oleate	1.25	2.4	3.6	7.3
methyl elaidate	1.30	2.3	3.4	5.8
Iodine no.				
methyl oleate	89.7	84.2	81.6	68.2
methyl elaidate	90.6	88.9	81.4	73.4

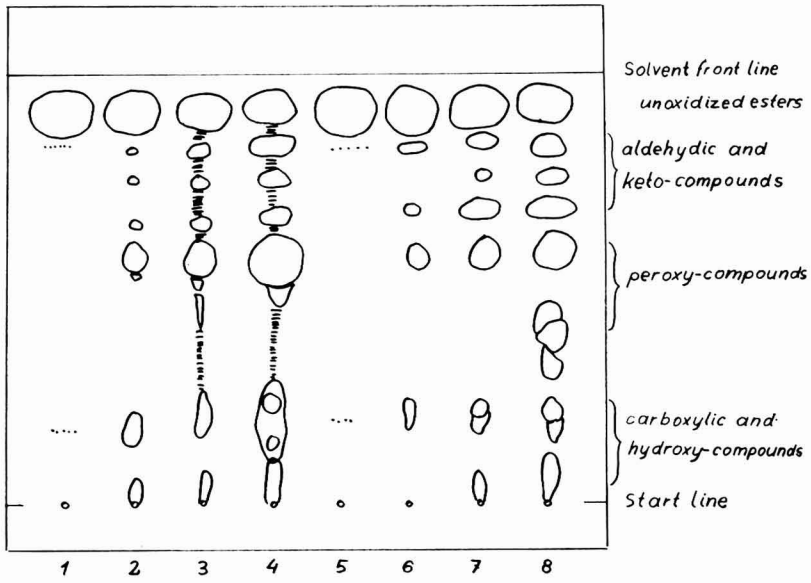


FIG. 3. The thin-layer chromatograms of unoxidized and oxidized samples of methyl oleate and methyl elaidate evoked by means of ammonium molybdate solution in sulfuric acid. (1) pure methyl oleate; methyl oleate after 15 days of oxidation at: (2) 40°C; (3) 50°C; (4) 60°C; (5) pure methyl elaidate; methyl elaidate after 15 days of oxidation at (6) 40°C; (7) 50°C; (8) 60°C.

higher than with methyl elaidate. The *a/m* facts agree with the determined course of peroxide number, acid number and iodine number changes.

Fig. 5 is an exemplary NMR spectrum of pure methyl elaidate. Table 3 shows the characteristics of this spectrum and ascribes individual bands to the proper proton groups in the molecule of examined compound.

Comparing the NMR spectra to unoxidized and oxidized samples, the changes of absorption band intensities were stated for the following proton groups:

(b) —HC=CH—

(e) —CH_2—C=C—CH_2—

(d) —CH_2—C(=O)—O—

In order to objectivate the *a/m* changes the following integral ratios of energy absorption bands were calculated: *b/h; e/h; d/h*. This assumption was allowed that the integral value of triplet (*h*), representing the

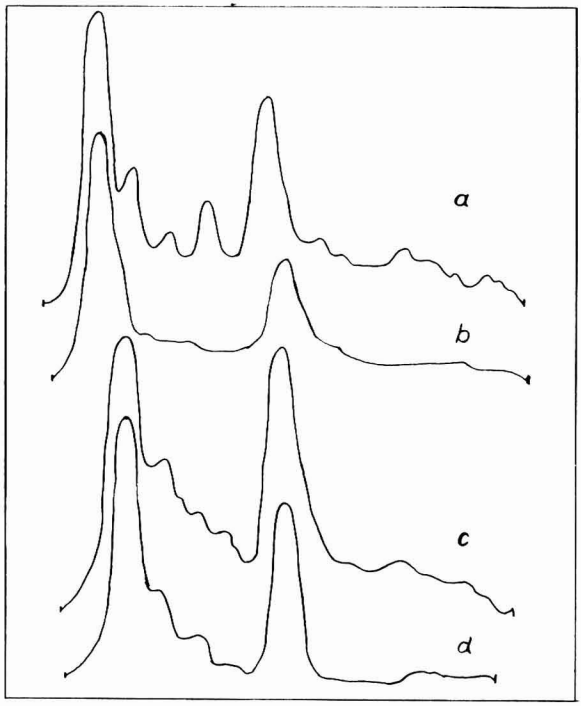


FIG. 4. The densitograms of studied esters methyl elaidate oxidized 15 days at: (a) 60°C; (b) 40°C; methyl oleate oxidized 15 days at: (c) 60°C; (d) 40°C.

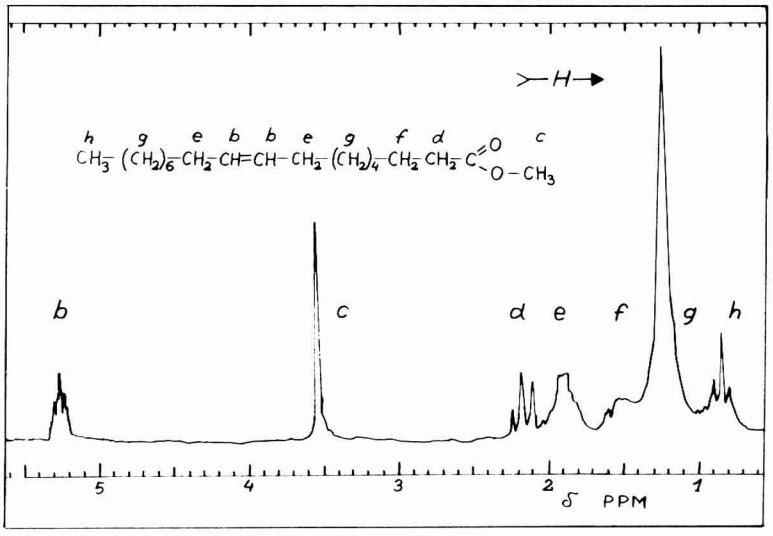
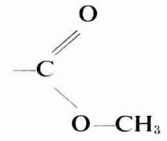
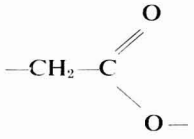
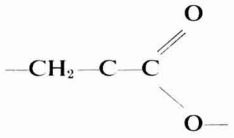


FIG. 5. NMR spectrum of pure methyl elaidate (operating conditions, see text).

TABLE 3

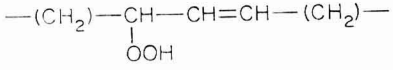
EVALUATION OF THE NMR SPECTRUM OF PURE METHYL ELAIDATE

Absorption band	Chemical shift (δppm)	Multiplicity	No. of protons	Chemical representation
b	5.28	Septet	2	—CH=CH—
c	3.55	Singlet	3	
d	2.19	Triplet	2	
e	1.92	Multiplet	4	—CH_2—C=C—CH_2—
f	1.52	Multiplet	2	
g	1.25	Singlet	20	—CH_2—
h	0.86	Triplet	3	—CH_3

terminal methyl group, is the reference value for the corresponding spectra, respectively.

Table 4 shows that the higher the temperature of oxidation of methyl oleate and methyl elaidate, the significantly lower the integral ratio values of *b/h*. It is a proof for the decrease of the quantity of protons connected with these carbon atoms, which participate in double bonds of the examinated substances.

With methyl oleate one observes the simultaneous diminution of the integral ratio values of *e/h*. It is effected with the lowering number of protons connected with the alpha-carbon atoms, neighboring to those of double bonds, i.e., with forming of the hydroperoxides in the initial stage of autoxidation (*6*):



From the changed *e/h* values one may conclude about the smaller aggregation of peroxidic compounds in the case of *trans*-isomer. Besides with

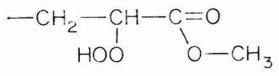
TABLE 4

THE CHANGES OF THE RATIOS *b/h*, *d/h* AND *e/h* OBSERVED AFTER 15 DAYS OF OXIDATION

Ratio	Unoxidized ester	Ester oxidized at (°C): 40	50	60
Methyl oleate				
b/h	0.63	0.57	0.46	0.45
d/h	0.67	0.69	0.74	0.78
e/h	1.34	1.19	1.10	1.03
Methyl elaidate				
b/h	0.65	0.64	0.63	0.61
d/h	0.67	0.66	0.65	0.65
e/h	1.31	1.26	1.20	1.18

methyl oleate one noted the higher *d/h* ratio values in the case of the ascending autoxidation temperatures, due to the progressing hydroperoxide destruction to the carbonylic products. It seems to agree with our TLC results, which indicate the forming of new carbonylic groups followed by the increase of activated methylene groups.

In the case of methyl elaidate the diminution of the integral ratio *d/h* values is a proof for the partial decrease of activated methylene groups. It may be explained with forming of the hydroperoxidic groups at the activated alpha-methylene position neighboring with the functional ester group.



This hypothesis agrees with the oxidation course proposed by Mercier and Serim (*5*) for the saturated acid methyl esters. The author suggests formation of the hydroperoxidic groups in alpha-position to ester group in the initial stage of autoxidation.

Figure 6 shows the fragments of iR spectra in the region 1100–900 cm^{-1} for the reference and oxidized (after 15 days of oxidation at 40, 50 and 60°C) samples. In this region we observe the absorption band (978 cm^{-1}) representing the deformation vibration of the C–H bondings, the mentioned carbon atom belonging to the disubstituted olefinic *trans* configuration.

In the iR spectra of the oxidized methyl elaidate samples the decrease in the intensity of this absorption band was observed. With methyl oleate one noted the growth in the mentioned band intensity. These facts

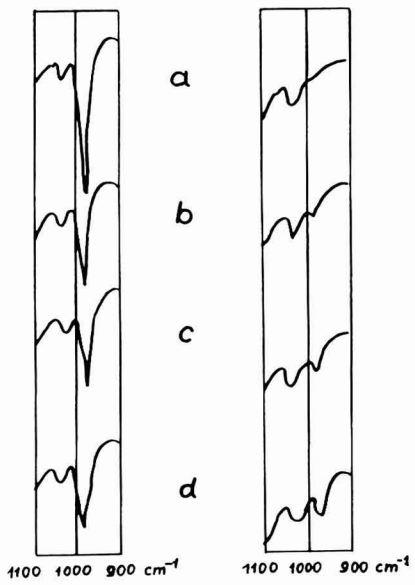


FIG. 6. Infrared spectra in the region 1100–900 cm^{-1} of the oxidized and reference samples of methyl oleate (right) and methyl elaidate (left). (operating condtions, see text).

suggest the progress of *cis–trans* isomerization evoked with the autoxidation at its initial stage. Pallotta, Piretti and Capella (*6*) found that the mixture of hydroperoxides formed during the autoxidation of pure methyl oleate at 80°C consisted of ca. 23% *cis*- and ca. 77% *trans*-isomers.

Growth in the intensity of 978 cm^{-1} band for the samples of methyl oleate oxidized for 15 days at 40, 50 and 60°C and diminution of the same band for the samples of methyl elaidate processed in the same way proves our suggestions that in the initial stage of autoxidation, the reaction courses of the studied esters appreciably differ, till they obtain the isomerization equilibrium.

CONCLUSIONS

As it proceeds from the gathered material, the differences in the first stage of oxidation of *cis*- and *trans*-9-octadecenoic acid methyl esters are effected with the differentiated structure of the oxidized substances. The angular structure of methyl oleate permits more effective forming of the peroxidic products. With *trans*-isomer of the examined ester this process runs more slowly. Nearly linear structure of the *trans*-isomer submitted to the oxidation gives the better steric packing of the ester molecules and consequently the deceleration of this process.

The *trans*-isomer is thermodynamically more stable than the *cis*-isomer and this causes the slower forming of peroxidic products. The isomerization of double bonds in the first stage of autoxidation induces the decay of the differentiation in further stages of the studied process.

SUMMARY

In our paper the studies on the initial stage of autoxidation of *cis*- and *trans*-9-octadecenoic acid methyl esters were reported. The applied analytical methods were: (1) high-resolution NMR spectroscopy; (2) absorption iR spectroscopy; (3) adsorption TLC. The complementary methods were: determination of peroxide number, acid number and iodine number in the course of autoxidation. Our results pointed to the fact that the oxidation of *trans*-isomer showed the more restrained character. We tried to suggest the influence of structure of the examined compounds and of the peroxides formed in the initial stage of autoxidation on the course of this process.

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Design and Construction of a Spectrophotometer Accommodating Long-Path Microcells

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INTRODUCTION

The primary demands on modern analytical chemistry can be summarized as follows: detecting and determining smaller amounts, and obtaining results faster and more automatically (*1*). In satisfying these demands strictly instrumental methods have been extensively used and remarkable results have been achieved. Frequently, however, the basic sensitivity and the selectivity of the instrumental method were not adequate. In many such cases the situation was remedied by utilizing a wet chemical step ahead of the instrumental finish in order to provide enrichment and separation.

One such combination which has been employed for quite some time is that of extraction with photometric determination. While this combination is very successful in solving many analytical problems, few deliberate attempts have been made to exhaust its full potential. Some possibilities for improvement, to enable the determination of even smaller amounts are of interest.

An evaluation of the existing possibilities can be made upon the examination of Lambert–Beer's law, $A = abc$, with the symbols denoting the parameters to which they are conventionally assigned. The important measuring principle is to have a high absorbance for a low effective concentration of the species to be determined. The effective concentration can usually be improved by enrichment via extraction as discussed in detail elsewhere (*2*).

From a purely instrumental point of view the minimum absorbance that can effectively be employed depends on the signal-to-noise ratio. The better this ratio, the smaller the concentration that can be determined with a certain reliability. Bettering this ratio involves the use of higher-quality components and improved design features in the optical and electronic portion of the instrument. With the components and design presently available the possibilities have almost been fully exhausted

unless one wishes to go to extremes financially. It is therefore desirable to concentrate on the two remaining parameters.

Improvement would result from increasing the absorptivity, *a*. This method of attack would include the introduction of new reagents or means of enhancing the absorptivity of compounds already in use for photometry. An enhancing technique has been developed that showed moderate success, namely, working with solutions containing sulfolane. According to unpublished results the cobalt thiocyanato complex has then a fourfold increase in absorptivity. In general, increasing the absorptivity implies synthesizing new substances. Such an approach has frequently brought success, but since the knowledge of exactly what structure is required for what purpose is limited, the research involved is very time-consuming and success is definitely not assured. In addition, absorptivity is not the only factor to be considered. The problems of selectivity, kinetics, availability, stability, freedom from interferences, and others must also be taken into account.

Only the length of the light path, *b*, remains to be examined. Obviously, increasing the path length brings about an increase in absorbance. However, under normal operating conditions with commercially available spectrophotometers, the increase in path length is invariably coupled with the requirement of a much larger volume of sample solution. What is needed is a special spectrophotometric cell providing a long light path but requiring only a small amount of sample solution for complete filling. Two principal ways exist to achieve this goal. One is to employ multiple reflection. This approach was examined, and while it could be established that the principle works, several difficulties were encountered. Most of the multiple reflection cell designs which were tested required a highly collimated beam, which is difficult to obtain without elaborate and expensive optics. In many such designs, the essential examination of cell contents is hindered or even impossible. In addition, such cells are exceedingly difficult to construct as true microcells. With these considerations, the other approach to increasing path length, namely, a mere physical expansion of cell length by using a capillary for the body of the cell seemed more promising. While this approach appears simple and obvious, considerably difficulties occur in reproducibly locating the cell in the light beam. With cells of normal diameter, the light beam is positioned within the cell, a rather simple task. With the small-diameter cell the situation is different, as the cell is placed within the beam. Then the relative position of cell and beam becomes quite critical. It was therefore felt that a single beam instrument with a single cell in a fixed position with regard to the light beam would prove best. It may be possible to precision-machine a cell carrier

to allow use of two cells, one for the reference and one for the sample; but the single-cell operation seemed to be much simpler, especially for an initial study of long-path spectrophotometry. Thus, at first the cell would be filled with reference or blank solution and 100% T set; then the reference solution would be withdrawn and the cell filled with the standard or sample solution and the photometric measurement effected. In actual experiments such a procedure proved less cumbersome than it may sound. Two ways of preparing the inside of the cell have been considered and tested. One approach was to siliconate the cell and thus make the inside walls unwettable. It was hoped that, after withdrawal of the reference solution, the cell would be virtually completely empty and ready for receiving the standard or sample solution. This was as expected, but tiny air bubbles formed that tended to cling to the wall of the small capillary when the cell was filled again. The path of the light, of course, was intolerably altered. It was found considerably more advantageous to have the inside walls as perfectly wettable as possible. Then the liquid fills the cell without a tendency to form bubbles. Rinsing is then necessary when switching from one liquid to another. If adequate amounts of sample solutions are available, rinsing with a few drops of sample suffices to fully remove the remaining traces of previous solution. If the amount of sample solution is restricted, rinsing with water and then ethanol, and ether or acetone, and finally drying by air suction is adequate. It must, however, be pointed out that the organic solvents should be of high purity, otherwise some traces of grease may coat the walls and render them unwettable. With these considerations in mind the basic features of design and construction of the instrument given in the following sections should be readily appreciated.

THE INSTRUMENT

General Layout

The optical portion of the instrument is totally based on conventional concepts. In the initial state of testing various possibilities of cell design, the optical components were simply a lamp, condensing lens, and interference filter. The simplicity of interference filter designs is, however, paid for by the necessity of having a considerable number of filters if a reasonable spectral range is desired. A simple monochromator assembly, namely the optical portion of a dismantled Bausch and Lomb Spectronic 20 was used in a special photometer, previously constructed in this laboratory (*3*). The results were satisfactory and the approach was employed again. The components can be obtained at a reasonable cost and are used in the present instrument in the order seen in Fig. 1.

Great latitude exists in the size and material for the instrument en-

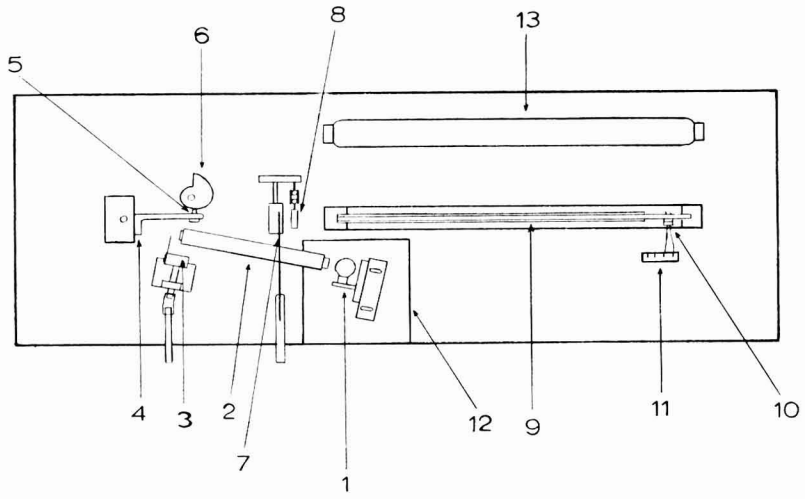

FIG. 1. General layout of the instrument: (1) lamp; (2) lens assembly; (3) control comb; (4) grating; (5) cam follower; (6) cam; (7) shutter and exit slit; (8) condensing lens; (9) cell and cell mount; (10) plug connection; (11) terminal; (12) lamp housing; and (13) inspection lamp.

closure. The apparatus described was built on a 1-in. plywood base 95 × 35 cm. These dimensions are deliberately large in order to provide room for modifications and addition that may become desirable with further developments. Aluminum plates are attached to this base where especially precise mounting of components is required. Heavy felt weather-stripping around the edge of the underside of the base prevents rocking of the instrument, damps vibrations, and sufficiently elevates the base so that wires for electrical connections can be located beneath it.

The sides (12 cm in height) and top of the instrument are cut from black Plexiglas and screwed to the plywood base. They are readily removed to facilitate repair and adjustment. Black plastic tape applied to the outside of the corners insures complete exclusion of ambient light at the Plexiglas joints. The top overlaps the sides and is secured to nylon posts driven into holes in the plywood base. Sponge-rubber tape is applied around the inside of the walls at their upper edges. The tape projects slightly above the rim and provides a tight, even seal between top and sides. The top is equipped with a door 52 cm long and 10 cm wide to allow direct access to the cell. The door panel rests on the projected portion of felt tape, cemented to the underside of the top around the door opening.

Of greatest importance is a wall that seals off the monochromator assembly from the rest of the instrument. The light beam leaves the air-

tight monochromator enclosure through a window. The reason for this partition is that particulate matter suspended in the optical path is of grave consequence. With the very narrow segment of the light beam being employed a small dust particle amounts to a considerable portion of the beam's cross-section, and thus severe fluctuations in the light intensity can result. With the wall installed as prescribed dust particles are excluded or at least not agitated by air currents.

Light Source and Collimating Assembly

The light source is a General Electric 1631X instrument lamp, attached to an adjustable bracket. The light source and adjustable mount are housed in an aluminum box fitted with felt tape around the edges. One end of the collimating lens assembly fits snugly into the housing and provides the only exit for the light. No ventilation openings are necessary since the heat generated is not excessive. The collimating assembly tube (a standard Spectronic 20 component) is securely mounted on an aluminum plate attached to the base.

Control Comb

The control comb is driven by a worm gear and adjustment is made by a knob located outside the instrument. The comb and gear are unchanged from a Spectronic 20 but are located at a different position in the optical path.

Grating and Cam Assembly

The Spectronic 20 diffraction grating, with mount and cam follower is secured in a cage-type housing between two steel slots. The bottom plate of the housing is slotted and secured to the base with screws. The slots allow slight shifting of the housing to achieve correct alignment. The cam follower is threaded into the end of the cam follower arm. This threaded shank allows for the adjustment required when calibrating the wavelength setting. The shaft of the cam driving the grating is seated in a mounting bracket with bronze bushing machined especially for the cam. A pointer to indicate the wavelength setting of the monochromator is also attached to this mounting bracket. The cam is equipped with the original Spectronic 20 wavelength scale. The wavelength setting is read through a window in the instrument top. The cam shaft projects through the top where a control knob is attached.

Monochromator Slits and Shutter

The monochromator entrance slit is located within the tube of the collimating lens assembly. The exit slit was constructed from two razor

blade edges held in position by a brass frame. The slit width is 1.5 mm at the present, but can be adjusted by shifting one of the razor blades. The frame is horizontally adjustable for proper optical alignment with the cell. The shutter was cut from brass sheet stock and slides within a dovetail in the frame of the exit slit. Shutter control is effected via a shaft extending through the wall of the instrument with an attached push–pull knob. Connected to the exit slit assembly between the slit and cell is a condensing lens that concentrates the light on the cell window. This achromatic lens is attached to a locking double ball joint clamp and enables accurate positioning of the lens and maximizes the light reaching the cell window.

Figure 2 shows details of the cell and cell-mounting system. The cell is constructed from Pyrex brand glass tubing. Two sizes of tubing have so far been employed for cell body construction; 3-mm o.d. and 0.6-mm wall, and 2-mm o.d. and 0.5 mm-wall. The body tubes are 40.0 cm in length and at the ends are fitted with 2-mm o.d. inlet and outlet tubes 1 cm long. A 1.5-cm long, 4-mm o.d. flared portion of each cell accommodates its photoreceptor, a Texas Instrument IN2175 or LS-400 photo-duo-diode. Both types of detectors are equipment with an integral lens. The light-sensitive semiconductor chip is located near the focal point of that lens. Light not entering within an extremely small angle about the optical axis misses the chip. This small angle of acceptance is especially desirable in eliminating interference by extraneous light.

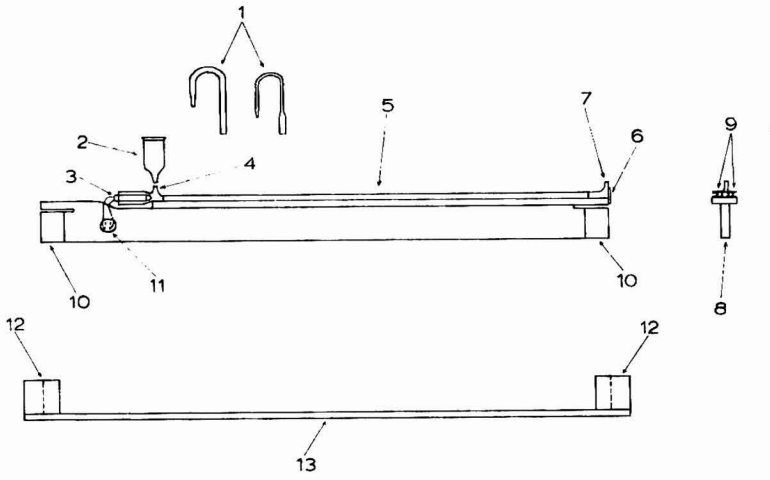

FIG. 2. Photometric cell and cell mount: (1) capillary tips; (2) 2-ml reservoir; (3) detector; (4) inlet tube; (5) cell (6) cell window; (7) outlet tube; (8) "T" rail; (9) "H" beam; (10) teflon tape; (11) plug connection; (12) mounting block; and (13) base plate.

The shape and small size of these devices (12 mm in length × 2 mm in diameter for the IN2175, and 16 mm in length and 2 mm in diameter for the LS-400) is of value in their alignment with the bore of the photometric cell. Both devices have been shown to possess the qualities desired in instruments previously constructed in this laboratory (*3–5*). With 21 V supplied by three mercury batteries of 7 V each, the LS-400 shows about 5 times greater sensitivity than the IN2175 and is therefore employed with the smaller (2-mm o.d., 0.5-mm wall) cell. Both photodiodes have adequate sensitivity throughout most of the visible range. The sensitivity, however, decreases drastically below 450 nm. The photoreceptor is wrapped in Teflon tape and permanently cemented into the flared end of the tube with epoxy adhesive. The opposite end end of the cell is fitted with a small glass window, fabricated from microscope cover-glass. The window is cut to the appropriate size (about 3 mm × 6 mm) and, except for a circular center portion, is etched with hydrogen fluoride to improve adhesion. The window is glued with epoxy to the end of the cell and the cell mount, flush with the tube end. A diaphragm consisting of a gray plastic wafer (4 mm × 12 mm) with a hole of the same diameter as the inside of the cell tube is cemented over the window so that the hole and tube bore coincide. The diaphragm prevents light from frontally entering the walls of the tube.

The tube is positioned between two "H" shaped plastic beams as used in model construction. The beams are 37.5 cm in length and cemented to the flat top of the "T" rail (see below), thereby clamping the cell firmly to the mount.

Cell Mount

The cell mount, constructed from ⅛ in. clear and colorless Plexiglas, supports the cell. The mount is made by cementing a Plexiglas strip 38 cm long and 0.8 cm wide to the top edge of a second strip, 48 cm long and 2.5 cm high to form the "T" rail. The mount is securely positioned with proper alignment in the following manner. Two mounting blocks are provided, each having a slot to receive the ends of the cell mount. For a snug fit in the slots, Teflon tape is wrapped around the ends of the "T" rail as shown in the diagram. The slotted blocks are fabricated from Micarta and glued to a plastic base plate providing a smooth mounting surface. The "T" rail and mounting unit are aligned with the light beam by maximizing detector response. The plastic baseplate with the mounting blocks is then secured to the instrument base with screws.

The leads from the photodetector are soldered to a plug connection,

secured in a hole through the cell mount. The mating plug is attached to a terminal in the instrument base.

Inspection Lamp

To enable the filled cell to be checked for air bubbles and particulate matter, an inspection lamp is provided. This is a 15-W fluorescent tube located alongside of the cell mount (Fig. 1). To provide appropriate cell illumination, the bottom of the "T" rail and the side furthest from the inspection lamp are painted with white enamel. With this arrangement, light is guided up and through the cell allowing inspection of its contents. The lamp is operated by a standard button switch and transformer as present in desk lamps.

Photodiode Circuit

The circuits described for phototitrators in previous papers (*3–5*) can serve perfectly well also here when used in connection with a sensitive galvanometer. To employ more rugged meters, and *especially* to allow for a log read-out, different circuits were required.

The circuitry employed for control and amplification of the photoreceptor as designed by G. O'Brien is shown in Fig. 3. The major components are four Fairchild integrated circuit operational amplifiers, A1–A4 (one μA725 and three μA741, respectively), and one Analog Device 751P logarithmic module. The module includes two silicon transistors (Q_1 and Q_2) and a temperature-compensating voltage divider (resistors RTC = 995 ohm and RG = 15K). Three 7-V mercury batteries provide the power for the photodiode and two further such batteries serve as the power supply for the operational amplifiers. The control elements are an on–off switch S1; a four-position mode switch, S2; two potentiometers, P1 and P2, to set zero and 100% *T*, respectively; and a potentiometer, P3, for voltage offset. The mode switch provides setting of $\%T$, $\%T\times2$, $\%T\times3$ and A (absorbance). The voltage offset control is equipped with a ten-turn counter dial. Potentiometers P4–P8 serve to internally calibrate the circuit functions. All components except the control elements are soldered to an etched circuit board. The entire circuit is enclosed in a Bud (#CU-3008) Minibox. The connection to the photodiode is made by a shielded cable with a free-floating ground on the photodiode end. The connection to the meter is effected by standard wire.

Signal Readout

The circuit just described provides an output between 0.000 and 1.000 volt as corresponding to 0 and 100% transmittance. The voltage

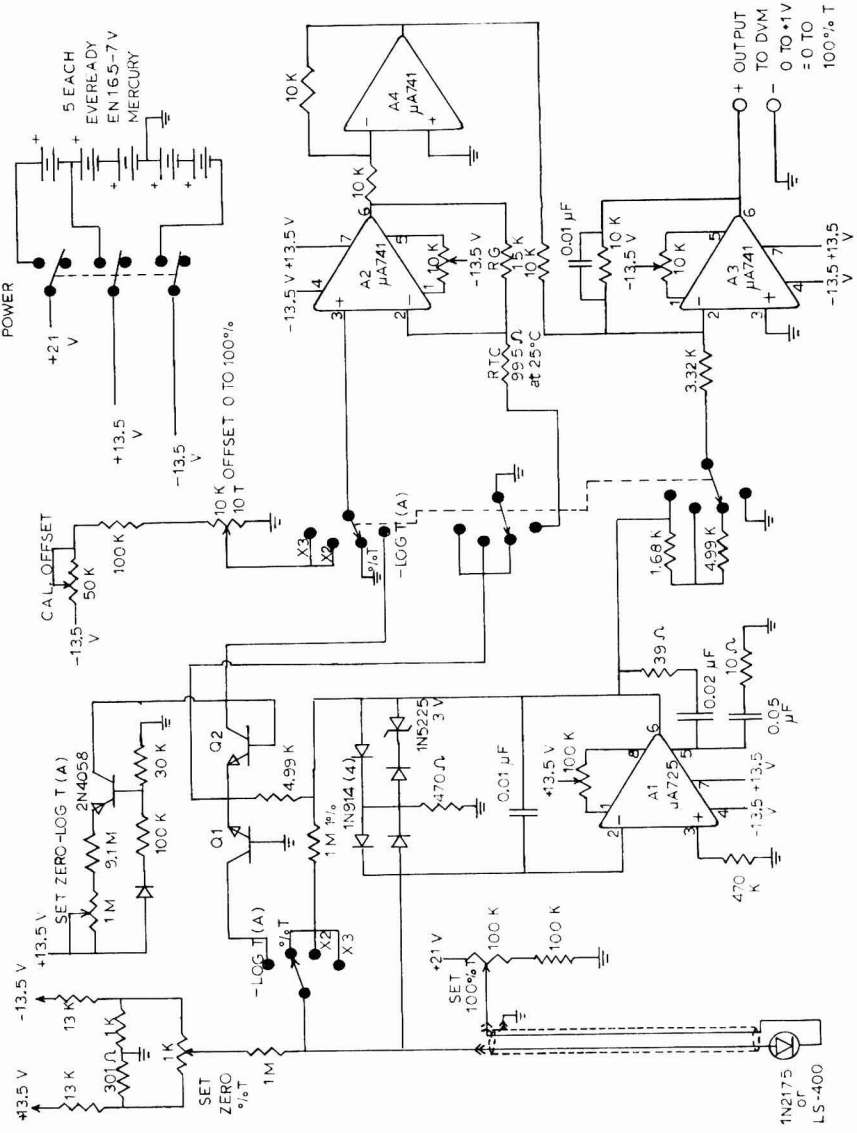

FIG. 3. Photodiode circuit.

can be read on a 0–1 voltmeter. However, such meters are not readily available and instead one may use an amperometer. A good quality meter with full scale current from 10 μA to 10 mA is satisfactory. When employing a current meter, it is necessary to use a resistor, R, in series with the meter. The value of this resistor in ohms can be computed from

$$R = (1/I_m) - R_m$$

where I_m and R_m are the full-scale meter current in amperes and the internal resistance of the meter in ohms, respectively. When available, a digital voltmeter is of the greatest convenience. In the present experiments a Keithley Multimeter was used. Such a meter allows the reading of very small signals when using a lower-range than the 0–1-V range.

Power Supply for Photometer Lamp

Power for the light sources may be provided by two or more 6-V automotive storage batteries connected in parallel. However, with the 1631X lamp employed in the instrument, frequent recharging of the batteries is required. Therefore, the use of an electronic power supply is more advantageous. The schematics of the circuit for a highly stable power supply especially designed by G. O'Brien for the use with the instrument is shown in Fig. 4.

The lamp switch, LS, activates the supply and the voltage applied to the lamp. At the supply terminals 5 to 7 V dc and 0–3.5 A are available. The normal load with the 1631X lamp is 6.5 V and 2.75 A. The lamp supply is equipped with an input, AR, for automatic intensity regulation. This input can be used in conjunction with another photodiode to provide additional stabilization via an essentially semidouble beam configuration. The entire circuit is enclosed in a Bud (#WA-1540) cabinet.

The power supply needs a 10-min warmup period after which it is fully stable with regard to long- and short-term drifts.

INSTRUMENT OPERATION

The instrument is used and monitored as any spectrophotometer. The description given here of its operation should point out the specifics especially in connection with the special circuits.

Standard Procedure

Filling the cell may be accomplished by using the inverted U attachment or the small (about 2-ml capacity) reservoir (Fig. 2). Either is attached to the inlet tube of the cell with a short section of surgical

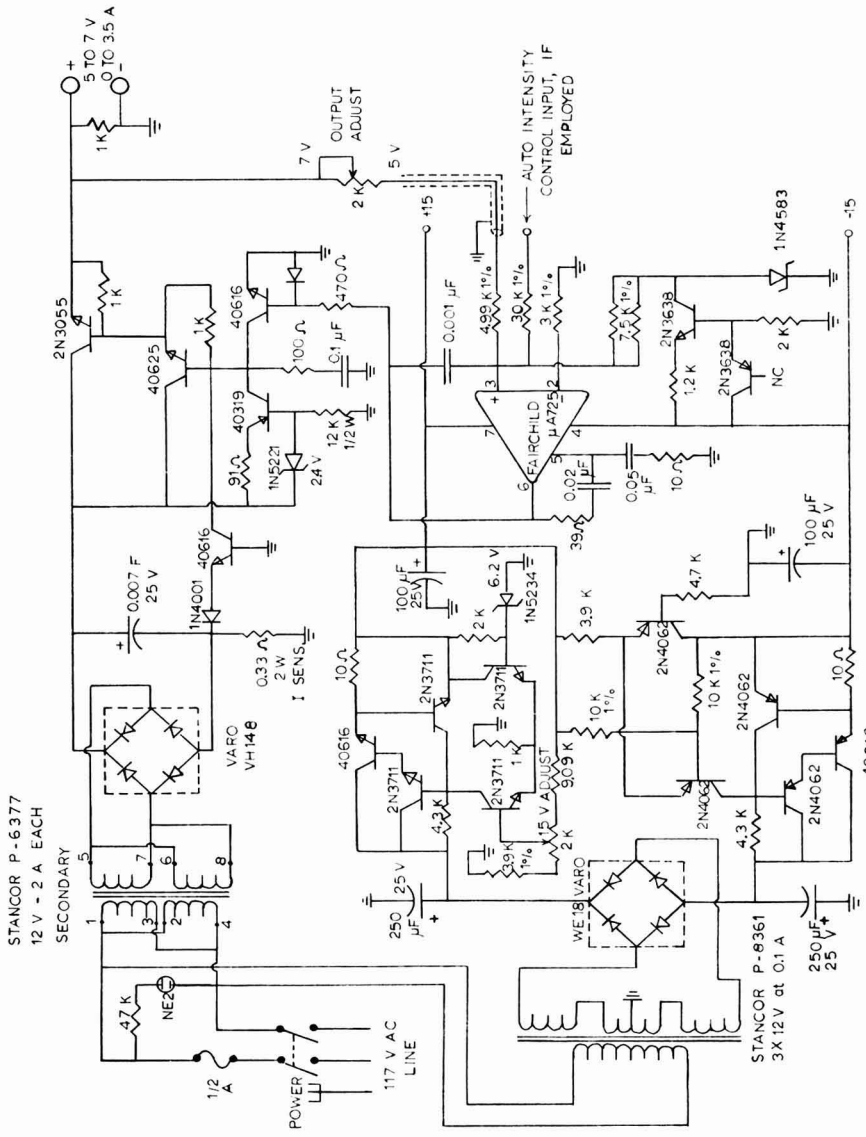

FIG. 4. Lamp power supply circuit.

rubber tubing. The cell outlet tube is connected via surgical tubing to a simple liquid trap made from an Erlenmeyer flask. The outlet of the trap is equipped with a further section of surgical tubing to which is added a small glass mouthpiece or the hypodermic syringe. Filling the cell is then accomplished by applying a slight amount of suction by mouth or syringe.

The following operational description holds for the instrument used in connection with the photodiode circuit described. If any other circuit is used appropriate changes must be made:

(1) Set the desired wavelength with the wavelength control.

(2) Switch on the meter.

(3) Switch on the photodiode circuit and set the mode selector to "%T".

(4) Close the optical shutter and adjust 0% transmittance (0.000 V.) with the zero-set control.

(5) Open the shutter and roughly set 100% transmittance (1.000 V) with the lamp voltage control and the light intensity control comb.

(6) Make the fine adjustment of 100% transmittance with the "100% *T* set" of the photodiode circuit.

(7) Close and reopen the shutter, to check the 0 and 100% transmittance settings, respectively.

When operating below 450 nm, where the sensitivity of the photodiode decreases, the photodiode circuit should be set to "%*T* × 2" or "%*T* × 3" position to obtain adequate meter readings. However, with the mode switch in these two positions the "100% *T* set" control is ineffective and 100% transmittance must be adjusted by carefully turning the knob of the light-intensity control comb.

After appropriate setting of 0 and 100% transmittance with the shutter and blank solution, the standards and sample are introduced as described for the blank solution. It is best to first wash the cell with two portions of the solution to be measured before filling to take the reading. The readings may be taken either as percent transmittance or as absorbance according to the position of the mode switch.

Voltage Offset Control

The voltage offset control enables shifting of the zero in the negative direction providing increased sensitivity by zero suppression. The control is effective only in the "%T×2" and "%T×3" positions. The scale length is increased by setting the number of additional scale units desired (from 0 to 100) on the voltage offset control counterdial and adjusting the light intensity with the supply voltage voltage control and intensity control comb to obtain a 100% scale reading. The effective

zero is then displayed downscale (negative) the number of scale units shown on the ten-turn counterdial. For readings below the meter zero the voltage offset is returned to the zero position and the effective zero and meter again coincide. When using a digital voltmeter with three and a half digit readout, a large scale range can be obtained by simply increasing the light intensity (on any of the % transmittance mode positions) to give a meter reading up to the limit (1.999 V).

Regardless of the mode of operation, the "0 $\%T$ set" should be adjusted with the mode selector switch in the "$\%T$" position to give a reading of 0.000 V.

PERFORMANCE TESTS

Wavelength Calibration

Interference filters with sharp maxima were used to calibrate the wavelength scale. An interference filter is placed in front of the cell window and the cam rotated to give maximum detector output. The peak transmission wavelength of the filter is then set on the indicator scale and the scale locked into place on the threaded cam shaft with the nut and lock washer. Fine adjustment of the setting is made using the treaded cam follower.

With the present instrument the calibration conducted in this manner was verified with six additional intereference filters with peak transmission wavelengths between 420 and 700 nm. All of these filters read within 3 nm of their labelled values.

Stability

With sufficient warmup of lamp and lamp power supply, the instrument showed only minor short term fluctuations (0.001 Volt). No significant long term drift was observed. With the door closed, bright external light has no effect on the instrument readings. Less than 0.2% transmittance change was observed in the zero setting (with the shutter closed) when the lamp power supply was switched off and on. With the Keithley Digital Multimeter the instrument showed readings of good stability even on the 0–10 mV scale (2–3% transmittance change).

Fluctuations can usually be traced to particulate matter suspended in the optical path outside or within the cell or to spikes in the line voltage. On a well-regulated line, the lamp power supply shows stability within 1 mV.

Removal and replacement of the cell and cell-mount unit generally results in changes of less than 0.2% transmittance.

The detector output can be maximized for each separate cell by

readjusting the condensing lens but such adjustment is not required to obtain an adequate detector response when the cell is changed. When the detector response for the cell with the large body tube (3 nm-o.d., and 0.6-mm wall) was maximized and the smaller cell (2 mm-o.d., and 0.5-mm wall) substituted the coincidence of the light beam with the cell bore remained adequate.

The linearity of the instrument's indicated response was tested with screens according to a method previously described (*4*). Linearity up to the absorbance value of 1.6 (that of the most absorbing screen at hand) was demonstrated.

Testing of Operational Modes

The various operational modes of the photodiode circuit were tested for their internal consistency and for accuracy relative to each other. The logarithmic mode was found to be accurate for absorbance readings up to 3. The voltage offset control was shown to provide an accurate and precise zero suppression when comparing the meter reading to the reading of the ten-turn counter dail.

THE EFFECTIVE PATH LENGTH

Before proceeding with this discussion it is important to differentiate clearly between the *effective path length* and the *actual cell length.* The *effective path length* refers to the b in Lambert–Beer's law, $A = abc$, and is the mean distance traversed by the light through the solution being measured. The *actual cell length* is the distance between the cell windows within the cell.

The effective path lengths of the cell were calculated from comparison of the absorbance values of solutions obtained with the instrument to those read on a Cary 16 Spectrophotometer. In this experiment the assumption was made that for the Cary 16 the actual cell length and effective path length are equal.

Figure 5 shows the results obtained when solutions of methylene blue were measured at 650 nm in the microcell of 40-cm actual cell length (Curve A) and the 10.0-cm cell of the Cary 16 (Curve B). From the ratio of the slope of these curves the effective path length of the microcell is calculated to be 48.2 cm. Similar measurements were made with solutions of bromocresol purple, buffered to pH 10, at 590 nm; fast gray at 545 nm; red G at 520 nm, and glycinecresol red, buffered to pH 5, at 415 nm. The effective path lengths were in the range 47–49 cm. The differences in effective path length over the actual cell length can be understood if the internal reflection due to diverging

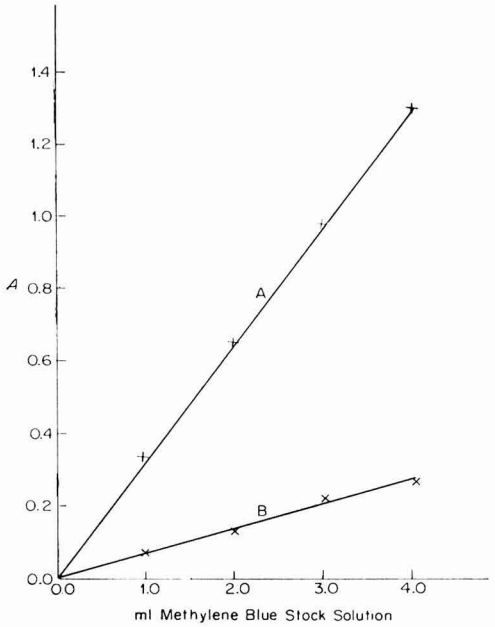

FIG. 5. Calibration curves for an aqueous solution of methylene blue on the long-path Instrument (curve A) and Cary 16 spectrophotometer (curve B).

light reflecting off the cell walls is considered. Since the incident light is not parallel, it is to be expected that changes in the refractive index due to changing the solution within the cell would cause a difference in effective path length by altering the angles at which entering light strikes the cell walls and thus increasing or decreasing the number of reflections occurring as the light passes down the tube.

The fact that the spectral bandwidth of the instrument (approximately 10 nm, constant) is about 10 times that of the Cary 16 at normal settings may also cause a variation in the measured effective path length. However, experiments in which the spectral bandwidth of the Cary 16 was increased by opening the slit have shown that this factor constitutes little of the observed variation if compounds with broad absorbance maxima are employed for measurement of the effective path length.

The effective path length may be appreciably varied by adjustments made within the optical path of the spectrophotometer.

The possible variation in effective path length, regardless of cause, is no problem at all if the general rule of photometric analysis is followed, namely, to establish the calibration and reference under conditions identical to those when measuring the sample.

ACKNOWLEDGMENTS

Great help was received from Mr. G. O'Brien, who designed and built (in part) the electronic circuits and supplied advice on pertinent matters. R. B. was recipient of a fellowship sponsored and funded by the J. T. Baker Chemical Co., Phillipsburg, N.J.

SUMMARY

An increase in sensitivity of spectrophotometric measurements results when small volumes of enriched solutions can be used. Commercial instruments do not allow application of this principle to the extent desirable. A simple single-beam, single-cell instrument is described that accomodates 40-cm cells with less than 0.8-ml solution required for complete filling and showing an effective path length of about 48 cm. The instrument can be built from readily available components and material at a reasonable effort and cost.

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Macro- and Microdetermination of Mercury(I) Using Potassium Permanganate as an Oxidant in Acid Medium in Presence of Fluoride Ions

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INTRODUCTION

In a previous investigation the reaction between Hg(I) and $KMnO_4$ in presence of fluoride ions in H_2SO_4 solution was studied both visually (*1*) and potentiometrically (*2*). The difficulty with sulfuric acid is that mercurous sulfate precipitates out and dissolves slowly during the titration, transforming on oxidation into the soluble $HgSO_4$.

The present investigation involves performance of the titration in nitric acid medium and application of the method for the microdetermination of Hg(I) both in nitric acid and sulfuric acid media.

EXPERIMENTAL METHODS

Preparation and Standardization of Solutions

The potassium permanganate solutions were prepared as given before (*3*).

Mercurous nitrate solutions were prepared by dissolving the solid in 1 liter of twice-distilled water containing 10 ml of AnalaR concentrated nitric acid. The solution was then filtered through a 1 G 3 sintered glass funnel, and standardized by titration with $KMnO_4$ (*2*).

Other solutions including ~9 *N* H_2SO_4, ~8 *N* HNO_3, ~2% NaF and ~0.25 *M* $CuSO_4$ were prepared by dissolving the C.P. materials in twice-distilled water.

Potentiometric Equipment

This is the same as previously described (*3*).

RESULTS AND DISCUSSION

A. Macrodetermination of Mercury (I)

i. The effect of acidity.

Table 1 shows that good end points (EP) are obtained at acidities

TABLE 1

EFFECT OF ACIDITY

	Acidity HNO_3 (N)	Expt. EP (ml)	Theoretical	Error (%; ±)	Inflection at EP mV/0.1 ml of titrant
Potentiometric [a]					
	0.08	6.97	6.88	1.30	29
	0.2	6.84	6.88	0.60	113
	0.4	6.82	6.88	0.87	176
	0.8	6.94	6.88	0.87	218
	1.2	6.92	6.88	0.60	240
	2.4	7.10	6.88	3.20	259
Visual [b]					
	0.12	5.76(turbid)	5.72	0.35	
	1.1	5.76	5.72	0.70	
	2.0	5.88	5.72	2.80	

[a] Titration of 10 ml of 0.0677 N Hg_2^{2+} with 0.123 N $KMnO_4$ using 50 ml of 2% NaF

[b] Visual titration of 10 ml of 0.0568 Hg_2^{2+} with 0.1241 N $KMnO_4$, using 50 ml of 2% NaF and 2 ml of $CuSO_4$.

ranging from 0.2–1.2 N HNO_3 both in the potentiometric and visual titrations on using 50 ml of 2% NaF solution. At lower acidities, e.g., 0.08 N or less the end points are attained later due to partial production of Mn(IV). Similarly at higher acidities, e.g., 2.4 N the end point is attained later, due to slight decomposition of $KMnO_4$ in presence of higher concentration of the acid. The titration curves shown in Fig. 1

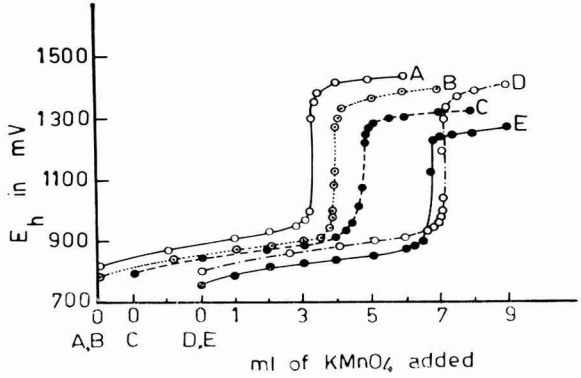

FIG. 1. Titration of 10 ml of 0.0677 N Hg_2^{2+} with 0.123 N $KMnO_4$: (A) 0.8 N HNO_3 and no NaF added. (B) 0.8 N HNO_3 and 50 ml of 2% NaF added. (C) 0.4 N HNO_3 and 50 ml of 2% NaF added. (D) 2.4 N HNO_3 and 50 ml of 2% NaF added. (E) 0.8 N HNO_3 and 50 ml of 2% NaF added at 50°C.

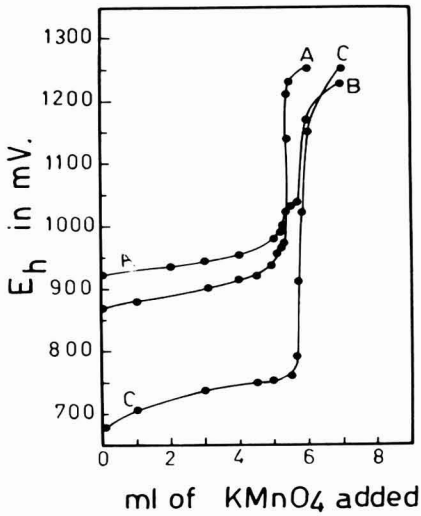

FIG. 2. (A) 6 ml of 0.06067 *N* Hg(I), 0.0854 *N* $KMnO_4$, in 0.8 *N* HNO_3, 50 ml of 2% NaF, total vol, 100 ml. (B) 6 ml of 3.03×10^{-3} *N* Hg(I), 4.27×10^{-3} *N* $KMnO_4$, in 0.8 *N* HNO_3 (containing traces of nitrous oxides), 25 ml of 2% NaF, total vol, 50 ml. (C) 6 ml of 6.07×10^{-4} *N* Hg(I), 8.54×10^{-4} *N* $KMnO_4$, in 1.2 *N* HNO_3, 10 ml of 2% NaF, total volume, 20 ml.

are more or less smooth and characterized by sharp inflections at the end points.

The values of the formal redox potentials of Hg^{2+}/Hg_2^{2+} system as evaluated from the titration curves are shown in Table 2A.

These data show that the redox potential remains approximately constant within the acidity range from 0.4–2.4 *N*. At concentrations lower than 0.4 *N* HNO_3 the potential increases indicating that the reaction performed at lower acidities depends on the $[H^+]$ of the solution in contradistinction to the reactions performed at higher ones. It is very

TABLE 2

REDOX POTENTIAL OF Hg^{2+}/Hg_2^{2+} COUPLE

A						
Normality of HNO_3	0.08	0.2	0.4	0.8	1.2	2.4
Conc of NaF (*M*)	0.238	0.238	0.238	0.238	0.238	0.238
Formal redox potential (V)	1.02	1.00	0.87	0.87	0.86	0.87
B						
Conc of NaF (*M*)	0.119	0.238	0.357			
Normality of HNO_3	0.8	0.8	0.8			
Formal redox potential (V)	0.88	0.87	0.86			

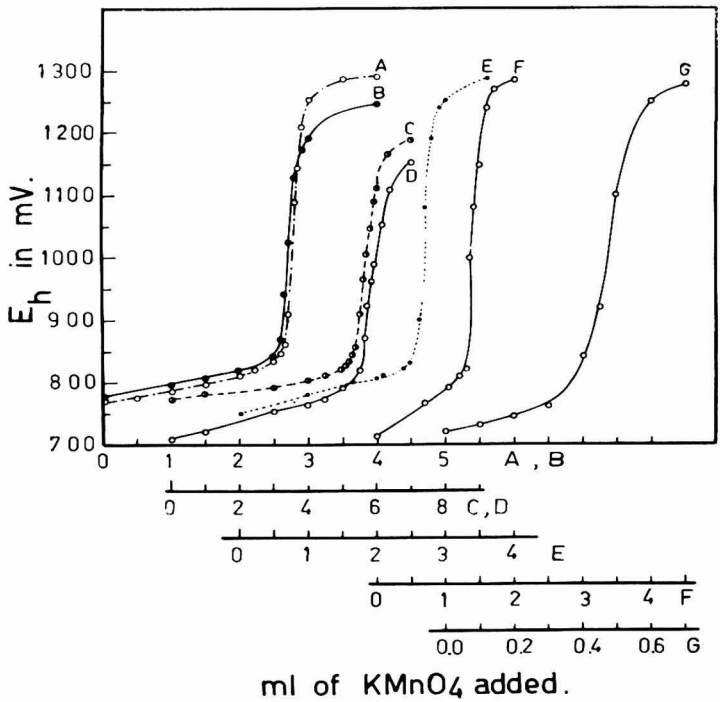

FIG. 3. Titration of Hg(I) with $KMnO_4$ in presence of 1% NaF and total vol, 50 ml: (A) 3 ml of 3.03 × 10^{-3} *N* Hg(I), in 0.9 *N* H_2SO_4, 3.42 × 10^{-3} *N* $KMnO_4$. (B) 3 ml of 3.03 × 10^{-3} *N* Hg(I), in 0.45 *N* H_2SO_4, 3.42 × 10^{-3} *N* $KMnO_4$. (C) 6 ml of 6.07 × 10^{-4} *N* Hg(I), in 0.45 *N* H_2SO_4, 8.54 × 10^{-4} *N* $KMnO_4$. (D) 6 ml of 6.07 × 10^{-4} *N* Hg(I), in 0.36 *N* H_2SO_4, 8.54 × 10^{-4} *N* $KMnO_4$. (E) 3 ml of 3.03 × 10^{-3} *N* Hg(I), in 0.36 *N* H_2SO_4, 3.42 × 10^{-3} *N* $KMnO_4$; 3 ml of 0.25 *M* $CuSO_4$. (F) 1.5 ml of 3.03 × 10^{-3} *N* Hg(I), in 0.36 *N* H_2SO_4, 3.42 × 10^{-3} *N* $KMnO_4$; 3 ml of 0.25 *M* $CuSO_4$. (G) 0.5 ml of 3.03 × 10^{-3} *N* Hg(I), in 0.36 *N* H_2SO_4, 3.42 × 10^{-3} *N* $KMnO_4$; 3 ml of 0.25 *M* $CuSO_4$.

probable that at low acid concentration hydrolysis takes place with the result that monovalent mercury is present as an oxygenated species and hence involve hydrogen ions in the process of oxidation. At the same time the higher redox potential of the Hg^{2+}/Hg_2^{2+} system would indicate a lower rate of reaction and may lead to errors resulting from the production of some higher oxidation state of manganese, mainly Mn(IV). As the acid concentration is increased hydrolysis of the mercurous ions is inhibited and hence the solution would contain only simple Hg(I) ions, the oxidation of which involves only transfer of electrons without the interference of hydrogen ions.

ii. The effect of fluoride ion concentration. The results shown in Table 3 indicate that the fluoride ion concentration can be varied from

TABLE 3

EFFECT OF NaF CONCENTRATION

Vol of 2% NaF added (ml)	EP (ml)		Error (%; ±)	Inflection at EP (mV/0.1 ml of titrant)
	Expt.	Theoretical		
Potentiometric [a]				
—	6.25	6.88	9.20	301
25	6.92	6.88	0.60	149
50	6.94	6.88	0.87	218
75	6.80	6.88	1.17	252
Visual [b]				
25	5.74	5.72	0.35	
50	5.72	5.72	Nil	
75	5.73	5.72	0.17	

[a] Titration of 10 ml of 0.0677 *N* Hg_2^{2+} with 0.123 *N* $KMnO_4$ in 0.8 *N* HNO_3.

[b] Visual titration of 10 ml of 0.0568 *N* Hg_2^{2+} with 0.1241 *N* $KMnO_4$, using 5 ml of 8 *N* HNO_3 and 2 ml of $CuSO_4$.

0.119 to 0.238 *M*, while the amount of nitric acid is kept constant at 0.8 *N* HNO_3, without appreciable errors. The inflection at the end point increases with increasing concentration of the fluoride ion present in the reaction medium.

From the titration curves the formal redox potential values of Hg^{2+}/Hg_2^{2+} system are evaluated as shown in Table 2B.

It is obvious that the redox potential of the Hg^{2+}/Hg_2^{2+} system does not change appreciably by the variation of fluoride ion concentration while keeping the acid concentration constant indicating that no interaction occurs between monovalent mercury and fluoride ions.

iii. The effect of $CuSO_4$. The results recorded in Table 4, show that the inflection at the end point increases from 152 to 209 mV/0.1 ml of

TABLE 4

EFFECT OF $CuSO_4$

Titration of 10 ml of 0.0677 *N* Hg_2^{2+} with 0.123 *N* $KMnO_4$ in 0.8 *N* HNO_3 and 1% NaF.

0.25 *M* $CuSO_4$ added (ml)	EP (ml)		Error (%; ±)	Inflection at EP (mV/0.1 ml of titrant)
	Expt.	Theoretical		
—	6.88	6.88	Nil	152
20	6.85	6.88	0.44	209

TABLE 5

EFFECT OF TEMPERATURE

Temp (°C)	EP (ml) Expt.	EP (ml) Theoretical	Error (%; ±)	Inflection at EP (mV/0.1 ml of titrant)
Potentiometric [a]				
25	6.88	6.88	Nil	273
40	6.84	6.88	0.60	176
50	6.85	6.88	0.44	94
60	6.68	6.88	3.00	60
Visual [b]				
25	5.73	5.72	0.17	
50	5.71	5.72	0.17	
75	Turbid	5.72	—	

[a] Titration of 10 ml of 0.0677 *N* Hg_2^{2+} with 0.123 *N* $KMnO_4$ in 0.8 *N* HNO_3 and 1% NaF.

[b] Visual titration of 10 ml of 0.0568 *N* Hg_2^{2+} with 0.1241 *N* $KMnO_4$ using 5 ml of 8 *N* HNO_3 and 50 ml of 2% NaF.

titrant by addition of $CuSO_4$ to the reaction mixture. Furthermore, the presence of $CuSO_4$ has not much influence on the accuracy of the results or on the character of the titration curve.

The formal redox potential of the Hg^{2+}/Hg_2^{2+} system amounts to 0.87 V in absence or presence of 20 ml of $CuSO_4$.

In the visual titration of relatively concentrated solutions of Hg(I), the blue color of Cu^{2+} ions conceals the pink color of MnF_4^- complex which vitiates the end point.

iv. The effect of temperature. The results shown in Table 5, indicate that Hg(I) can be titrated with $KMnO_4$ safely at temperatures up to 50°C, provided the optimum conditions of acidity and F^- ion concen-

TABLE 6

EFFECT OF Hg(I) CONCENTRATION

Titration 0.0677 *N* Hg_2^{2+} with 0.123 *N* $KMnO_4$ in 0.8 *N* HNO_3 and 1% NaF.

Hg(I) (ml)	Hg(I) (mg)	EP (ml) Expt.	EP (ml) Theoretical	Error (%; ±)	Inflection at EP (mV/0.1 ml of titrant)
5	56.8	3.45	3.44	0.29	253
10	113.6	6.94	6.88	0.87	218
15	170.4	10.24	10.32	0.79	175
30	340.8	20.55	20.64	0.44	74
Visual	113.6	6.88	6.88	Nil	(No $CuSO_4$)
	227.2	20.60	20.64	0.2	(+6 ml of $CuSO_4$)

tration are applied. At higher temperatures, e.g., 60°C, early end points are attained due to partial formation of Mn(II). Also the inflections at the end points decrease with increasing temperature.

v. The effect of concentration of Hg(I). On applying the optimum conditions, the titration curves for different concentrations of Hg(I) possess almost the same character. Good results are obtained for amounts of Hg(I) up to 340 mg as recorded in Table 6.

B. Application of the Method for the Microdetermination of Hg(I)

Table 7B, lists the results of determining small amounts of Hg(I) in presence of nitric acid and NaF under the optimum conditions.

It was essential that the nitric acid be purified from any traces of nitrous oxides that interfere in the reaction especially at low concentrations of Hg(I). Amounts of Hg(I) as low as 0.127 mg can be titrated

TABLE 7

DETERMINATION OF MILLIGRAM AMOUNTS OF Hg(I)

No.	Hg(I) (mg) Taken	Found	Difference (± mg)	Inflection at EP (mV/0.05 ml of titrant)	Total vol (ml)
A. In H_2SO_4					
1 [a]	73.03	73.03	Nil	120	100
2	3.65	3.65	Nil	80	50
3	1.83	1.83	Nil	90	50
4	0.73	0.75	0.02	80	50
5 ($CuSO_4$)	0.73	0.75	0.02	140	50
6 ($CuSO_4$)	0.913	0.917	0.004	180	50
7 ($CuSO_4$)	0.304	0.307	0.003	160	50
8 ($CuSO_4$)	0.73	ppt	—	—	20
B. In HNO_3					
1 [b]	73.03	73.03	Nil	120	100
2	73.03	73.05	0.02	130	75
3	73.03	73.06	0.03	150	50
4	3.65	3.65	Nil	50	50
5	3.65	3.67	0.02	60	20
6	0.73	0.75	0.02	60	50
7	0.73	0.75	0.02	80	20
8	0.38	0.38	Nil	80	50
9	0.13	0.13	Nil	70	50

[a] Nos. (1) 0.06067 *N* Hg(I), 0.0854 *N* $KMnO_4$; (2,3) 3.03×10^{-3} *N* Hg(I), 2.14×10^{-3} *N* $KMnO_4$; (4–8) 6.07×10^{-4} *N* Hg(I), 8.54×10^{-4} *N* $KMnO_4$.

[b] Nos. (1–3) 0.0607 *N* Hg(I), 0.0854 *N* $KMnO_4$; (4,5) 3.03×10^{-3} *N* Hg(I), 4.27×10^{-3} *N* $KMnO_4$; (6–9) 6.07×10^{-4} *N* Hg(I), 3.42×10^{-4} *N* $KMnO_4$; $CuSO_4$ is present in all titrations performed.

safely in 0.8 *N* HNO_3 in the presence of 1% NaF and a total volume of 50 ml, the inflection at the end point amounts to 70 mV/0.05 ml of titrant.

Table 7A, shows the results when using H_2SO_4 instead of HNO_3 at acid concentrations ranging from 0.36 to 0.90 *N*. It was found that, when using a total volume of 50 ml, 1% NaF, and optimum acidity, good results are obtained without the appearance of any precipitate due to Hg_2SO_4. Amounts of Hg(I) as low as 0.3 mg can be determined accurately with an inflection amounting to 160 mV/0.05 ml titrant in presence of $CuSO_4$.

In conclusion it is preferable to use sulfuric acid as a medium in the microdetermination of Hg(I) rather than HNO_3 because any traces of nitrous oxides present in the nitric acid interfere with the potentiometric titrations of dilute solutions of Hg(I) leading to erroneous results. Also the addition of $CuSO_4$ to the titration mixture is essential in order to accelerate the reaction and to shorten the time needed to attain the equilibrium.

It is worthy of mention, that a volume of 20 ml is not suitable for the titration owing to the precipitation of Hg_2SO_4, which reacts slowly with dilute permanganate solutions, leading to erroneous results.

SUMMARY

Mercurous mercury can be titrated potentiometrically and visually with $KMnO_4$ in the presence of fluoride and nitric acid. The optimum conditions are 0.2–1.2 *N* HNO_3 in presence of 1% NaF at temperatures not exceeding 50°C.

The method can be applied for the microdetermination of amounts as small as 0.13 mg of Hg(I) both in HNO_3 and H_2SO_4. The latter acid is preferable.

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MICROCHEMICAL JOURNAL 17, 612–618 (1972)

Oxidation of Glycerol, Mannitol and Some Saccharides with Co(III)

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INTRODUCTION

Due to its high redox potential, Co(III) acetate has recently been developed as a volumetric oxidant for the determination of some ions and organic compounds. All papers published on the use of Co(III) compounds as oxidizing agents have been summarized in a review which appeared recently (*1*). In the present paper, the investigation has been extended to the study of its reaction in both nonaqueous and aqueous media, at laboratory and elevated temperature with glycerol, mannitol and some saccharides with a view to understand the mode of their stepwise oxidation, establishing the conditions for quantitative reactions and comparing its oxidizing behavior with that of Pb(IV) acetate and some other oxidants (*2–6*). This paper continues a series (*7, 8*) in the study of oxidation of organic compounds.

EXPERIMENTAL METHODS

Co(III) acetate solution was prepared and standardized according to the procedure reported earlier (*9*). All other chemicals used were of analytical grade purity and the solutions (0.01 *M*) were prepared by direct weighing and dissolving in 95, 50 and 25% acetic acid.

PROCEDURE

A known volume (1–4 ml) of about 0.01 *M* solution of the reductants was treated with a known excess volume of Co(III) acetate solution (1–4 ml of approximately 0.09 *M*) in the same medium at 20 and 40°C or more, and was kept for some time (Table 1) allowing the reaction to take place. The unconsumed Co(III) acetate was reduced with

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TABLE 1

MEDIUM, 95% ACETIC ACID

Reaction temp (°C)	Reaction time (hr)	Equivalents of Co(III) consumed/mole of							
		Glucose	D-Galactose	L-Sorbose	Saccharose	Lactose	Maltose	Mannitol	Glycerol
20	1	2.00	1.70	2.00	NR [a]	0.90	NR	NR	NR
	2	3.60	3.55	3.60	NR	1.00	NR	NR	NR
	3	3.70	5.20	5.00	NR	1.00	NR	NR	NR
	4	4.80	6.20	5.65	NR	1.00	0.90	1.80	NR
	5	5.90	6.60	5.80	NR	—	—	—	NR
	6	6.00	6.60	6.00	NR	—	1.90	1.90	NR
	8	6.55	6.60	6.65	NR	—	2.00	1.90	NR
	16	6.70	8.30	7.30	NR	2.60	—	2.70	0.70
	18	6.70	8.30	7.40	NR	3.00	—	—	—
	20	6.70	8.30	7.80	NR	3.80	2.00	3.80	0.90
	22	7.30	8.30	7.90	NR	—	—	3.90	—
	24	8.00	8.65	8.00	NR	—	2.00	4.70	1.90
	28	—	—	—	—	4.01	2.00	5.70	2.00
40	1	6.00	6.25	6.80	0.70	6.00	4.30	9.80	3.70
	2	7.30	8.40	8.40	1.00	6.00	5.80	10.00	4.10
	3	8.30	8.60	8.40	1.40	6.00	5.90	10.00	4.60
	4	8.70	9.00	9.30	2.10	6.00	6.00	10.00	4.90
	5	—	—	—	—	8.00	6.00	—	5.10
	6	—	—	—	—	8.00	6.40	10.00	5.90
	7	—	—	—	—	11.00	—	—	6.00
	8	—	—	—	—	12.00	9.80	—	6.00

[a] NR = no reaction.

a known excess volume of standard ferrous sulfate solution and the unreacted ferrous sulfate was in turn titrated potentiometrically with a defined potassium bichromate solution. Since Co(III) acetate is comparatively less stable at higher temperatures as well as in the presence of higher percentages of water, blank experiments were simultaneously performed under similar conditions.

RESULTS AND DISCUSSION

It is clear from Table 1 that with Co(III), attack of glucose, D-galactose, L-sorbose and lactose in 95% acetic acid at room temperature (20°C) is extremely slow. Despite excess volumes of Co(III), maltose and mannitol remain unattacked up to a period of 3 hr, glycerol up to 16 hr while saccharose is not attacked even up to 24 hr under similar conditions. It is also evident from Table 1 that once a reaction starts, it continues progressively with the increase in reaction time and does not stop completely at any particular stage. Though by elevating the reaction temperature, the reaction rate is enhanced, yet complete oxidation of the compounds up to CO_2 could not been achieved. Eight-electron exchange in case of glucose, D-galactose and L-sorbose can be attributed to the formation of acetic acid and formic acid as reported earlier with chloramine-T (*6*), according to the schematic reaction

$$C_6H_{12}O_6 + 4H_2O + 8Co(CH_3COO)_3 = 4HCOOH + 9CH_3COOH + 8Co(CH_3COO)_2.$$

With glucose, D-galactose and L-sorbose similar results were also achieved when 50% acetic medium was used at 40°C (Table 2). It is evident from Tables 1 and 2 that in case of glucose, D-galactose and L-sorbose there are some stages where the reaction seems to stop for a reasonable period of time. With glucose 6- and 10-electron changes remain unaltered for intervals of 1 and 3 hr, respectively. The same is true for D-galactose where a 10-electron change remains extended over a period of 3 hr while with L-sorbose a 7-electron exchange continues for 1 hr in 50% acetic acid medium.

Saccharose, even at 40°C and in 50% acetic acid medium was not very effectively attacked and in addition no particular stage of oxidation could be achieved. One- and 2-electron changes do not infer to any particular step of oxidation.

With lactose various pronounced stages of oxidation detected with Co(III) correspond to 4-, 6-, 8- and 12-electron changes (Table 1) which remain unaltered for the periods of 8, 4, 2 and 2 hr, respectively. In 95% acetic acid medium, maltose exhibits a similar behavior with a 2-electron change extended over a period of 22 hr at room tempera-

TABLE 2

MEDIUM, 50% ACETIC ACID

Reaction temp (°C)	Reaction time (hr)	Equivalents of Co(III) consumed/mole of							
		Glucose	D-Galactose	L-Sorbose	Saccharose	Lactose	Maltose	Mannitol	Glycerol
40	1	6.30	2.20	0.75	NR [a]	6.90	2.00	9.90	1.90
	2	6.60	6.10	6.10	2.30	6.90	6.90	10.00	2.00
	3	7.90	7.40	7.00	3.10	7.10	6.90	10.00	2.00
	4	9.80	9.80	7.00	4.70	7.00	7.00	10.00	3.90
	5	9.90	9.80	7.60	—	8.00	10.10	9.90	4.10
	6	10.80	9.80	8.00	5.70	10.10	10.00	9.90	6.00
	7	—	—	—	—	11.90	10.00	—	6.00
	8	—	—	—	—	12.10	12.00	—	6.00

[a] NR = no reaction.

ture and 6-electron change for 3 hr in 50% acetic acid at 40°C, while a 10-electron change spreads over an interval of 4 hr in 50% acetic acid at 40°C. Due, probably, to a mixed type of oxidation taking place in almost all the reactions, no concrete mechanism could be given for the various steps of the oxidation of this compound. Using copper(III) compounds as oxidizing agents a 48-electron change has been reported both for lactose and mannitol, but the experimental conditions employed in this work (*5*) were drastic and were completely unfavorable for testing them with Co(III) system.

Mannitol at room temperature (20°C) and in 95% acetic acid medium behaved very much similar to other compounds. Unexplainable stages of 2- and 4-electron changes extended over a period of 4 and 2 hr, respectively (Table 1). However at 40, 50 and 60°C and in 95, 50 and 25% acetic acid a fairly reproducible and stable stage of 10-electron change has been observed (Table 3), which schematically corresponds to the formation of formic acid and formaldehyde:

$$CH_2OH\text{–}(CHOH)_4\text{–}CH_2OH + 10Co(CH_3COO)_3 + 4H_2O = 2HCHO + 4HCOOH + 10CH_3COOH + 10Co(CH_3COO)_2.$$

With regard to this step though Co(III) acetate resembles with Pb(IV) acetate, but an 18-electron change reported earlier with copper(III) oxidation (*3*) could not be detected under the present experimental conditions.

In the case of glycerol a fairly stable stage of 2-electron change continues for an interval of 4 hr in 95% acetic acid medium at 20°C and for 1 to 3 hr in 50% acetic acid at 40°C (Table 4). It can be predicted that likewise under the effect of bromine (*2*) secondary alcoholic group

TABLE 3

MANNITOL

Medium (%)	Reaction temp (°C)	No. of electrons exchanged	Period between which the electron change remains constant (hr)
Acetic acid			
95	40	10	1–6
50	40	10	1–6
25	40	10	2–6
50	50	10	1–4
50	60	10	1–4
Acqueous	40	10	2–6

TABLE 4

GLYCEROL

Medium (%)	Reaction temp (°C)	No. of electrons exchanged	Period between which the electron change remains constant (hr)
Acetic acid			
95	20	2	24–28
50	40	2	1–3
95	40	4	1–2
50	40	4	4–5
95	40	6	6–8
50	40	6	6–8

present in glycerol is attacked and in turn converted into a keto group according to the scheme:

$$CH_2OH\text{–}CHOH\text{–}CH_2OH + 2Co(CH_3COO)_3 = CH_2OH\text{–}CO\text{–}CH_2OH + 2CH_3COOH + 2Co(CH_3COO)_2.$$

At 40° and in 95 and 50% acetic acid media 4-electron change remains unaltered (Table 4) for 1 hr in each case. It can be assumed that at this step glycerol is converted into formic acid and formaldehyde:

$$CH_2OH\text{–}CHOH\text{–}CH_2OH + 4Co(CH_3COO)_3 + H_2O = 2HCHO + HCOOH + 4CH_3COOH + 4Co(CH_3COO)_2.$$

Similarly at 40°C in 95 and 50% acetic acid media a reproducible 6-electron change remaining stable between the 6–8 hours of the reaction periods (Tables 3 and 4) corresponds to the conversion of glycerol into formaldehyde and CO_2:

$$CH_2OH\text{–}CHOH\text{–}CH_2OH + 6Co(CH_3COO)_3 + H_2O = 2HCOOH + CO_2 + 6CH_3COOH + 6Co(CH_3COO)_2.$$

With regard to these two steps, behavior of Co(III) acetate is fully in harmony to the one reported with Pb(IV) acetate earlier (*3*). However Beck (*4*) without describing the course of the reaction has reported 4-, 6-, 8- and 12-electron changes in the case of glycerol using copper (III) as oxidant. Though the first two steps found with copper(III) resemble those with Co(III), the last two stages corresponding to 8- and 12-electron changes could not be detected.

SUMMARY

Oxidation studies of glucose, D-galactose, L-sorbose, saccharose, lactose, maltose, mannitol and glycerol have been carried out with Co(III) acetate as

reagent at room temperature, as well as at 40, 50, and 60°C using 95, 50, and 25% acetic acid and aqueous media. Stepwise oxidation in almost all the compounds has been observed for well-defined periods. Glycerol is oxidized to the stages of 2-, 4-, and 6-electron exchange, while at higher temperatures mannitol is oxidized exchanging 10 electrons. Glucose, D-galactose and L-sorbose undergo an 8-electron change. Saccharose is not effectively attacked.

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MICROCHEMICAL JOURNAL 17, 619–623 (1972)

Book Reviews

Inorganic Titrimetric Analysis: Contemporary Methods. By WALTER WAGNER AND CLARENCE J. HULL. **Treatise on Titrimetry, Vol.** 1. Edited by JOSEPH JORDAN. Dekker, New York, 1971. xii + 225 pp. $13.50.

Titrimetry today is still as viable and as important an analytical tool as envisioned over a century ago by Friedrich Mohr in his *Lehrbuch der chemisch-analytischen Titrirmethode,* a work that stands as a classic in the literature of chemical analysis. New series on titrimetry are timely and will be welcomed by many analysts. Professor Jordan, as editor in chief of such a series, has in the introductory volume provided no inkling of the overall organization. Volume 2 is announced to have the title "New Developments in Titrimetry."

Volume 1 carries no introduction to the treatise, but a Preface by Dr. Jordan, rather than the authors, delineates the arrangement and purpose of the volume. This reviewer judges that the book fails to live up to the stated aims.

Each chapter considers one family of the periodic table (main group and subgroup). For each element, synoptic surveys of "classical methods" and "contemporary methods" are followed by an "outline of recommended contemporary methods." For classical methods, usually 2 to 12 lines of text appear, frequently with the citation of Volume 1 of the 6th ed. of *Standard Methods of Analysis,* published in 1962 and edited by N. H. Furman. No other compendium or no specialist monograph appears to be cited. For each classical method, often a single descriptive sentence is provided with no indication of its merit or degree of acceptance in current analytical practice. The dichotomy between classical and contemporary methods seems temporal and is made somewhere in the period 1950–1955. As a result, chelometric titrations are treated in some chapters as classical and in others as contemporary. For each contemporary method, the synoptic survey again often consists of sentence or two with a single literature citation. In the "outline of contemporary methods" that follows, procedural descriptions are often less detailed than might be expected in, say, *Analytical Abstracts.* The Preface states "the reader is advised to consult the pertinent literature." Unfortunately some references are to journals that are not in the collections of many university or industrial libraries. If Doctors Wagner and Hull secured and read some of the relatively obscure Russian and Chinese papers, readers would welcome more details being shared with them.

For many chapters, this reviewer does not appreciate the recommendation of some contemporary over classical methods and does not understand the criteria applied in the selection of some contemporary ones from the periods ranging from the mid 1950's to 1968.

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Membrane Structure. Vol. 7. By DANIEL BRANTON AND DAVID W. DEAMER. Springer-Verlag, New York, 1972. 70 pp. $13.50; subscription price $10.50.

This monograph is part of a continuing series of handbooks published under the general title of "Protoplasmatologia," and dealing with problems and findings in cell biology. It is a little book, but the authors have taken the subject of the structure of cellular membranes and explored it extensively. The various theories of membrane structure are discussed, evaluated and judged according to the most recently available technical methods. An extensive up-to-date bibliography covering numerous scientific disciplines gives a unique, broad approach to the investigation of membrane organization.

In the analysis of the many theories on membrane structure a variety of molecular models are considered and rejected. Some of the tools used in the analyses vary from X-ray diffraction, and infrared spectroscopy to nuclear magnetic resonance. The skill of the authors lies in the utilization of diagrams which ably illustrate the points they wish to make. The freeze-etching technique, in combination with the electron microscope most clearly indicates membrane structure at the molecular level.

The book is a well-written, lucid examination of the various theories of membrane structure, with a conclusion as to which structure most probably occurs. It is a lean book, with no extra fat, and should be useful for all those wishing to be brought up to date in the field.

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Encyclopedia of Industrial Chemical Analysis. Volume 15. Edited by F. D. SNELL AND L. S. ETTRE. Wiley (Interscience), New York, 1972. xv + 574 pp. $45.00 ($35.00 by subscription).

Ion Exchange Resins through Mercaptans—This the trek through the chemical alphabet for the latest volume of this major reference work for practicing chemical analysts. Intermediate topics include Iron (and its alloys and compounds), Isocyanic Acid Esters, Lactic Acid and its Derivatives, Lanthanum, Lead, Lime and Limestone, Liquefied Petroleum Gas, Lithium (and its compounds), LSD, Lubricants, Magnesium (and its alloys and compounds), Malonic Acid and its Derivatives, Manganese (and its alloys and compounds), Margarine, and Matches.

This encyclopedia has as its goal "the discussion of general analytical techniques and the analysis of industrial chemical products." The editors in the latest volume deviate from this aim with the appearance of an 11-page article on *N,N*-diethyl-*d*-lysergamide, better known as LSD from the German *L*yserg *S*äure *D*iäthylamid. The editors state ". . . LSD is not an industrial chemical product. However, the current problems in the control of drug addiction induce us (to deal) . . . with the identification and determination of this substance. We felt this . . . important since we know of no publication where the available analytical methods of this substance are compiled." The separation, identification, and determination of this hallucinogen includes solvent extraction, liquid, thin-layer and gas chromatography, simple color tests, visible, ultraviolet and infrared spectrophotometry, spectrofluorometry, and mass spectrometry.

With the treatment of most metals throughout the work, material has been pre-

sented on the analysis of key alloys and commercially available compounds. Surprisingly, this excellent arrangement is not followed for lead, and the only cross references provided are to lead azide and lead styphnate (considered under Explosives). This reviewer holds the assessment of lead compounds and pigments as important. Is it possible that some author missed the deadline and the information will appear in a latter volume? "Plumbic and Plumbous Compounds" would be a reasonable title!

For the practice of microchemistry and microanalysis many articles in this volume, as in previous volumes, provide salient information that is applicable directly to *real* samples. For inorganic practice, the articles for key metals can be recommended. The 31-page article on Isocyanic Acid Esters summarizes most effectively, and especially through the use of tables and spectral curves, their properties, reactions, determination, and analysis. Organic isocyanates have become of commercial importance only since World War II with their extensive application in the polymer industry. The 24-page article on Mercaptans delineates the determination of the thiol function, including the presentation of gas chromatographic and amperometric titrimetric procedures.

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Quantitative Measurements and Chemical Equilibria. By ERNEST H. SWIFT AND ELIOT A. BUTLER. Freeman, San Francisco, 1972. XVII + 719 pp. $14.50.

It is surprising that the choice of subject matter for a modern freshman and sophomore quantitative analysis text has changed so little over the past decade. This book represents yet another entry to an already saturated textbook market. Unfortunately the entry is flawed by several drawbacks. Some material incorporated by the authors probably belongs in a general chemistry or introductory chemistry text. In addition the authors have an annoying tendency to change voice and address the reader/student directly. The book is organized into five sections and includes 30 procedures and experiments as well as seven useful appendices.

The first section discusses the general principles of quantitative measurements over the space of nine chapters but contains superfluous material, such as the historical background of quantitative analysis (Chapter 1). In the discussion of terms and units (Chapter 2)—also superfluous in a text of this nature—the authors err in defining atomic and molecular weights in terms of gram-atomic and gram-molecular weights and confuse the reader concerning the exact number of equivalents per mole in a substance like phosphoric acid. A clear and well-written section dealing with evaluation of experimental data follows in Chapter 3. The remaining material in Section I lays the foundation for gravimetry and titrimetry.

Section II treats equipment and techniques in detail, but contains some unnecessary material. For instance, stirring rods and dropping pipettes are available commercially, and time in the freshman chemistry is usually too valuable to spend making these items by hand. Perhaps it is a sign of the times that the reader/student is warned against coming into the chemical laboratory in sandals (Hippie-type professors, take note!)

In Gravimetric Measurements and Methods—Section III—the lengthy discussion of the principles of operation of the analytical equal-arm balance seems mis-

placed since most university and industrial chemical laboratories now employ the more convenient single pan balance. The principles of titrimetric measurements (Section IV) are treated in great detail with copious literature references (albeit few from the last decade). The concept of gravimetric titrations employing small polyethylene wash bottles for dispensing titrant is novel and probably deserves greater popularity than it now enjoys. The fifth and last section treats electrical and optical methods. There are far too few experiments included here and notably absent are discussions of ion-selective electrodes and atomic absorption analysis.

The authors are quite thorough where it counts but the organization of material is confusing to the reader. The reviewer would have preferred seeing the procedures and experiments collected in one section of the book rather than distributing them throughout the text. A laboratory manual containing *only* procedures and experiments (with a sturdy vinyl cover to withstand laboratory conditions) might have been an even better approach.

Despite the diligent and rather detailed treatment given to principles and theory of gravimetric and titrimetric analysis, the student has at times been forgotten. There is a paucity of worked numerical examples and a lack of illustrative diagrams for gas chromatography, liquid–liquid extraction, and ion-exchange separations. In summary the book has shortcomings which detract from its overall appeal and which prevent its recommendation by the reviewer.

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Dating Techniques for the Archeologist. By H. N. MICHAEL AND E. K. RALPH. M.I.T. Press, Cambridge, MA, 1971. XI + 227 pp. $12.50.

The reader may well ask the question as to why a review of a book of the above title occurs in a microchemical journal. This question is answered readily by the simple fact that archeological dating may be done by microanalytical techniques and these are referred to in the text.

The text is written by eight contributors each discussing his field of specialization. The first section goes promptly into the technique of ^{14}C dating. The format of this section is followed subsequently in each of the sections. This comprises an explanation of the principle of the technique followed by the mechanical and chemical procedures for obtaining and preparing the sample. These techniques are described in detail, the text being directed to workers in the field. The final measurement is then described in detail, including the application of statistics in arriving at the age of estimation. Estimation of the validity of the results obtained are then discussed. Under the heading "Laboratory Methods" detailed setups are shown by drawings and halftones for carrying out the procedures. Finally, a list of references is cited.

Other topics discussed in a similar manner are dendrochronology (tree ring counting), archaeomagnetic dating, thermoluminescence dating of pottery, fission track dating, potassium argon dating, and obsidian hydration.

The tree ring dating makes use, at least at present, of no particular chemical techniques. It is well known that since the weather varies from year to year changes occur in the width of the tree rings. A particular sequential arrangement of wide and narrow rings becomes a recognizable pattern in structural lumber permitting the correlation of the dates between different sites.

When clay is heated to a high temperature it is demagnetized. As it cools it is remagnetized along the lines of the earths magnetic field at that time. Thus the declination, inclination and total field intensity of the earth's magnetic field can be obtained provided the clay is in its original position. This permits the changes in the earth's magnetic intensity to be plotted for several thousands of years. Subsequently this information may be used for dating newly found specimens. This technique is called "Archaeomagnetic Dating." This is explored in depth in the text.

When an X-ray beam strikes a piece of glass it turns brown, a KCl crystal turns purple. This is due to dislocation of electrons and storage of some of the irradiated energy. If heated, this energy will be given off as "thermoluminescence" along with the normal incandescent light. On cooling and reheating the extra energy will not be present. Thus, if a fired piece of ceramic is buried it will absorb energy year by year due to radioactive materials in the ceramic and its surroundings. The amount of glow obtainable on heating is a measure, then, of the time elapsed between firing and the present. This assumes that one can estimate the rate of energy absorbed per year. How this is done is given in the chapter with the title "Thermoluminescent Dating of Pottery."

If a mineral contains uranium, then spontaneous fission will take place of ^{238}U. As the atom splits, the fragments plow through the material at high speed, leaving tracks. These tracks can be brought out in a substance like obsidian by etching with HF. By counting the number of tracks, one may estimate how long ago it was that the volcanic glass was formed. This is a new technique and is still being developed. For this reason the chapter on "Fission Track Dating" is short.

The so called "potassium–argon clock" is based on the accumulation of radiogenic ^{40}Ar produced in the radioactive decay of ^{40}K. Most often it is applied to date lava flows. At that time the rock is completely degassed setting the clock to zero. This subject is discussed in a chapter on "Potassium–Argon Dating."

The last chapter is an extensive one on obsidian hydration dating. This is also a relatively new technique. When obsidian is formed it is heated to a high temperature and dehydrated. As it ages it becomes hydrated, water diffusing into the obsidian at a fairly continuous and determinable rate. One then cuts the specimen and after polishing may then measure and photograph the hydration rim.

It is of interest to the analytical chemist that by X-ray spectrometry, neutron activation and conventional analytical techniques, volcanic glasses from different parts of the world have been analyzed and characterized by their trace metal components. In this manner specimens which have been moved in prehistoric times in order to make artifacts can be traced back to their point of origin.

The text is intended as a workbook for those engaged in acheological dating and is written with this in mind. However, it is of general informational interest to the analytical chemist and may serve as a source book for the microanalyst suggesting applications of new techniques developed to archaeological problems.

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INFRARED SPECTRA OF INORGANIC COMPOUNDS (3800 - 45 cm^{-1})

By RICHARD A. NYQUIST
RONALD O. KAGEL
Both at Chemical Physics Research Laboratory
The Dow Chemical Company
Midland, Michigan

Here is the first comprehensive collection of inorganic compound spectra covering both the mid (3800-200 cm^{-1}) and far (200-45 cm^{-1}) infrared regions. Approximately 875 spectra have been recorded, in a manner that minimizes the possibility of ion exchange between sample and window support material. The book provides numerical and alphabetical indices to the infrared spectra, as well as the compound name and empirical structure of each spectrum.

The spectra are arranged according to both compound class and cation to permit systematic study, and vibrational frequency correlation charts and tables are included as an aid to the analyst. Experimental techniques are demonstrated, with examples of ion exchange between sample and window support materials, and the book provides spectra-structure correlation charts and many anion frequency assignments to assist the reader in identifying unknown inorganic samples. Bands arising from crystalline lattice modes are observed in the region below 700 cm^{-1}.

Chemists, geologists, and research workers in government and spectroscopy laboratories and industry will find this collection of inorganic compound spectra an invaluable reference source.

1971, 504 pp., $20.00

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