This Number Completes Volume 12

Volume 12, Number 4, December 1967

Microchemical Journal

devoted to the application of microtechniques in all branches of science

Editor-in-Chief: Al Steyermark

Published under the auspices of the American Microchemical Society by

ACADEMIC PRESS

New York and London

NEW DEVELOPMENTS IN
ULTRASTRUCTURE RESEARCH

Volume 2 of **ULTRASTRUCTURE IN BIOLOGICAL SYSTEMS**
Edited by **Albert J. Dalton** and **Françoise Haguenau**

Ultrastructure of the Kidney

Edited by **Albert J. Dalton**
Public Health Service NCI
National Institutes of Health, Bethesda, Maryland

Françoise Haguenau
Laboratoire de Médecine expérimentale
Collège de France, Paris, France

The monograph series on "Ultrastructure in Biological Systems" was begun to fill a need for summary statements of the current status of particular areas of ultrastructural research in which significant and rapid advances are being made. The ultrastructure of the mammalian kidney is certainly one of these areas. This second volume in the series presents the view of the contributors who, while acquainting the reader with the research being carried on in these areas, have also brought into focus the many problems still awaiting solution. This volume will be of use to medical school departments of anatomy, pathology, urology; undergraduate departments of biology, zoology and physiology and individual investigators in the field of ultrastructure research.

CONTENTS:

1967, 240 pp., $14.00

AP 1922

Academic Press
NEW YORK AND LONDON
111 FIFTH AVENUE, NEW YORK, N.Y. 10003
BERKELEY SQUARE HOUSE, LONDON W.1

Microchemical Journal

devoted to the application of microtechniques in all branches of science

Volume 12, Number 4, December 1967

Published under the auspices of the American Microchemical Society by

ACADEMIC PRESS
New York and London

CONTENTS

Published quarterly by Academic Press Inc.,
111 Fifth Avenue, New York, New York 10003
In 1967, Volume 12 (4 issues) will be published. Price: $18.00.
In 1968, Volume 13 (4 issues) will be published. Price: $22.00
(Information about reduced price for personal subscriptions placed by members is available from the American Microchemical Society)
All correspondence and subscription orders should be sent to the office of the Publishers
Publication Office at Worcester, Mass.
Second class postage paid at Worcester, Mass.

Microchemical Journal, Vol. 12, No. 4

Briefs

New Titrimetric Microdetermination of Glycols. V. N. P. SRIVASTAVA AND O. C. SAXENA, *Chemical Laboratories, University of Allahabad, Allahabad, India.*

New titrimetric determination of glycols in micro amounts has been described by oxidizing with gold chloride in alkaline medium. Oxidation of ethylene glycol, 1,2-propylene glycol, and trimethylene glycol has been effected by means of an excess of gold chloride in the presence of a known excess of sodium hydroxide. The remaining excess of gold chloride is titrated back by acidifying and adding a known excess of potassium ferrocyanide against a standard solution of ceric sulphate using *N*-phenyl anthranilic acid as indicator. Ranges in which ethylene glycol, 1,2-propylene glycol, and trimethylene glycol have been estimated vary from 0.6365 to 0.1294 mg, 3.147-0.6348 mg, and 2.618–0.3289 mg, respectively.

Microchem. J. **12**, 435 (1967).

Applications Involving the Iodide Ion I. A New Potentiometric Method for the Micro- and Semimicrodetermination of Silver. Analysis of Binary and Ternary Mixtures. H. KHALIFA AND B. ATEYA, *Cairo University, Faculty of Science, Giza, Egypt, U.A.R.*

A new method is given for the accurate micro- and semimicrodetermination of silver involving precipitation of silver as the sparingly soluble silver iodide and back titration of excess iodide with standard mercuric solution using the silver amalgam as indicator electrode. By its aid amounts of silver ranging from 30 milligrams down to 6.5 micrograms were determined with fair accuracy. Binary mixtures with copper were successfully analysed. Ternary mixtures have been analyzed by aid of the additional volumetric methods making use of potassium cyanide as masking agent. The minimum amount of silver was determined with fair accuracy and precision in presence of a cluster of cations of Cu(II), Ba, Ca, Sr, Zn, Co(II), Ni(II), Cr(III), Cd, In, and Mg amounting in total to 22 milligrams. The same amount was determined with requisite accuracy in presence of 56 milligrams of Cu(II).

Microchem. J. **12**, 440 (1967).

Photometric Determination of Gallium, Indium and Thallium Employing Solochrome Cyanine R. ARUN P. JOSHI AND KAILASH N. MUNSHI, *Chemistry Department, University of Nagpur, Nagpur, India.*

The formation of violet color between solochrome cyanine R (SCR) and gallium (III), indium(III) and thallium(III) have been studied. The analytical data for the spectrophotometric determination of these metals with SCR have been calculated.

Range for adherence to Beer's law, sensitivity, and the values of molecular extinction coefficient, suggest SCR as a sensitive reagent for the spectrophotometric determination of Ga(III), In(III) and Tl(III).

Microchem. J. **12**, 447 (1967).

Determination of Yeast Viability. FREEMAN R. SWIFT, *2 Floyd Court, Englewood Cliffs, New Jersey 07632.*

A relatively quick (same day) and accurate growth method for determining the percent viability of yeast cultures is detailed.

Microchem. J. **12**, 454 (1967).

Conductometric Determination of Graphite in Propellants. GEORGE NORWITZ AND HERMAN GORDON, *Frankford Arsenal, Philadelphia, Pennsylvania 19137.*

A conductometric method is proposed for the determination of graphite in propellants. The method is applicable to the semimicro and macro range. A sample containing up to 0.7 mg of graphite is dissolved in 10 ml of nitric acid by heating on the steam bath. If tin is present, 3 drops of hydrofluoric acid are added to prevent the precipitation of metastannic acid and occlusion of organic matter. The solution is filtered through a small Gooch crucible wrapped in aluminum foil to prevent contamination by tarry matter from the adapter. The Gooch is washed with hot dilute nitric acid (1:1), water, acetone, and again water in that order. It is then heated at 900°–1000°C in a ceramic tube in a current of oxygen, and the carbon dioxide is determined conductometrically using a Leco Conductometric Carbon Determinator. Such factors as the best means for dissolving the sample, possible segregation, preparation of calibration curve, mechanism of the combustion, and interferences are considered.

Microchem. J. **12**, 458 (1967).

Amperometry with Two Polarizable Electrodes. XV. Chelometric Determination of Small Amounts of Bismuth(III). JAN VORLÍČEK AND PETR PETÁK, *Ore Research Institute, Prague 4, Czechoslovakia.*

Amperometry with two polarizable electrodes and employing chelometry is used for the determination of microgram quantities of bismuth(III). There is no interference from a large number of ions.

Microchem. J. **12**, 466 (1967).

I. Study of the Reactivity of Bromine in the System Acetic Acid-Water. JIŘÍ ŠEVČÍK AND JAROSLAV ZÝKA, *Department of Analytical Chemistry, Charles University, Prague, Czechoslovakia.*

The behavior of bromine, in acetic acid, as dependent on its concentration, temperature, and light, with regard to the stability of the above element in the medium

used, and to the products formed, was studied. In this first part of the study, experiments with the use of various techniques of measurements (volumetric methods, potentiometry, polarography, chronopotentiometry, conductivity, spectrophotometry, and gas chromatography), are described. The discussion and scheme of the reaction are presented in the second part of this study.

Microchem. J. **12**, 472 (1967).

II. Study of the Reactivity of Bromine in the System Acetic Acid-Water. JIŘÍ ŠEVČÍK AND JAROSLAV ZÝKA, *Department of Analytical Chemistry, Charles University, Prague, Czechoslovakia.*

The experimental results of the study of the reactivity of bromine in the acetic acid-water system (described in Part I of this study) are discussed in the present communication from the point of view of the reaction products formed, and a scheme of the respective reactions is proposed.

Microchem. J. **12**, 491 (1967).

Centimilligram Determination of Organic Nitrogen with Sealed Tube Combustion. KEIICHIRO HOZUMI AND KOUICHIRO UMEMOTO, *Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan.*

The new technique involving sealed tube combustion and the measurement of nitrogen collected over 50% KOH solution under reduced pressure has been extended to the centimilligram determination of organic nitrogen. The volume of water delivered from a piston buret to replace the volume of nitrogen was corrected by measuring the difference of the two volumes which had been functional to the inside diameter of the combustion tube. The natural capillary rise of the KOH solution in the combustion tube and the volume adhering to the inside wall of the same tube were also precisely estimated to insure better approach to the actual volume of nitrogen. A standard deviation of $\pm$ 0.17% was obtained with different types of organic samples ranging from 30-80 μg.

Microchem. J. **12**, 512 (1967).

Rapid Distillation Separation of Microgram Quantities of Fluoride. HISASHI KUBOTA, *Analytical Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.*

A modification of the Willard-Winter separation is described which gives good recovery of microgram quantities of fluoride which is determined spectrophotometrically with the alizarin-complexone reagent.

Microchem. J. **12**, 525 (1967).

Spectrophotometric Determination of Nitrogen Dioxide in Nitroglycerin. MAE I. FAUTH AND ANNIE C. RICHARDSON, *Analytical Applications Branch, General Research Division, Research & Development Department, Naval Ordnance Station, Indian Head, Maryland 20640.*

The method is based on the colorimetric determination of nitrogen dioxide using Saltzman's reagent. The system obeys Beer's Law and levels of nitrogen dioxide as low as 0.1 μl are detectable.

Microchem. J. **12**, 534 (1967).

New Titrimetric Microdetermination of Antipyrine. O. C. SAXENA, *Chemical Laboratories, University of Allahabad, Allahabad, India.*

New titrimetric microdetermination of antipyrine has been described by oxidizing with gold chloride in alkaline medium.

Microchem. J. **12**, 542 (1967).

Methods for the Isolation and Characterization of Constituents of Natural Products. V. Separation of 2,6-Dinitrophenylhydrazone Pyruvamides into Classes and Resolution of the Individual Members. D. P. SCHWARTZ AND C. R. BREWINGTON, *Dairy Products Laboratory, Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Washington, D. C. 20250.*

Class separation of the 2,6-dinitrophenylhydrazone derivatives of pyruvamides of *n*-aliphatic primary and symmetrical secondary aliphatic amines is described. Both quantitative column adsorption and qualitative thin-layer adsorption chromatographic procedures are presented.

Methods for the separation of a homologous series of the primary amine derivatives and a partial series of secondary amine derivatives by thin-layer partition chromatography is also described. Approximately 1.5×10^{-4} μmole of derivative can be utilized in this procedure. Reversed phase separation of the higher primary amine derivatives by thin-layer chromatography is also presented.

Microchem. J. **12**, 547 (1967).

Pharmaceutical Applications of Internal Reflectance Spectroscopy. R. J. WARREN, I. B. EISDORFER, W. E. THOMPSON, AND J. E. ZAREMBO, *Smith Kline & French Laboratories, Philadelphia, Pennsylvania.*

The application of internal reflectance spectroscopy to pharmaceutical analysis is described. Samples which ordinarily cannot be analyzed by conventional infrared transmission techniques are handled easily, and provide information which otherwise might be impossible or extremely difficult to obtain. Examples of the application of internal reflectance spectroscopy to wax coatings, tablet coatings, metabolite deter-

mination, gas-layer chromatography (GLC) and thin-layer chromatography (TLC) fractions, trace impurities and sediments, vehicles, multidose vials and tissue studies are given.

Microchem. J. **12**, 555 (1967).

A Thirty Minute Direct Oxygen Analysis. HUGH J. BARTON AND CLYDE W. NASH, *Rohm and Haas Company, Bristol, Pennsylvania 19007.*

The method for direct oxygen analysis as described by Steyermark, has been modified through the use of special flow control valves and a combustion and sweep rate of 20 ml per minute. A microanalyst can complete an oxygen analysis in 30 minutes with no sacrifice of precision and accuracy.

Microchem. J. **12**, 568 (1967).

Microdetermination of Carbon, Hydrogen and Nitrogen by Thermal Conductivity Measurement. GERALD KAINZ AND EUGEN WACHBERGER, *Analytical Institute of the University of Vienna, Vienna, Austria.*

A previously described method for the determination of carbon, hydrogen and nitrogen has been improved with respect to procedure and apparatus. The sample is burned in a measured amount of pure oxygen. Excess oxygen is absorbed on copper. Nitrogen oxides are reduced. Water is absorbed on a calcium chloride-quartz mixture, and carbon dioxide, on a layer of molecular sieve 5A. Nitrogen is detected first, and the signal integrated on an integrator with digital read out. Carbon dioxide is then desorbed through heating and is similarly quantitated. Finally, water is desorbed and determined. Alternate peak height methods were evaluated, but were not accurate enough for micro elemental analysis. The necessary conditions for accurate integration are discussed.

Microchem. J. **12**, 584 (1967).

The Determination of Phosphorus in Organic Compounds on the Centimilligram Scale. A. J. CHRISTOPHER AND T. R. F. W. FENNELL, *Royal Aircraft Establishment, Farnborough, Hants, England.*

Open tube digestion with a perchloric-sulphuric acid mixture followed by spectrophotometric measurement, at 315 mμ, of the phosphovanadomolybdate complex provides an accurate, precise, and rapid method for the determination of phosphorus in organic compounds on the centimilligram scale. An alternative finish by production of molybdenum blue and measurement at 735 mμ may be used with some loss of precision and speed.

Certain fluorocompounds are not amenable to open tube digestion. These materials may be mineralized by sealed tube digestion with a nitric-sulphuric acid mixture or by hot flask combustion.

Microchem. J. **12**, 593 (1967).

The Microdetermination of Sulfur in Organic Compounds Containing Phosphorus. R. B. BALODIS, A. COMERFORD, AND C. E. CHILDS, *Parke, Davis and Company, Research Laboratories, Ann Arbor, Michigan.*

An oxygen-flask procedure is described for the microdetermination of sulfur in phosphorus-containing compounds. The phosphate formed is masked with ferric ions and the sulfate is titrated directly with barium chloride.

Microchem. J. **12**, 606 (1967).

New Titrimetric Microdetermination of Osmium. O. C. SAXENA, *Chemical Laboratories, University of Allahabad, Allahabad, India.*

The microdetermination of osmium is accomplished by the addition of an excess of potassium ferrocyanide followed by titration of the excess reagent with ceric sulfate. A number of elements interfere with the determination.

Microchem. J. **12**, 609 (1967).

MICROCHEMICAL JOURNAL **12**, 435-439 (1967)

New Titrimetric Microdetermination of Glycols

V. N. P. SRIVASTAVA AND O. C. SAXENA

Chemical Laboratories, University of Allahabad, Allahabad, India

Received March 21, 1967

INTRODUCTION

Literature concerning the volumetric estimation of glycols is rather sparse. Kedrinskii and Skornyakova (*1*) attempted to determine ethylene glycol and propylene glycol by oxidizing with potassium dichromate. Schaefer (*2*) determined glycol in dilute solutions containing oxidizable impurities where they calculated the results by assuming that the reaction goes only to 97.9%. Cannon and Jackson (*3*) determined small amounts of 1,2-propylene glycol in ethylene glycol by oxidizing with periodic acid, and removed the aldehydes by distillation. Charles and Hatch (*4*) determined 1,2-propylene glycol in ethylene glycol mixtures with silver nitrate. Cardone and Compton (*5*) suggested the determination of diethylene glycol by oxidation with potassium dichromate. Hess and co-workers (*6*) developed a method based on the fact that $AgIO_3$ is nearly insoluble in dilute nitric acid whereas $AgIO_4$ is soluble. Yurist and Firsova (*7*) determined ethylene glycol by acetylating and back titrating the remaining NaOH with acid.

The present method deals with the determination of glycols by oxidation with gold chloride in the presence of excess alkali. A known excess of gold chloride is added to either a solution of ethylene glycol, 1,2-propylene glycol, or trimethylene glycol in the presence of a large excess of sodium hydroxide; this is heated for three hours on a hot plate (the reaction mixture should not be allowed to evaporate). The gold chloride solution is reduced to metallic gold corresponding to the glycol oxidized. Probably the following reactions occur between gold chloride and glycols in alkaline media:

$$4\left[\begin{array}{l}CH_2OH\\|\\CH_2OH\end{array}\right] + 4AuCl_3 + 12NaOH \rightarrow 8HCHO + 4Au + 12NaCl + 10H_2O + O_2$$

$$4\left[\begin{array}{l}CH_2OH\\|\\CHOH\\|\\CH_3\end{array}\right] + 4AuCl_3 + 12NaOH \rightarrow 4HCHO + 4CH_3CHO + 4Au + 12NaCl + 10H_2O + O_2$$

$$4\left[\begin{array}{l}CH_2OH\\|\\CH_2\\|\\CH_2OH\end{array}\right] + 4AuCl_3 + 12NaOH \rightarrow 4HCHO + 4CH_3CHO + 4Au + 12NaCl + 10H_2O + O_2$$

In the case of ethylene glycol only formaldehyde (*8*) is formed during its oxidation as it gives a positive test with sodium nitroprusside.

The remaining gold chloride is estimated (*9*) by acidifying the solution and titrating back by adding known excess of potassium ferrocyanide against a standard solution of ceric sulphate using *N*-phenyl anthranilic acid as indicator.

The results agree with those obtained by a standard method (*10*); similar and precise values were found.

EXPERIMENTAL

Chemicals Employed

Gold chloride (Palmston's grade sample); ethylene glycol (B.D.H. grade sample); 1,2-propylene glycol (B.D.H. grade sample); trimethylene glycol (B.D.H. grade sample); sodium hydroxide (Merck, grade sample); potassium ferrocyanide (B.D.H. AnalaR, grade sample); sulphuric acid (AnalaR B.D.H. grade sample); ceric sulphate (Technical B.D.H. grade sample); sodium carbonate (B.D.H. AnalaR grade sample); *N*-phenyl-anthranilic acid (B.D.H. grade sample); and ferrous ammonium sulphate (B.D.H. AnalaR grade sample).

A ceric sulphate (in $8N$ H_2SO_4) solution is standardized against a standard solution of ferrous ammonium sulphate (in $1N$ H_2SO_4) using *N*-phenyl-anthranilic acid as indicator.

PROCEDURE

Since the method is the same for the determination of either ethylene glycol, 1,2-propylene glycol, or trimethylene glycol, only glycol will be mentioned in the following procedure:

To a solution of glycol, a known excess of a standard solution of gold chloride is added. Thereafter the reaction mixture is treated with an excess of sodium hydroxide (standard) solution and kept on a hot plate for three hours. After cooling the reaction mixture to room temperature, the metallic gold is filtered and washed thoroughly with distilled water. The filtrate and the washings are collected, acidified, and titrated back by adding a known excess of a standard solution of potassium ferrocyanide against a standard solution of ceric sulphate using *N*-phenylanthranilic acid as an indicator. A brownish red color appears sharply at the end point.

RESULTS

The results of determinations on the three compounds are shown in Tables 1-3. Ranges in which ethylene glycol, 1,2-propylene glycol, and trimethylene glycol have been estimated vary from 0.1294 to 0.6365 mg; 0.6348-3.147 mg; and 0.5289-2.618 mg, respectively.

SUMMARY

This paper has described a new method for the microdetermination of glycols. This was done by an oxidation-reduction reaction in which gold chloride in an alkaline medium was used to reduce a glycol. The method gives accurate results if

TABLE 1
DETERMINATION OF ETHYLENE GLYCOL

Amounts used						
Ethylene glycol 0.0104*M* (ml)	NaOH 0.8*N* (ml)	$AuCl_3$ 0.0162*N* (ml)	$K_4Fe(CN)_6$ 0.0391*N* (ml)	$Ce(SO_4)_2$ 0.0087*N* (ml)	$Ce(SO_4)_2$ 0.0087*N* (ml)	Ethylene glycol (found) (mg)
—	—	—	2.5	11.24	—	—
—	—	2	2.5	7.90	3.34	—
0.2	25	2	2.5	8.14	0.24	0.1294
0.4	25	2	2.5	8.36	0.46	0.2481
0.6	25	2	2.5	8.60	0.70	0.3776
0.8	25	2	2.5	8.86	0.96	0.6178
1.0	25	2	2.5	9.08	1.18	0.6365

TABLE 2
DETERMINATION OF 1,2-PROPYLENE GLYCOL

Amounts used						1,2-Propylene glycol (found) (mg)
1,2-Propylene glycol 0.0417*M* (ml)	NaOH 0.8*N* (ml)	$AuCl_3$ 0.0162*N* (ml)	$K_4Fe(CN)_6$ 0.0391*N* (ml)	$Ce(SO_4)_2$ 0.0087*N* (ml)	$Ce(SO_4)_2$ 0.0087*N* (ml)	
—	—	—	2.5	11.24	—	—
—	—	4	2.5	4.56	6.68	—
0.2	25	4	2.5	5.52	0.96	0.6348
0.4	25	4	2.5	6.52	1.96	1.2960
0.6	25	4	2.5	7.42	2.86	1.8910
0.8	25	4	2.5	8.46	3.90	2.5780
1.0	25	4	2.5	9.32	4.76	3.1470

TABLE 3
DETERMINATION OF TRIMETHYLENE GLYCOL

Amounts used						Trimethylene glycol (found) (mg)
Trimethylene glycol 0.0348*M* (ml)	NaOH 0.8*N* (ml)	$AuCl_3$ 0.0162*N* (ml)	$K_4Fe(CN)_6$ 0.0391*N* (ml)	$Ce(SO_4)_2$ 0.0087*N* (ml)	$Ce(SO_4)_2$ 0.0087*N* (ml)	
—	—	—	2.5	11.24	—	—
—	—	4	2.5	4.56	6.68	—
0.2	25	4	2.5	5.36	0.80	0.5289
0.4	25	4	2.5	6.12	1.56	1.0310
0.6	25	4	2.5	6.94	2.38	1.5730
0.8	25	4	2.5	7.72	3.16	2.0890
1.0	25	4	2.5	8.52	3.96	2.6180

glycols are present in traces. This method is unique in that the reaction proceeds to one step only, i.e., to the formation of aldehydes. The reaction between gold chloride and glycol takes place in the ratio of 1:1.

ACKNOWLEDGMENT

The authors are grateful to Dr. Bal Krishna, Dr. M. P. Singh and Dr. M. N. Srivastava; and to the University Grant Commission and C.S.I.R. (Government of India) for providing financial assistance.

REFERENCES

1. KEDRINSKII, V. V., AND SKORNYAKOVA, V. F., Determination of ethylene and propylene glycol by oxidation with potassium dichromate. Materials on Cracking and Chemical Treatment of Products Obtained. *Khimteoret.* **2**, 201-205 (1935).

2. SCHAEFER, WILLIAM E., Determination of glycol or glycerol in dilute solutions containing oxidizable impurities. *Ind. Eng. Chem., Anal. Ed.* **9**, 449-450 (1937).
3. CANNON, W. A., AND JACKSON, L. C., Determination of small amount of 1,2-propylene in ethylene glycol. *Anal. Chem.* **24**, 1053-1055 (1952).
4. JORDON, CHARLES B., AND HATCH, VIRGIL O., Determination of 1,2-propylene glycol in ethylene glycol mixtures. *Anal. Chem.* **25**, 636-638 (1953).
5. CARDONE, MARIO J., AND COMPTON, JOHN W., Dichromate oxidation of diethylene glycol. *Anal. Chem.* **25**, 1869-1874 (1953).
6. HESS, ELEEN R., JORDON, CHARLES B., AND ROSS, HALXEY K., Determination of ethylene glycol in water. *Anal. Chem.* **28**, 134-135 (1956).
7. YURIST, I. M., AND FIRSOVA, YU. F., Determination of ethylene glycol and its monoethyl ethers. *Zh. Analit. Khim.* **11**, 205-207 (1956).
8. CLARKE, HANS THACHER, A Hand Book of Organic Analysis—Qualitative and Quantitative." p. 127 (1956).
9. SAXENA, O. C., A new volumetric method for the estimation of Gold. *Indian J. Appl. Chem.* **1**, 1074 (1966).
10. SHUPE, I. S., Periodate reaction applied to cosmetic ingredients. Determination of glycerol, ethylene glycol, and propylene glycol. *J. Assoc. Offic. Agr. Chemists* **26**, 249 (1943).

MICROCHEMICAL JOURNAL 12, 440-446 (1967)

Applications Involving the Iodide Ion

I. A New Potentiometric Method for the Micro- and Semimicrodetermination of Silver. Analysis of Binary and Ternary Mixtures

H. KHALIFA AND B. ATEYA

Cairo University, Faculty of Science, Giza, Egypt, U.A.R.

Received March 22, 1967

INTRODUCTION

Interest in this work arose because of the high selectivity of the method using iodide ions for the potentiometric determination of mercuric ions, and mercuric ions for determining iodide ions even with very low concentrations. Furthermore, in titrating either of the ions with the other, the potential breaks are large and sharp enough for detecting the end point with high accuracy and precision, using silver amalgam as indicator electrode.

We used this procedure, previously investigated by Salem (*9*), for investigating a new accurate method for the micro- and semimicrodetermination of silver in pure and impure media, because iodide precipitates silver so quantitatively that any excess iodide can be back-titrated with mercuric ions without separation.

The literature, dating back to 1936, refers to direct potentiometric determination of silver with iodide using the silver-silver iodide electrode as recommended by Kolthoff (*6*). This procedure has some drawbacks, namely the tedious precautions, the relatively high error of $+ 3.2\%$, the prolonged time of 1-1.5 hours necessary for a single titration, and the less sharp breaks obtained when heat is applied to shorten the time required for a titration.

The recent method developed by Negoiu (*7*) is only suitable for macro amounts of silver (10.8 mg/ml).

Adam (*3*) determined milligram amounts of silver by titration with

standard sodium diethyl dithiocarbamate solution, and silver metal as indicator electrode.

Pribil (*8*) determined potentiometrically silver concentrations down to 0.001 *M*, using Fe(III)-EDTA to reduce silver ion to the metal, but he observed interference from Hg(I), Hg(II), Au(III), Pt(IV), and Pd(II).

The method of Shigeru (*10*) using any of the potassium halides for titration is suitable only for determining high contents of silver.

Thiocyanate was employed by Deliyannis (*2*) to titrate silver ions. However, the presence of traces of ferric iron renders the titration impossible; and when two or more ions are present, the end point corresponds to the total.

The present method has the advantage of being simple, rapid, and extremely reliable.

EXPERIMENTAL

The water used was always twice distilled from all-glass equipment. The chemicals were all of the requisite purity. They included potassium iodide, nitrates of silver, mercury(II), calcium, strontium, barium, copper, cadmium, cobalt(II), nickel, magnesium, chromium(III), zinc, indium, disodium salt of ethylenediaminetetraacetic acid (EDTA), eriochrome black T, and pyrocatechol violet.

SOLUTIONS

The 0.05 *M* mercuric nitrate solution was prepared and standardized as mentioned above. Lower concentrations were prepared by appropriate dilution. The 0.0920 *M* potassium iodide solution was prepared by dissolving the calculated amount in water, and standardized potentiometrically against the mercuric solution. Lower concentrations were prepared by appropriate dilution. The 0.0928 *M* silver solution was prepared from AnalaR silver nitrate and standardized following Volhard's procedure. The 0.061 *M* EDTA solution was prepared and standardized by recommended procedures.

PROCEDURES

The procedure for the determination of silver alone involved back titration of a mixture of silver and excess iodide ions with standard mercuric solution. The same procedure is used to determine submicrogram amounts of silver in the presence of a variety of cations which do not interfere with such low silver concentrations. However, when milligram

amounts of silver were determined in the presence of a variety of cations, the cations were masked with EDTA.

For analysis of binary mixtures containing microgram amounts of silver and milligram amounts of copper, the silver was determined by the above procedure, and the copper, in another sample of the mixture, was determined iodometrically. If milligram amounts of silver and copper were present, the copper was masked with EDTA before the silver determination.

Analysis of ternary mixtures involved the use of EDTA prior to the determination of silver. In another sample of the mixture, the total ions except silver were determined by direct titration with EDTA using a suitable indicator and pH. In a third sample of the mixture, to which a slight excess of cyanide was added, lead was determined volumetrically by direct titration with EDTA.

When copper is present, iodometric determination is preferred. When cobalt is present, lead is separated as the sulfate prior to the determination of cobalt.

TABLE 1
DETERMINATION OF MILLIGRAM AMOUNTS OF SILVER

	Ag			
No.	Taken (mg)	Found (mg)	% Error	(mv/0.1 ml) Titrant
1[a]	30.033	30.160	+ 0.42	361
2[a,c]	19.414	19.414	0.00	107
3[a]	15.016	15.080	+ 0.43	336
4[a,d]	19.414	19.370	− 0.22	138
5[a,e]	9.700	9.620	− 0.82	133
6[a,f]	9.707	9.650	− 0.58	178
7[b]	7.508	7.508	0.00	221
8[b]	3.754	3.754	0.00	206
9[b]	1.877	1.877	0.00	249

[a] Nos. 1-6: 0.092 *M* I^- × 0.05 *M* Hg^{2+}.

[b] Nos. 7-9: 0.023 *M* I^- × 0.025 *M* Hg^{2+}.

[c] Determined in presence of 35.7 mg of a mixture of 0.05 *M* of each of Ca, Sr, Ba, Mg, Co, Ni, Cr(III), Cd, Zn, and In ions; and 10 ml of 0.061 *M* EDTA.

[d] Determined in presence of 5.6 mg copper(II) and 3 ml of EDTA.

[e] Determined in presence of 17.85 mg of the above mixture and 5 ml of EDTA.

[f] Determined in presence of 25 mg copper(II) and 8 ml of EDTA.

RESULTS AND DISCUSSION

In Table 1 are listed the results of determining milligram amounts of silver. Results of determining microgram amounts are recorded in Table 2. These results show that the present method is extremely reliable for the determination of silver amounts in the submicro to the macro range, whether in pure solutions or in presence of many other cations.

TABLE 2
DETERMINATION OF MICROGRAM AMOUNTS OF SILVER

	Ag			
No.	Taken (μg)	Found (μg)	% Error	(mv/0.1 ml) Titrant
1[a]	312.60	311.30	— 0.42	134
2[a]	260.53	258.71	— 0.70	153
3[a]	208.40	208.40	0.00	144
4[a]	156.32	156.20	—0.08	147
5[a]	130.20	130.20	0.00	133
6[a]	104.20	103.78	— 0.40	122
7[a]	78.00	77.00	— 1.30	127
8[a]	52.11	52.07	— 0.07	119
9[a]	26.05	25.90	— 0.57	119
10[b]	52.13	51.78	— 0.67	116
11[b]	39.03	38.96	— 0.18	111
12[b,c]	26.03	25.89	— 0.54	66
13[b]	13.02	13.03	+0.08	105
14[b]	6.51	6.508	0.00	110
15[b,d]	6.51	6.48	— 0.46	65
16[b,d]	6.51	6.51	0.00	94

[a] Nos. 1-9: $(4.6 \times 10^{-4}\ M\ I^-) \times (5 \times 10^{-4}\ M\ Hg^{2+})$ in final total volume of 40 ml.

[b] Nos. 10-16: $(2.3 \times 10^{-4}\ M\ I^-) \times (2.5 \times 10^{-4}\ M\ Hg^{2+})$ in final total volume of 20 ml.

[c] Determined in presence of 56 mg copper(II) without EDTA.

[d] Nos. 15-16: determined in presence of 10 mg of the above mixture without EDTA.

The data in Table 2 indicate that by aid of the present method micro amounts of silver down to 6.51 μg can be determined with fair accuracy and high precision. Large potential breaks are obtained with such very low dilutions and the time required for a single determination does not exceed 15 minutes.

The constancy of the electrode potentials at, and after, the end point,

and the stoichiometry of the reaction in presence of silver iodide indicates that mercuric ions have no influence upon the silver iodide precipitate, in the sense that they cannot shift the dissociation of AgI towards production of its ions. This is in harmony with the fact that the solubility of HgI_2, as referred to by Kohlrausch and Rose (*4*), amounts to 0.0004 g per liter at 18°C, corresponding to 8.8×10^{-7} mole per liter, and the solubility of AgI from the solubility product value of 8.5×10^{-17} amounting to 9.2×10^{-9} mole per liter, show lower solubility of silver iodide than that of mercuric iodide.

In determining large amounts of silver as one extreme, iodide solution should be of comparatively high concentration, a condition which allows interference by foreign cations. This problem was overcome by masking these cations with EDTA which do not react with silver.

The presence of EDTA in the titration mixture decreases the potential break at the end point by about 50%. However, the conditions still permit the detection of the expected end points with reasonable breaks amounting to an average of 130 mv per 0.1 ml of 0.06 *M* titrant. Such a phenomenon is attributed to the pronounced decrease in the mercuric ion concentration brought about by chelation between EDTA and the slight excess of Hg^{2+} ions added just beyond the end point, where the potential set by the mercury electrode is given by the relation

$$E = E^0_{Hg} + RT/nF \ln (Hg^{2+}).$$

In absence of EDTA (Hg^{2+}) amounts to 10^{-5} *M*, whereas in its presence (Hg^{2+}) amounts to about 10^{-11} *M*, as computed from the instability constant values of $\sim 10^{-22}$ and 10^{-25} (*4*) for HgY^{2-} and HgI_2 respectively, and the solubility of 8.8×10^{-7} mole/liter of HgI_2 (*5*). The difference between the above two concentrations corresponds to a difference in potential of 178 mv which accounts for the smaller potential breaks obtained in the presence of EDTA (see Table 1).

In determining micro amounts of silver as the other extreme, the use of very dilute iodide solutions prevents interference from foreign ions. Between the two extremes, EDTA may or may not be used, depending on whether its use appreciably influences the position of the expected end point.

Representative results of analyses of binary mixtures of silver and copper are listed in Table 3. The above data indicate that the present

TABLE 3
ANALYSES OF BINARY MIXTURES

Cu			Ag		
Taken (mg)	Found (mg)	% Error	Taken (mg)	Found (mg)	% Error
56.00	56.00	0.00	6.51×10^{-3}	6.48×10^{-3}	— 0.46
56.00	56.00	0.00	2.60×10^{-2}	2.59×10^{-2}	— 0.38
24.79	24.98	0.36	9.707	9.605	— 1.05
4.96	4.96	0.00	19.414	19.371	— 0.22

method is extremely reliable as applied to analysis of mixtures of widely varying percentages of silver and copper. This method can be used as a simple and rapid procedure for analysis of alloys of both metals.

In determining milligram amounts of silver and copper the excess iodide present is sufficient to reduce cupric to cuprous ions. This problem was overcome by using EDTA which decreases the oxidation potential (*1*) of the cupric-cuprous system, rendering impossible the reaction between cupric and iodide ions.

Representative results of analyzing ternary mixtures are shown in Table 4.

In analyses of the above mixtures we unified the amount of silver to test the reproducibility and to calculate the standard deviation, which as revealed, is negligible.

The above procedure can be extended to analysis of similar ternary mixtures containing, instead of lead, any metal ion behaving similarly towards cyanide.

TABLE 4
ANALYSES OF TERNARY MIXTURES

Silver		Lead		Metal	
Taken (mg)	Found (mg)	Taken (mg)	Found (mg)	Taken (mg)	Found (mg)
19.763	19.580	31.827	31.827	9.807 Zn	9.820
19.763	19.763	15.913	15.820	22.494 Cd	22.595
19.763	19.763	31.827	31.827	7.695 Ni	7.606
19.763	19.763	15.913	15.860	6.894 Co	6.894
19.763	19.763	31.827	31.956	14.868 Cu	14.868

SUMMARY

A new method is given for the accurate micro- and semimicrodetermination of silver involving precipitation of silver as the sparingly soluble silver iodide and back titration of excess iodide with standard mercuric solution using the silver amalgam as indicator electrode. Amounts of silver ranging from 30 milligrams down to 6.5 micrograms were determined with fair accuracy. Binary mixtures with copper were successfully analyzed. Ternary mixtures have been analyzed by aid of the additional volumetric methods using potassium cyanide as masking agent. The minimum amount of silver was determined with fair accuracy and precision in presence of a cluster of cations of Cu(II), Ba, Ca, Sr, Zn, Co(II), Ni(II), Cr(III), Cd, In, and Mg amounting in total to 22 milligrams. The same amount was determined with requisite accuracy in presence of 56 milligrams of Cu(II).

REFERENCES

1. BELCHER, R., GIBBONS, D. AND WEST, T. S., The effect of EDTA on the Ferrous/Ferric and cuprous/cupric systems, *Anal. Chim. Acta*, **12**, 107-114 (1955).
2. DELIYANNIS, A. A., Potentiometric titration of silver, mercurous and mercuric ions with thiocyanate as precipitating agent, *Chim. Chronika (Athens, Greece)* **20**, 80 (1955).
3. HULANIKI, A., Potentiometric titrations of metals by means of diethyldithiocarbamate, *Acta Chim. Acad. Sci. Hung.* **27**, 41-47 (1961).
4. KOHLRAUSCH, F. AND ROSE, F., *In* "Comprehensive Treatise on Inorganic and Theoretical Chemistry" (J. W. Mellor, ed.), Vol. 4, p. 911, Longmans, Green Co., London, 1946.
5. KOHLRAUSCH, F. AND ROSE, F., "Comprehensive treatise on inorganic and theoretical chemistry" (J. W. Mellor ed.), Vol. 4, p. 911. Longmans, Green Co., London, 1946.
6. KOLTHOFF, I. M. AND LINGANE, J. J., The potentiometric iodide-silver titration of extreme dilution, *J. Am. Chem. Soc.* **58**, 2457 (1936).
7. NEGOIU, D. AND ELGRIGORE, R., A new potentiometric determination of silver, *Ser. Stiint. Nat.* **10**, 225-228 (1961).
8. PRIBIL, R., SIMON, V. AND DOLEZAL, J., Reductimetric determination and proof of silver, *Anal. Chemik.* **1952**, 90-95
9. SALEM, T., Potentiometric determination of iodide with (Hg II), *Inst. D'Egypt* **2**, 38 (1950).
10. YOKOSUKO, S., Determination of high percentage of silver by potentiometric titrations, Nippon kinzoku gakkaishi, **20**, 31-34 (1956), *Japan. Sci. Rev., Mining Met.* **1**, 65 (1957).

MICROCHEMICAL JOURNAL **12**, 447-453 (1967)

Photometric Determination of Gallium, Indium, and Thallium Employing Solochrome Cyanine R

ARUN P. JOSHI AND KAILASH N. MUNSHI

Chemistry Department, University of Nagpur, Nagpur, India

Received March 23, 1967

INTRODUCTION

Sodium salt of *O*-sulfohydroxydimethylfuchsone dicarboxylic acid, commonly known as Solochrome Cyanine R (SCR), forms colored chelates with metal ions and its use as a chromogenic reagent for beryllium (*4*, *7*, *8*) has been recommended. Determination of fluorides with Zr-SCR lake (*3*, *6*) has also been discussed.

Babko and Kish (*2*) described its use as a reagent for photometric determination of indium; and the use of SCR in the spectrophotometric determination of gallium has been recently suggested (*1*, *5*).

The present paper describes a systematic investigation of the chromogenic property of SCR in the spectrophotometric determination of gallium(III), indium(III) and reports for the first time spectrophotometric determination of thallium with SCR.

EXPERIMENTAL

Instruments. Beckman Model B spectrophotometer was used for absorbance measurements. It was operated by 110 volts AC current which is further stablized by a constant voltage transformer. 1-cm glass cells were used and absorbance measurements were done against distilled water blank. For pH measurements, a Beckman direct reading pH meter with a glass calomel electrode system, was used. The colorimetric measurements were done using a Klett-Summerson photoelectric colorimeter which was connected to 220 AC main. 1-cm diameter pyrex tube and filter No. 54 was used.

Materials. Solutions of Ga(III) and In(III) were prepared by dissolving their nitrates (Johnson Matthey) in distilled water acidified with dilute nitric acid. Thallic oxide (Johnson Matthey) was dissolved

in 5 *M* hydrochloric acid. A known weight of SCR (B.D.H.) was dissolved in distilled water. The solutions of required concentration were obtained by suitable dilution.

Conditions of Study. All measurements were taken at 25°C. The pH of all the solutions was maintained at 3.5 by adding suitable quantities of HCl or NaOH solutions. Total volumes were maintained at 25 ml.

RESULTS AND DISCUSSION

Rate of Color formation. Color appears instantaneously when SCR is added to the metal solution. But colorimetric measurements show that full color intensity is achieved only after the mixtures stand for 30 minutes. However, all the mixtures were kept for one hour before absorption measurements were made.

Stability of Color at room temperature. The mixtures containing 5 ml of metal ion ($2 \times 10^{-4}M$) and 15 ml of SCR ($2 \times 10^{-4}M$), diluted to 25 ml were kept at room temperature and the effect of time was studied by measuring the absorbance values. It was found that the color intensity was constant even after 12 hours, a stability quite adequate for spectrophotometric determination.

Effect of temperature. The color intensity was found to be stable between 10° to 40°C.

Order of addition of the reagent. It was found that there is no appreciable change in absorbance values when the order of addition of reagents was altered. However, in all cases SCR solution was added to the metal ion solution.

Wavelength of maximum Absorption. The λ_{max} of SCR at pH 3.5 was found to be 520 mμ. The λ_{max} of the chelates was obtained by mixing the reagent and metal ion in various ratios and taking the complete absorption spectra. They are 570 mμ (for Ga(III)-SCR chelate) 560 mμ (for In(III)-SCR chelate) and 560 mμ (for Tl(III)-SCR chelate) at pH 3.5.

However, the absorbance values were recorded at 580 mμ in all the cases, where the differences in the absorbance values of the chelates and of the reagent alone were appreciable.

Influence of pH. A series of mixtures containing SCR and metal ion in the ratio of 4:1 were prepared at different pH values. The absorbance values were measured at 580 mμ and the results are plotted graphically in Fig. 1-3.

It is observed that the absorbance of Ga(III)-SCR chelate is constant

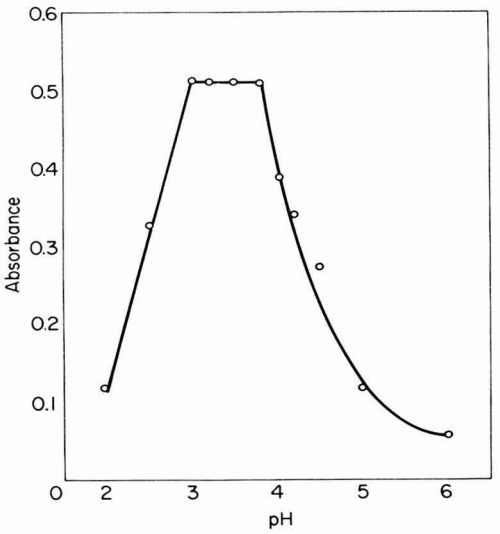


FIG. 1. Influence of pH on the absorbance of Ga(III)-SCR chelate at 580 mμ. 8 ml of 2 × $10^{-4}M$ SCR + 2 ml of 2 × $10^{-4}M$ Ga(III) solution. Total volume 25 ml.

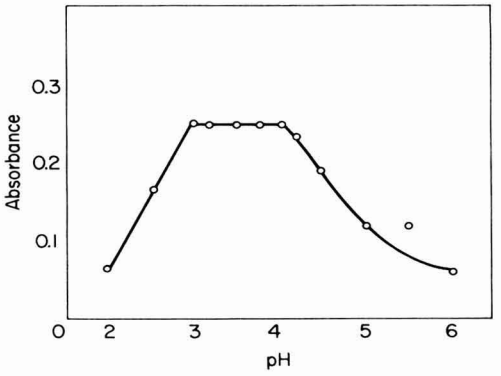


FIG. 2. Influence of pH on the absorbance of In(III)-SCR chelate at 580 mμ. 8 ml of 2 × $10^{-4}M$ SCR + 2 ml of 2 × $10^{-4}M$ In(III) solution. Total volume 25 ml.

between pH 3-3.8, that of In(III)-SCR chelate between pH 3-4, and for Tl(III)-SCR chelate between pH 3.5-4.

Adherence to Beer's law. The linearity between the absorbance of the chelates and the metal ion was tested by varying metal ion concentration.

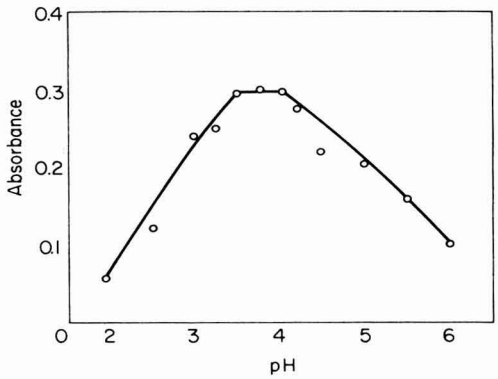


FIG. 3. Influence of pH on the absorbance of Tl(III)-SCR chelate at 580 mμ. 8 ml of 2 × $10^{-4}M$ SCR + 2 ml of 2 × $10^{-4}M$ Tl(III) solution. Total volume 25 ml.

The final concentration of SCR in case of Ga(III) was 8 × $10^{-5}M$ and in case of In(III) and Tl(III) was 2 × $10^{-4}M$. All observations were done at 580 mμ and at pH 3.5. The range of adherence to Beer's law and range for effective photometric determination is given in table below.

Effect of reagent concentration. 3 ml of 2 × $10^{-4}M$ metal ion solutions were taken in each flask and various amounts of excess of SCR was added. Total volume was maintained to 25 ml. Absorbance readings were recorded at 580 mμ. It was found that the reagent in all the cases must be at least 6 times more than the metal ion to have maximum color development. The Figs. 4-6 show the results.

Sensitivity. The values of practical as well as the Sandell sensitivity are given in Table 2.

TABLE 1
RANGE FOR ADHERENCE TO BEER'S LAW AND PHOTOMETRIC DETERMINATION OF SCR CHELATES

Metal ion (III)	Range of concentration for adherence to Beer's law (ppm).	Range of concentration for effective photometric determination.
Ga	0.28-5.0	0.5-3.5
In	0.46-3.7	0.8-2.5
Tl	0.82-4.9	1.0-4.0

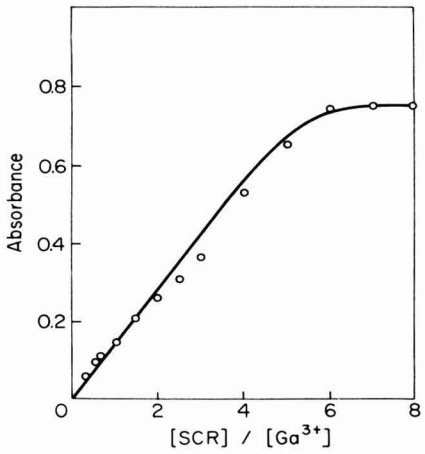


FIG. 4. Effect of excess of reagent on the absorbance of Ga(III)-SCR chelate at 580 mμ (pH 3.5) Ga(III) concentration $2.5 \times 10^{-5}M$.

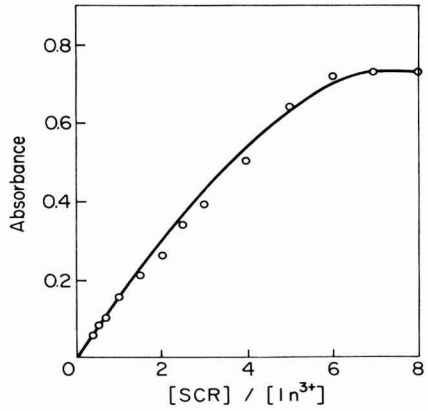


FIG. 5. Effect of excess of reagent on the absorbance of In(III)-SCR chelate at 580 mμ (pH 3.5) In(III) concentration $2.5 \times 10^{-5}M$.

Molar absorption coefficient. To a constant concentration of metal ions ($2 \times 10^{-5}M$) 10, 15 and 20 ml of ($2 \times 10^{-4}M$) SCR was added. Total volume was made up to 25 ml. The average value of molar absorption coefficient calculated at 580 mμ and at pH 3.5 is shown in Table 3.

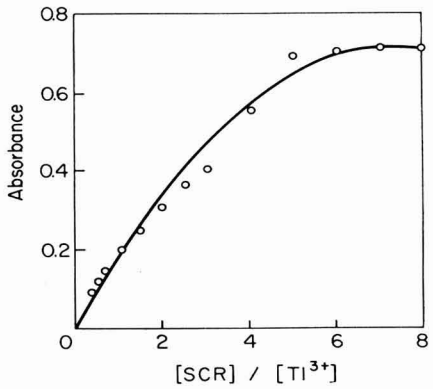


FIG. 6. Effect of excess of reagent on the absorbance of Tl(III)-SCR chelate at 580 mμ (pH 3.5) Tl(III) concentration $2.5 \times 10^{-5} M$.

TABLE 2
SENSITIVITY OF COLOR REACTIONS

System	Practical Sensitivity ($\mu g/cm^2$)	Sandell Sensitivity ($\mu g/cm^2$)
Ga(III)-SCR	0.07	0.007
In(III)-SCR	0.30	0.030
Tl(III)-SCR	1.20	0.120

TABLE 3
VALUES OF MOLECULAR EXTINCTION COEFFICIENT

System	Molecular extinction coefficient
Ga(III)-SCR	20,600
In(III)-SCR	10,260
Tl(III)-SCR	10,200

From the values of sensitivity and molecular extinction coefficient SCR can be suggested as a quite sensitive reagent for the spectrophotometric determination of gallium whereas it can also be used for the determination of indium and thallium.

SUMMARY

The formation of violet color between SCR and Ga(III), In(III), and Tl(III) have been studied. The analytical data for the spectrophotometric determination of these metals with SCR have been calculated. Range for adherence to Beer's law,

sensitivity, and the values of molecular extinction coefficient, suggest SCR as a sensitive reagent for the spectrophotometric determination of Ga(III), In(III) and Tl(III).

ACKNOWLEDGMENTS

The authors are thankful to Prof. R. H. Sahasrabudhey, Head of the Chemistry Department, Nagpur University, for providing necessary facilities and to the Government of India for providing R.T.S. Scholarship to one of them (APJ).

REFERENCES

1. AKHMEDLI, M. K. AND GLUSHCHENKO, E. L., Reagents for spectrophotometric determination of gallium. *Zb. Analit. Khim.* **19**, 556 (1964).
2. BABKO, A. K. AND KISH, P. P., Reagent for photometric determination of *In. Akad. Nauk. Ukr. RSR, Inst. Teploenerg., Zb, Prats* 1323 (1961).
3. MEEREGIAN, S., Rapid determination of fluoride with Zirconium-Eriochrome cyanine R Lake, *Anal. Chem.* **26**, 1161 (1954).
4. MOHILNER, P. R., Spot test for Beryllium based on color reaction with Eriochrome Cyanine R. *Anal. Chem.* **35**, 1103 (1963).
5. SAKELLARIDIS, P. V. AND RONJOGATIS, B. S., Spectrophotometric determination of Ga(III) in presence of In(III). *Chim. Chronika (Athens, Greece)* **29**, 113 (1964).
6. SARMA, P. L., Spectrophotometric determination of fluoride by Zirconium Eriochrome Cyanine R. *Anal. Chem.* **36**, 1684 (1964).
7. UMEMOTO, S., Spectrophotometric determination of Beryllium with Eriochrome Cyanine R. *Bull. Chem. Soc. Japan* **29**, 845 (1956).
8. WEST, P. W. AND MOHILNER, P. R. Estimation of Beryllium with Eriochrome Cyanine R using ring oven technique. *Anal. Chem.* **34**, 558 (1962).

MICROCHEMICAL JOURNAL **12**, 454-457 (1967)

Determination of Yeast Viability

FREEMAN R. SWIFT
2 Floyd Court, Englewood Cliffs, N.J. 07632

Received March 23, 1967

INTRODUCTION

There are two general methods of determining the viability of yeast cultures. One, the chemical method, is dependent on the differential ability of living and dead cells to take up various vital stains. Usually a standardized preparation of methylene blue is used under rigidly controlled conditions of pH, temperature, and timing. For at all reproducable results, however, this method demands an experienced technician who can distinguish, with confidence, between lightly-stained and unstained cells. Dead cells take up the stains readily, while living ones do not. Even so, there is an infinite gradation between the two which makes the method unsatisfactory at best.

The alternative, or growth method, takes, of necessity, more time, but if as much care in handling the sample is used as with the chemical method, nowhere nearly the experience and background is required of the technician to obtain comparable or superior results. It can be easily shown that sudden changes in environment dramatically effect living cells by temporarily slowing down the development of strong cells and actually killing or making useless weak ones (*1*). Therefore, such changes should be avoided.

EXPERIMENTAL

Weigh out an aliquoit of the sample to be tested (0.5 g, if enough is available). Pour a few drops of sterile distilled water into a 150 ml sterile, wide-mouthed, glass-stoppered dilution bottle. Roll them about to dampen the sides of the bottle and shake out all that will come easily. Drop the weighed yeast sample into the dampened bottle and set it aside at 30°C for half an hour to reach incubator temperature. This assumes that the sample has come from refrigerator storage, and is not one of the relatively few cultures which are fatally injured by too rapid dilution (one which

shows the dilution effect (*1*) in plate testing) or, as with some of the beer or enzyme producing yeasts, cannot be grown to advantage at 30°C. Such yeasts require special handling. This will be dealt with later. An already warm sample will, of course, not require this special warm-up treatment. At the end of half an hour contact the sample with 50 ml of sterile saturated calcium sulfate solution (also at 30°C) and set it back into the incubator for another half hour. Shake it vigorously at intervals, but do not use glass beads or other mechanical aids to put the sample into suspension. If a mechanical shaker is available in the incubator, it may be used, but vigorous hand shaking three or four times during the soaking period is usually all that is required. The calcium sulfate has no apparent effect on the yeast except to increase contrast and make later handling easier.

The half gram in 50 ml gives a 10^2 dilution. At the end half an hour soaking, transfer 1.0 ml of the 10^2 dilution to 9.0 ml of sterile distilled water in a test tube for a 10^3 dilution. Transfer 1.0 ml of the 10^3 dilution to 9.0 ml of distilled water for a 10^4 dilution. Put one drop of that suspension onto each field of a Thoma ruled hemocytometer and examine it. There should be between 250 and 350 cells well distributed throughout an entire ruled area, all 25 large squares. If too few are found, add a definite amount more from one of the lower dilutions and count again. If too many are found, add sterile distilled water in definite increments until the desired count is obtained. Then duplicate the final suspension, only this time use a thin malt gelatin solution[1] in place of the distilled water as the final dilutent. Set the mount away at 37°C for 5 minutes to allow all of the cells to settle out. Then mount and count it; this time use as high magnification and bright light as practical. Also, this time, be sure of the orientation of the hemocytometer so that a recount will surely check the proper field.

If two cells lie so close together that one could be a bud of the other, count each as an independent cell. If a clump is encountered which can be surely resolved count its individual cells. If not, count it seperately as a clump. Record the count for each field separately. Then set the mount on two bits of glass rod or a few glass beads in the bottom of a petri dish. Run a few mls of warm distilled water under it. Cover the dish and set it away at 30°C for an arbitrary three hours. At the end of that time, most of the cells will be found to be in the late two- or early four-cell stage. Even somewhat slow cells will be swollen and look different from

the dead cells which have not moved. The latter appear by contrast more opaque, granular, and somewhat shriveled. If a bud has been counted as an independent cell that combination will be in the three- or five-cell, instead of the four- or eight-cell stage; this shows that the bud was a little slower than the mother and is an independent cell.

As a culture ages or otherwise deteriorates, its original rate of development slows down. So, if after three hours incubation, an annoying number of very small buds or obviously alive but still unbudded cells is encountered, set the mount back at 30°C for another half hour or even another hour and reexamine.

Before making the final count, a few very important precautions should be taken. Especially in hot, steamy weather, remove the hemocytometer from its petri dish several minutes before putting it onto the microscope stage. This allows for evaporation of any condensation which may develop on top of the cover slip, due to the saturated atmosphere. Carefully protect the mount from microscope lamp heat which may melt the gelatin and start streaming in the mount. If that occurs, even momentarily, it at least reduces the reliability of the result and usually necessitates a completely new start.

In making the second count it is necessary to count only the smallest category, either the dead cells or with a very far gone sample, the living cells. For all practical purposes, by the end of three or at the very most five hours of incubation under these conditions, any cell which has not produced at least one bud can be considered dead. So maintaining the convention, no bud no life, there is a minimization of the personal equation in counting.

DISCUSSION

The number of replicates necessary in this work is governed purely by what is wanted from the answer. One double-field mount gives two answers which can be averaged. This is usually sufficient for routine work with a built in standard of experience to warn of an accident. On the other hand, three double-field mounts, or better three completely independent weighings give a safe control for a completely unknown sample.

Some unusual or special cultures, especially among the beer- and enzyme-producing yeasts, require much more careful handling than ordinary distillers and bakers yeast. (*1*). Those which are drastically effected by too rapid dilution should be contacted at first by only 8 ml

of dilutent or less and the rest added in six 8 ml and one 10 ml increments at 5 minute intervals during the soaking treatment. Other yeasts are actually hurt by any prolonged exposure to 30°C. These, of course, must be grown at their own lower optimum temperature and will require longer than the times given here to develop. It is well, in dealing with such special yeasts as these, to work out an individual schedule and time table for each one before beginning routine work with it.

Once this is done, this method will handle samples with a small fraction of one percent of viable cells left as readily as samples in prime condition. One can even learn to cut corners once a background knowledge of a sample has been acquired as a safety guard. It is not always necessary to carry one of these runs straight through at one sitting. One can set up a hemocytometer mount in the late afternoon and put it into the refrigerator instead of directly into the incubator. Then, if one can get a night worker to move it to the incubator at five o'clock or six o'clock in the morning, it will be ready to count and report by 9 a.m. Of course, if this is done, the top of the petri dish should be removed for the first hour or so in the incubator to allow the mount to warm up more quickly to incubator temperature.

The main advantage of this method, as outlined here, are that the final count is made before the dead cells or slow cells can be overgrown by developing colonies, and highly developed skill in interpreting results is not required. Clean, but not necessarily sterile, hemocytometers are all that is required because of the short time of the test.

[1] Thin malt gelatin. 33.0 g Dehydrated Difco Malt wort broth; 2.5 g glycerol; 60.0 g gelatin; and 1000.0 ml Distilled water. Dispense in tubes and sterilize 15 lbs for 15 minutes.

SUMMARY

A relatively quick (same day) and accurate growth method for determining the percent viability of yeast cultures is detailed.

REFERENCE

1. GUILLILARD, R. B., Determination of yeast viability. *J. Inst. Brew.* **65**, 424-429 (1959).

MICROCHEMICAL JOURNAL **12**, 458-465 (1967)

Conductometric Determination of Graphite in Propellants

GEORGE NORWITZ AND HERMAN GORDAN

Frankford Arsenal, Philadelphia, Pennsylvania 19137

Received March 27, 1967

INTRODUCTION

Graphite in propellants is ordinarily determined by dissolution of the sample in nitric acid or a mixture of sodium hydroxide solution and hydrogen peroxide, followed by filtration through a Gooch, washing with acetone, drying at 200°C., weighing, ignition at 600°-650°C., and calculation of the loss in weight as graphite (*12, 13, 16*).

The above methods are satisfactory when relatively large size samples are used (5 g). For smaller size samples the errors of the procedures are large (a significant error is that caused by the change in weight of the asbestos between weighings). Frequently in the analysis of foreign propellants and in the analysis of residues, only a small amount of sample is available. Also, for reasons of safety, it is advisable in certain situations to use a small size sample.

In view of the need for a method for the accurate determination of graphite using a small size sample, this installation undertook the development of a conductometric method, applicable to a semimicro and macro scale.

EXPERIMENTAL

Apparatus

1. Leco Conductometric Carbon Determinator (*11*).

2. Ceramic combustion tube (with tapered end) and manganese dioxide purification tube as shown in Figure 1. The combustion tube is a Leco tube (528-325) (length, 24 inches; inner diameter, $\frac{7}{8}$ inch; outer diameter, $1\frac{1}{8}$ inch). The rubber stopper at the inlet end of the combustion tube is protected by aluminum foil. The manganese dioxide tube is 6 inches long and is made of tubing 18 mm in diameter.

3. Gooch crucible, Coors 1, Series 291 (diameter 16 mm at top

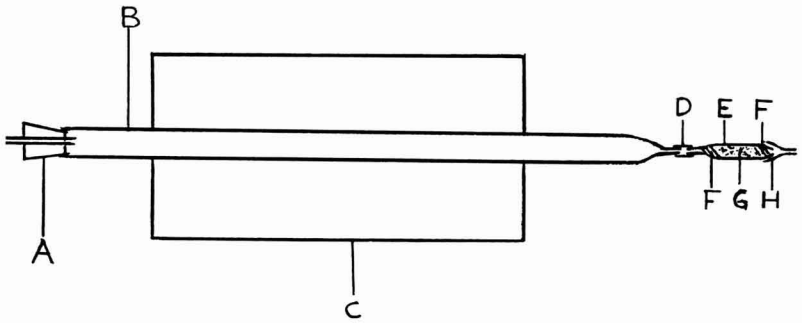


FIG. 1. Apparatus for combustion of graphite. A: Rubber stopper protected by aluminum foil; B: Ceramic combustion tube with tapered end; C: Electric heater; D: Tygon tubing; E: Manganese dioxide tube; F: Glass wool; G: Manganese dioxide; H: Ground glass joint (14/35).

and 13 mm at bottom; height, 50 mm; volume, 6 ml), Coors Porcelain Company, Golden, Colorado.

4. 500-ml suction flask.

5. Rubber adapter prepared by making a hole in a rubber stopper (that fits the suction flask), using a No. 9 cork borer. The stopper is then cut in half, perpendicularly to the hole. The bottom half of the stopper is used.

6. Aluminum foil.

7. Steam bath.

Reagents

Barium hydroxide solution (*11*). Bubble CO_2-free air through 16 liters of distilled water for 45 minutes. Dissolve 18 g of $Ba(OH)_2 \cdot 8H_2O$ in 500 ml of CO_2-free water and filter into the CO_2-free 16 liters of water. Dissolve 2 g of gelatin in 500 ml of warm CO_2-free water and add 5 ml of 2-ethyl hexanol (octyl alcohol). Add to the large volume and bring up to 18 liters with CO_2-free water. Shake thoroughly, insert a stopper, and allow to stand for at least a week.

Procedure

Prepare an asbestos pad in the Gooch crucible, dry at 110°C for 10 minutes, and heat in the hottest flame of a Meker burner for 30 minutes. The purpose of the heating at 110°C is to prevent the water vapor from

blowing out the pad. The crucible, once prepared, can be used for many runs.

Heat the electric furnace to about 900°-1000°C. Weigh the sample which contains preferably 0.3-0.7 mg of graphite (the maximum size sample that can be used is 0.3 g). Transfer to a 30-ml beaker and add 10 ml of nitric acid. If tin is present, add 3 drops of hydrofluoric acid. Heat on the steam bath until the sample is in solution (about 20 to 35 minutes.)

Add about 200 ml of tap water to the 500-ml suction flask to act as a trap for the nitric acid fumes. Insert the adapter into the suction flask.

Wrap the Gooch crucible tightly with a piece of aluminum foil (approximately $2\frac{1}{2}$ inches high and 3 inches long) so that the foil extends about $\frac{3}{4}$ inch beyond the bottom of the crucible. Insert the Gooch crucible and foil into the rubber adapter and push in tightly.

Filter the solution through the Gooch. Wash the beaker twice and the crucible three times with hot dilute nitric acid (1:1) (temperature about 80°C), and be sure to fill the crucible to the top after each decantation or washing. Wash the beaker twice and the crucible three times with hot water, and again be sure to fill the crucible to the top during each decantation and washing. Discard the filtrate. This must be done because nitric acid can produce a vigorous reaction with the acetone to be used in the next step.

Wash the beaker twice and crucible four times with acetone, and fill the crucible to the top each time. Wash the beaker twice and the crucible three times with water. Wipe the outside of the crucible with a cotton cloth moistened with acetone. Place the crucible into the original beaker and dry the crucible by heating the beaker and the crucible on the hot plate for 10 minutes.

Fill the absorption cell with barium hydroxide solution and adjust the flow of oxygen to about 100 ml/minute. Turn the decade dial to zero and bring the lines on the cathode screen together. Allow the oxygen to flow until the lines cease to move apart significantly. Bring the lines together, open the tube, quickly push the crucible into the hot zone, and quickly connect up the system. After 25 minutes, rotate the decade dial so that the lines coincide, and make a reading.

Calculate the mg of graphite present by consulting a calibration curve prepared by use of propellants whose graphite content has been accurately

determined gravimetrically by use of a 5-g sample (*12*, *16*). The range of the calibration curve should be 0-0.7 mg of graphite.

Calculate the percent of graphite as follows:

$$\% \text{ graphite} = \frac{\text{mg of graphite}}{\text{g of sample} \times 10}$$

RESULTS AND DISCUSSION

Conductometric Measurement of Carbon Dioxide

Various apparatus have been proposed for the conductometric determination of carbon dioxide (*2-7*, *9*, *14*, *15*). The apparatus used in the present investigation was the Leco Conductometric Carbon Determinator (*11*). This apparatus has previously been used for the determination of carbon in steels and other metals by combustion of the entire sample. The apparatus consists of a bridge circuit which incorporates two glass conductivity cells containing barium hydroxide solution. The carbon dioxide and excess oxygen bubble through a glass frit in the measuring cell. The change in conductance unbalances the bridge, and pushes apart a cathode ray indicator beam into parallel lines. The bridge is then balanced by adjusting a variable resistance until the lines coincide.

The change in conductance of the solution is a result of the following reaction:

$$CO_2 + Ba^{++} + 2OH^- \longrightarrow BaCO_3 + H_2O$$

In applying the conductometric method to the analysis of propellants, the sample is dissolved and the solution is filtered through a small Gooch, which is then ignited to about 900°-1000°C inside a tube heated by an electric furnace. The gases from the combustion are passed through manganese dioxide to remove oxides of sulfur and oxides of nitrogen.

The only combustion tube that was found to be satisfactory was a ceramic tube with a tapered end. The rubber stopper used in the inlet end was protected by aluminum foil. Experiments were conducted with a specially made quartz tube with ground glass joints. It was found, however, that this combustion tube was unsatisfactory because the Gooch adhered to the inside of the tube during the heating to 900°-1000°C.

The electric furnace used in this laboratory had silicon carbide heating elements. Attempts were made to use an induction heater after removing the asbestos pad and mixing it with low-carbon iron; however, the results obtained were unsatisfactory.

Dissolution of the Sample and Filtration

The best means for dissolving the sample was with 10 ml of nitric acid. With samples containing tin, however, when nitric acid alone was used, the results were high and erratic as a result of occlusion of organic matter by the metastannic acid. Part of this organic matter is held so tenaciously that it is not completely washed out with acetone. Metastannic acid is known as a strong occludor of inorganic compounds (*10*), but apparently no information is available concerning its occlusion of organic compounds. The error due to the occlusion of organic matter by the tin was eliminated by the addition 3 drops of hydrofluoric acid to complex the tin and thus prevent its precipitation. Some experiments were conducted to see if a mixture of hydrochloric and nitric acids could be used to dissolve the samples containing tin (tin will not precipitate from this medium); however, the use of this acid mixture was abandoned because dissolution took too long.

It was found that the temperature for the dissolution of the sample was somewhat critical. Too high a temperature (the boiling point of nitric acid is 121°C) caused low results. In order to regulate the dissolution temperature it is necessary to use a steam bath.

After the filtration through the Gooch, the beaker and crucible are washed with hot nitric acid solution (1:1), and then they are both washed with water and acetone. The nitric acid wash should not be omitted since washing with water (without the prior nitric acid wash) causes precipitation of nitrocellulose.

The only Gooch crucible that would fit the combustion tube was a Coor No. 1, Series 291 crucible.

Initially it was found that high and erratic results were frequently obtained because of contamination of the bottom and the sides of the crucible by the tar produced from attack of the rubber adapter by the hot nitric acid or acetone. This situation was finally remedied by wrapping the outside of the crucible with aluminum foil before it was inserted into the adapter. The most convenient adapter was a specially made one-hole rubber stopper.

Possible Segregation

The authors never encountered evidence of segregation of the graphite in propellants, even with the use of small size samples. The reason for the lack of segregation is the fact that the graphite is finely ground and

well incorporated with the other constituents of the propellants, or it is evenly coated on the propellants in the manufacturing process.

Preparation of Calibration Curve

The only feasible means for preparing the calibration curve was to use standard samples of propellants whose graphite content had been carefully determined by the gravimetric nitric acid method, with use of a large sample (5 g) (*12, 16*).

Ordinarily only artificial graphite is used in propellants; natural graphite is undesirable for this purpose because of its high iron and silica content (*1, 8*). The carbon content of artificial graphite is usually 97-98%.

Mechanism of the Combustion Reaction

The combustion of graphite is a slow reaction. It was found that 25 minutes were required for combustion to take place (this includes the sweep-out time).

All indications were that the carbon was completely combusted to carbon dioxide. Placing a layer of platinum powder (a catalyst for the conversion of carbon monoxide to carbon dioxide) on the bottom of the crucible before the filtration did not give better recovery of carbon dioxide.

Interferences

All the ordinary compounds found in propellants such as nitrocellulose, nitroglycerine, dinitrotoluene, diphenylamine, 2-nitrodiphenylamine ethyl centralite, phthalates, triacetin, RDX, HMX, oxamide, and TNT dissolve in the nitric acid or subsequent acetone wash. Inorganic salts and metal organic salts dissolve in the nitric acid and do not interfere. The only interference is carbon black. The method is not applicable to the determination of carbon black since some carbon black may pass through the Gooch as a colloid. Also, some types of carbon black, especially channel blacks, are attacked by nitric acid.

Results for Graphite in Propellants

The results obtained for graphite in three propellants by use of the method are shown in Table 1. The results show satisfactory precision

TABLE 1

RESULTS FOR GRAPHITE IN NITROCELLULOSE-BASE PROPELLANTS

Sample	% Graphite Found Gravimetrically	% Graphite Found Conductometrically
A[a]	0.09	0.08
		0.08
		0.10
		0.12
		0.11
		0.11
		Ave. 0.10
B[b]	0.18	0.19
		0.19
		0.18
		0.18
		0.18
		0.20
		Ave. 0.19
C[c]	2.20	2.27
		2.24
		2.12
		2.15
		2.14
		2.03
		Ave. 2.16

[a] Contains (%): 18.16 nitroglycerin, 0.39 dinitrotoluene, 0.99 diphenylamine, 0.75 dibutyl phthalate, 0.14 sodium sulfate, 0.48 calcium carbonate, 1.03 potassium nitrate.

[b] Contains (%): 9.64 nitroglycerin, 0.60 dinitrotoluene, 0.89 diphenylamine, 5.12 dibutyl phthalate, 0.11 sodium sulfate, 0.57 calcium carbonate.

[c] Contains (%): 35.11 nitroglycerin, 0.59 dinitrotoluene, 0.86 diphenylamine, 0.66 dibutyl phthalate, 0.11 sodium sulfate, 0.28 calcium carbonate.

and compare well the results obtained by the gravimetric method with the use of a 5-gram sample.

SUMMARY

A conductometric method is proposed for the determination of graphite in propellants. The method is applicable to the semimicro and macro range. A sample containing up to 0.7 mg of graphite is dissolved in 10 ml of nitric acid by heating on the steam steam bath. If tin is present, 3 drops of hydrofluoric acid are added to prevent the precipitation of metastannic acid and the occlusion of organic matter. The solution is filtered through a small Gooch crucible wrapped in aluminum foil to prevent contamination by tarry matter from the adapter. The Gooch is

washed with hot dilute nitric acid (1:1), water, acetone, and again water; it is done in that order. It is then heated at 900°-1000°C in a ceramic tube in a current of oxygen, and the carbon dioxide is determined conductometrically with the use of a Leco Conductometric Carbon Determinator. Such factors as the best means for dissolving the sample, possible segregation, preparation of calibration curve, mechanism of the combustion, and interferences are considered.

REFERENCES

1. Bebie, J., "Manual of Explosives, Military Pyrotechnics, and Chemical Warfare Agents," p. 76. Macmillan, New York, 1943.
2. Bennet, E. L., Harley, J. H., and Fowler, R. M., Conductometric method for determination of carbon in steels. *Anal. Chem.* **22**, 445-48 (1950).
3. Bolliger, H. R., and Treadwell, W. D., Micro conductometric determination of carbon, especially in aluminum. *Helv. Chim. Acta* **31**, 1247-59 (1948).
4. Cain, J. R., and Maxwell, L. C., An electrolytic resistance for determining carbon in steels. *Ind. Eng. Chem.* **11**, 852-60 (1919).
5. Fowler, R. M., Guldner, W. G. Bryson, T. C., Hague, J. L., and Schmitt, H. J., Determination of carbon in ferrous alloys, *Anal. Chem.* **22**, 486-88 (1950).
6. Gardner, K., Rowland, W. J. and Thomas, H., Estimation of small amounts of carbon in steels, *Analyst* **75**, 173-9 (1950).
7. Goodwin, R. D., Automatic recording of carbon dioxide by conductometry, *Anal. Chem.* **25**, 263-65 (1953).
8. "Graphite (for Use in Ammunition)", Joint Army-Navy-Air Force Specification, JAN-G-155, June 1954.
9. Hale, C. H., and Hale, M. N., Carbon on cracking catalyst, *Anal. Chem.* **23**, 724-26 (1951).
10. Hillebrand, W. F., Lundell, G. E. F., Bright, H. A., and Hoffman J. I., "Applied Inorganic Analysis," p. 288. Wiley, New York, 1953.
11. "Instruction Manual for Operating Leco No. 515 Conductometric Carbon Determinator," Laboratory Equipment Corp., St. Joseph, Mich.
12. "Military Standard, Propellants, Solid: Sampling, Examination, and Testing," Military Standard, MIL-STD-286A, January 1963, Method 309.1.2.
13. Parpaillon, M., and Rigal, S., Determination of graphite in explosives by the potassium hydroxide-peroxide method. *Mem. poudres*, **37**, 305-17 (1955).
14. Still, J. E., Dauncey, L. A., and Chirnside, R. C., Conductometric determination of carbon in metals. *Analyst* **79**, 4-17 (1954).
15. Treadwell, W. D., and Zurrer, T., Conductometric determination of carbon dioxide and methane in highly dilute gas mixtures. *Helv. Chim. Acta.* **17**, 869-77 (1934).
16. Welcher, F. J., "Standard Methods of Chemical Analysis," Vol. 2B, p. 1378. Van Nostrand, New York, 1963.

MICROCHEMICAL JOURNAL **12**, 466-471 (1967)

Amperometry with Two Polarizable Electrodes. XV. Chelometric Determination of Small Amounts of Bismuth (III)[1]

JAN VORLÍČEK AND PETR PETÁK

Ore Research Institute, Prague 4, Czechoslovakia

Received March 31, 1967

INTRODUCTION

Bismuth(III) ions form a very solid complex with EDTA at pH 1-2 in the medium of nitric acid; the visual indication to xylenol orange is possible in this medium and this was used analytically (*1*). When following the courses of the titration curves at the biamperometric indication with the Bi(III)-EDTA titration up to the equivalence point, almost no current changes occur; only after the first addition of EDTA, a vigorous current increase was registered. These current changes are so definite as to permit a reliable Bi(III) determination.

Vydra and Vorlíček (*2*) have investigated the courses of the titration curves of Bi(III) determined in the medium of nitric acid from pH 2.5 to the 1*M* HNO_3 concentration. The authors have found the optimum conditions for the bismuth determination in the concentration range of 0.52-10.4 mg Bi(III) in the medium of nitric acid from pH 1.5 to 1.5*N* HNO_3 at the applied voltage around 1.5 V. The Bi(III) determination can be carried out in the presence of Mg, Co, Sr, Ba, U(VI), Mn(II), Ag, Al, Tl(I), Pb, Cd, Zn, Ni, La, Th, Sc, Ga, F^-, Cl^-, SO_4^{2-}, ClO_4^-, Fe(III) does not interfere in the ratio Bi:Fe = 1:10, Cu:Fe = 1:1. It interferes with In, Zr, Tl(III) and Mo.

The preceding experiences directed us to the study of the possibilities of the determination of very small amounts of bismuth with EDTA titration, as the velocity of the current stabilizing, i.e., the velocity of the proper electrode actions, have indicated the validity of these assumptions.

[1] See Vorlíček, J., Fara, M., Vydra, F., *Microchem. J.* **12**, 409 (1967).

EXPERIMENTAL AND RESULTS

Reagents. The following reagent-grade chemicals were used: nitric acid, hydrochloric acid, perchloric acid.

The volumetric solutions of 0.0005 and 0.00005*M* EDTA were prepared by dilution of a stock solution. They were standardized against biamperometric titrimetric determination using Fe(III) solution (*3*). The volumetric solutions were stored in firmly closed polyethylene flasks. It was proved, during three months' observation, that no change of the factor volumetric solutions occurred.

The standard Bi(III) solution was prepared by dissolving bismuth nitrate in 0.1*M* HNO_3. The value of this prepared solution, 1 ml = 10 μg Bi(III), was standardized colorimetrically (yellow complex with thiourea). The value was checked regularly (*4*).

Apparatus. For biamperometric titrations, the same instruments were used as in our previous paper (*3*). Stationary Pt-Pt electrodes with a platinum working area $5 \times 7 \times 0.2$ mm, were used. When not in use, the electrodes were immersed in water. As an indication apparatus, a a mirror galvanometer from flame photometry apparatus (type Zeiss Jena, Model III) with the sensitivity of about 1 μA per 1,000 divisions of the scale, was used. The automatic record of the titration curves was made with a registration microammeter type EZ 2 (producer: Laboratorní přístroje, Prague, ČSSR). An automatic burette was used for titration (producer: Labora, Prague, ČSSR) with accuracy in measuring 0.001 ml, and additional standard microburettes of a 5.0 ml volume with graduations of 0.01 ml.

The evaluation of the titration curves was carried out graphically. All data given in this paper are arithmetical means of three determinations.

*Determination of Bi(III) with 0.0005*M *and 0.00005*M *EDTA.* As we have already mentioned in the introduction, the course of the titration curves at Bi(III) determination with the solution EDTA is given by the "irreversible system." This signifies that the current up to the equivalence point shows hardly any changes; and only after the first excess of EDTA does a vigorous current increase occur.

Many determinations from 100 to 500 μg were carried out with the volumetric solution 0.0005*M* EDTA. Some of the titration curves are shown in Fig. 1. It is obvious that these curves can be easily reproduced and reliably subtracted. We have worked with the 100 ml volume of

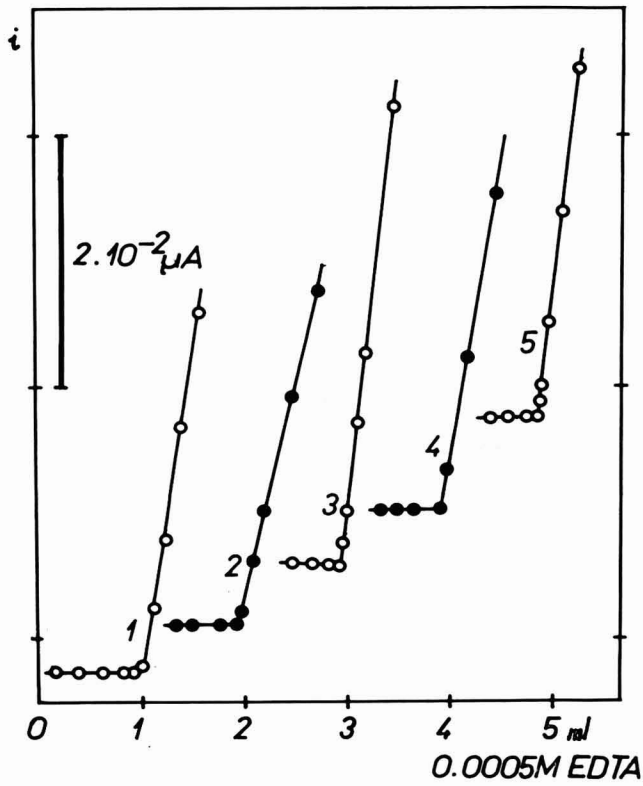


FIG. 1. The course of the titration curves in the determination of Bi(III) with 0.0005*M* EDTA. Total amount, 100 ml; pH, 1.2; temperature 20°C; applied potential, 1.4 V; Pt-Pt stationary electrodes. Curve 1, 100 μg Bi(III); 2, 200 μg; 3, 300 μg; 4, 400 μg; 5, 500 μg.

solution at pH 1.2, and applied voltage of 1.4 V at normal temperature about 20°C. The error did not exceed ± 1% relative.

The Bi(III) concentration at the interval from 10 to 50 μg was determined by the volumetric solution EDTA 0.00005*M*. The titration curves are given in Fig 2. The titration was performed in the volume of about 50 ml solution at pH 1.2 and applied voltage 1.4 V. The titration curves are consistent with the preceding results; also they show a very good reproducibility and reliable subtraction of the equivalence point.

Figure 3 pictures an actual record of the automatic registration of

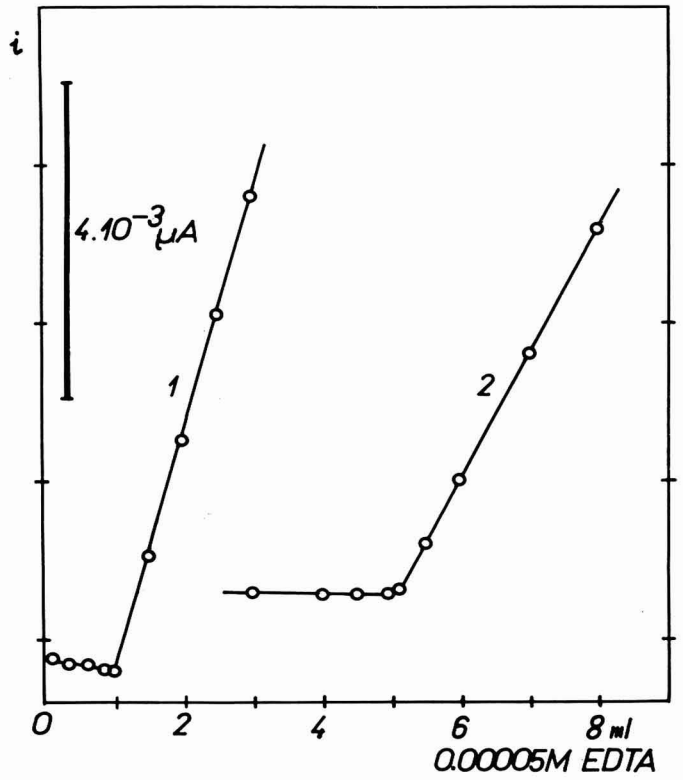


FIG. 2. The course of the titration curves in the determination of the Bi(III) with 0.00005 *M* EDTA. Total amount 50 ml; pH 1.2; temperature 20°C; applied potential 1.4; Pt-Pt stationary electrodes. Curve 1, 10 μg Bi(III); 2, 50 μg.

the titration curve of Bi(III) obtained with 0.005*M* EDTA. The reaction velocity of the electrode reaction of Bi(III) and EDTA allows the complete automation of the Bi(III) determination. The error of the determination is within ± 0.1% relative.

It was observed that the Bi(III) determination is, to a certain extent, affected by the presence of Cu(II) ions. This effect is due to a "passive" coating of the electrode systems: the cathode is coated by the deposited metal and this is the reason why the titration curve after the equivalence point is not reliable for subtracting the equivalence point. This "passive" coating on the electrode can easily be removed by hot (60°C) nitric acid,

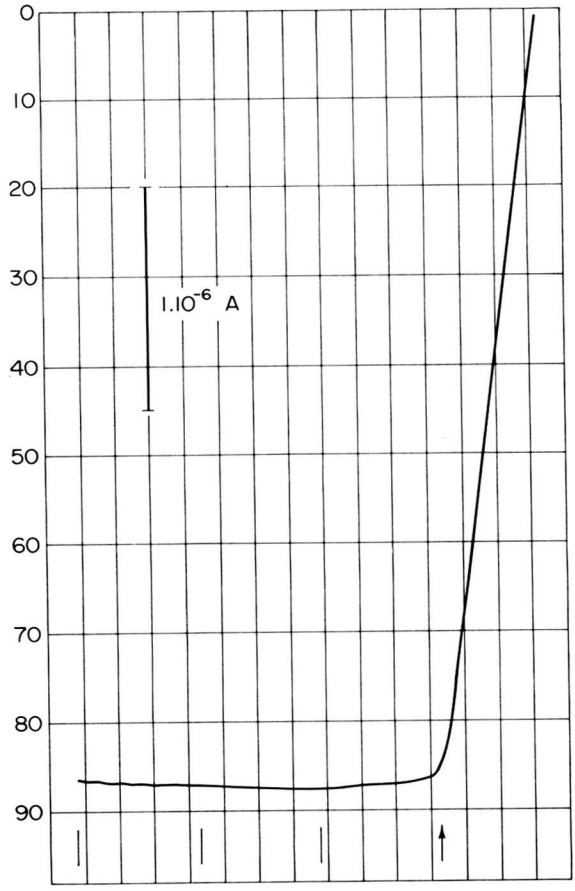


FIG. 3. Automatic registration of a titration curve with 0.005*M* EDTA. Total amount, 100 ml; speed of the registration paper, 180 mm/min; flow rate of the volumetric solution from burette, 1.0 ml/min.; concentration Bi(III), 3 ml 0.005*M*.

(1:1) dilution. After this step the titration curves resume a normal course.

Practical applications. In regard to these experiences concerning the selectivity of the Bi(III) determination, including the interfering effects, this method was applied to the bismuth determination in some types of mineral raw materials, for instance galenites, tetraedrites, etc. Because of a considerable excess of lead, decomposition of the samples was carried out by a mixture of perchloric acid and hydrofluoric acid. The Bi(III)

determination can be made either by direct EDTA titration, or after the addition of a standardized Bi(III)-EDTA solution for samples with low Bi(III) coating content. Concentrations as small as 10^{-4}Bi(III) were determined in the samples of galenite; and a more detailed working procedure is given elsewhere (*5*).

SUMMARY

It was found that it is possible to determine very small amounts of Bi(III) (10 μg) by means of volumetric solution of 0.0005 to 0.00005 *M* EDTA. Biamperometric indication to the electrode system of two stationary platinum electrodes was used. The Bi(III) determination is feasible in the presence of many ions, for example, Mg, Co, Sr, Ba, Be, U, Mn, Ag, Al, Tl, Pb, Cd, Zn, Ni, La, Th, Sc, Ga, F^-, Cl^-, SO_4^{2-}, ClO_4^-, Fe up to the ratio 1:10, Cu up to the ratio 1:1. The determination interferes with In, Zr, Tl(III) and Mo. The high selectivity of the Bi(III) determination in a considerably acid medium of HNO_3 allows a practical application of the (Bi(III) determination in many minerals and ores.

REFERENCES

1. PŘIBIL, R., "Komplexometrie II," VEB Deutsche Verlag, Leipzig, 1960.
2. VYDRA, F. AND VORLÍČEK, J., "Amperometry with two polarizable electrodes. XI. Determination of bismuth by EDTA Titration. *Talanta* **13**, 603 (1966).
3. VORLÍČEK, J., AND VYDRA, F., Amperometry with two polarizable electrodes. V. Determination of small amounts of Fe(III) by chelometric titration. *Microchem. J.* **9**, 1 (1965).
4. SANDELL, E. B., Colorimetric Determination of Traces of Metals," 3rd. ed. Wiley (Interscience), New York, 1959.
5. VORLÍČEK, J., PETÁK, P., ŠULCEK, Z., EDTA titration of Bi(III) in ores and concentrates by biamperometric indication. *Chemist-Analyst* (in press).

MICROCHEMICAL JOURNAL 12, 472-490 (1967)

II. Study of the Reactivity of Bromine in the System Acetic Acid–Water

JIŘÍ ŠEVČÍK AND JAROSLAV ZÝKA

Department of Analytical Chemistry, Charles University, Prague, Czechoslovakia

Received April 3, 1967

INTRODUCTION

The number of studies devoted to the behavior of bromine in acetic acid (*5, 14, 19, 25, 27, 29, 31, 32*), in some cases with regard to volumetric solution of this element in the solvent named, is not large. The purpose of these studies is to find an answer to problems relating to the form of bromine in solution (*1, 2, 3, 4, 6, 7, 11, 17*), and the stability of the bromine concentration is studied in relation to the possible equilibria (*22, 23, 26, 28*). The instability of the titer of volumetric bromine solutions (*18, 20, 24, 29*) has been ascribed in the majority of cases to the volatility of bromine in solution and, to increase the stability of the titer, it has been recommended to store the solutions in the cold and dark. However, we did not succeed in achieving a constant titer value by means of this procedure, and after the first experiments with bromine, obtained by means of coulometric generation, it has proved beyond doubt that the decrease of the bromine concentration is not only caused by its volatility (bromine was lost even from sealed vessels in which there was no phase boundary), but that the reaction of the bromine with the medium must also be taken into account.

In the present work, attention was given to the ternary mixture Br_2-H_2O-CH_3COOH, i.e. a volumetric bromine solution in aqueous acetic acid, and an explanation was attempted of the causes of loss of bromine, of the products formed, and of the reaction course as a whole.

The experiments were divided into several parts: mutually varied bromine; acetic acid and water concentrations in dependence on the bromide-ion concentration; temperature, illumination; pH value; and method of preparation of the volumetric solution. Several methods were used to study the assumed reaction: volumetric determination with poten-

tiometric indication; polarographic methods; chronopotentiometric measurement; conductivity measurement; spectrophotometric, and gas-chromatographic methods (i.e. methods which could offer any information whatever on the problem studied).

In the present study, it has been proven that in an acetic-acid medium a reaction takes place between bromine and the medium; the products formed were identified (i.e. tribromide ions, bromoacetic acid, carbon dioxide, methane, methylbromide, and methyl acetate) and a reaction scheme has been proposed, including an ionic as well as a radical mechanism. Moreover, it has been shown that the reaction is more rapid with the rising temperature and time of illumination. It was found that the most rapid loss of bromine takes place in 35% acetic-acid solution due to dissociation and solvatation of the acid. The bromine loss is slowest in anhydrous acetic acid containing dissolved bromide ions.

The first part of this study, i.e. communication I., is a detailed description of the experiments performed, the methods of measurement of the bromine decrease, and the formation of reaction products by means of various methods. The second part of the study, i.e. communication II, is a discussion of the experimental results and a justification of the reaction scheme proposed.

EXPERIMENTAL

Reagents

1. Bromine, reagent-grade.
2. Glacial acetic acid purified by distillation (*8, 12, 13, 15, 21*), redistilled with CrO_3 or with an addition of acetic anhydride.
3. KBr, reagent-grade.
4. The water used was either distilled or redistilled with $KMnO_4$.

1×10^{-3} N $N_2H_4 \cdot H_2SO_4$ solution was prepared according to Vulterin and Zýka (*30*). 1×10^{-3} M $Hg(NO_3)_2$ was prepared according to the method of Číhalík (*9*).

Apparatus

The potentiometer used was the Acidimetr AK (Dělba, Prague) with a Pt-foil indicator electrode and SCE, modified for concentrated acetic acid according to Číhalík and Šimek (*10*). Automatic potentiometric titrations were carried out by means of a set-up composed of the following instruments: Radiometer, Copenhagen; Auto-burette ABU 1a; Titrator TTT

1C, Titrigraph SBR 1c in connection with an electrode pair by the same firm (Pt foil, Type P 101 and SCE Type 401). Polarographic measurements were done with the polarograph Type LP 60 by Laboratorní Přístroje, Prague, with dropping-mercury electrode or rotating platinum electrode by Radiometer, Type M 22. The Polarecord E 261 by Metrohm, Herisau, with platinum and SCE electrodes was used for chronopotentiometric measurement, the Type E-211 Coulometer by the same firm was used to prepare a solution of coulometrically generated bromine.

Conductivity measurements were done with the Conductometer CDM 2d by Radiometer, with measuring cells types PP 1042 and CDC 104 by the same firm. The Spekol (Carl Zeiss, Jena) was used to measure in the visible spectral region, in the 380-750 nm range, the cells were of glass with a ground lid, layer thickness 1.00 cm. Ultraviolet spectra were studied with the Ultraviolet Spectrophotometer SP 800 by Unicam; it recorded extinction curves from 190 to 700 nm. The cells were of silica with a layer thickness of 1.00 cm and with a facility for thermostating. The Type SP-825 Programme Controller and Type SP-830 Automatic Cell Changer were added to the instrument.

Gaseous products were identified by means of the Model-C gas chromatograph (Carlo Erba) with heat-conductivity and flame-ionisation detectors, column filled with active carbon (Supersorbon Hrušov), and 9% silicone oil on Celite. Conditions and data for the columns are given together with the results.

Temperatures were kept constant with the Ultra-Thermostat U 10 by Mechanik Prüfgeräte, Medingen.

Methods and Procedures

The reaction mixture was prepared and studied as follows: acetic acid, or the binary mixture acetic acid–water, was made free of oxygen by passing through a stream of nitrogen, and elementary bromine was added to 75 ml of this solvent by an Agla microburette (the metallic needle being replaced by a ground-joint glass capillary). The solution was agitated, as the globule of elementary bromine formed. This dissolves very slowly and then the volumetric bromine solution was divided for individual measurements in such a way, that according to the method to be used, a cell was filled for spectrophotometric measurement, a known volume was measured for the volumetric bromine determination, a polarographic vessel was filled, and a conductivity cell and a vessel for chronopotentio-

metric measurement were filled. The respective bromine solution volumes for the various measurements were measured at periods of one minute (in the order in which the methods are listed above). The individual continuous measurements were started in the same order, so that the chronopotentiometric record was shifted every five minutes with respect to the time t_0 (moment of bromine addition).

A second part of the volumetric solution remaining in the stock bottle was divided into seven test tubes in portions of 5 ml each, and under equal conditions of temperature and illumination, the test tubes were sealed with rubber stoppers of the type used in gas chromatography, and immersed in a thermostat. At selected time intervals, the stopper was first pierced to take a sample for gas chromatographic measurement, and after the test tube was opened, samples were taken for volumetric investigation of changes in the bromine solution titer.

This arrangement permitted the bromine solutions to be studied by means of a number of different methods as described; the initial conditions were maintained similarly.

Investigation of Bromine Solutions by Means of Volumetric Methods

One ml of the reaction mixture was taken from the individual test tubes at certain intervals of time, diluted with 10 ml 35% acetic acid and 10 ml H_2O, and titrated with 1×10^{-3} N $N_2H_4 \cdot H_2SO_4$ with potentiometric indication, using platinum and SCE electrodes and a magnetic or blade-type stirrer.

When studying titer changes of the volumetric bromine solution in solutions of 100-90% acetic acid, the comparison electrode was modified (*10*). The volumetric hydrazine sulfate solution was added either from a burette calibrated in divisions of 0.02 ml, or from an autoburette Radiometer in connection with the Radiometer Titrigraph, by means of which the entire potentiometric curve was recorded. The bromide ions formed were determined with 1×10^{-3} M $Hg(NO_3)_2$ with potentiometric indication, using the same electrode pair as with the bromine determination. One ml of the solution was added to 10 ml carbon tetrachloride and 10 ml water and, after intensive agitation, the bromide ions were determined in the aqueous layer. In those cases, where at the beginning of the reaction (before the addition of bromine) bromide ions were present in the solution, changes of the bromide ion concentration were not studied volumetrically since the increments were relatively low.

All titrations were carried out at room temperature, whereas test tubes filled with the reaction mixture were stored in a thermostat at the temperature selected.

Polarographic Investigation of Solutions of Bromine in Acetic Acid

The polarographic method with dropping mercury electrode and saturated mercurisulphate electrode was used to measure the i/E as well as i/t relations. The LP-60 polarograph with recorder was used. The optimum conditions were found to be: range 0-2 V, abscissa 200 mV/minute, record speed 40 mm/minute, and drop time 2.1 seconds dampening 1 without compensation current.

The polarographic vessel, of 15 ml volume (Fig. 1) permitted measure-

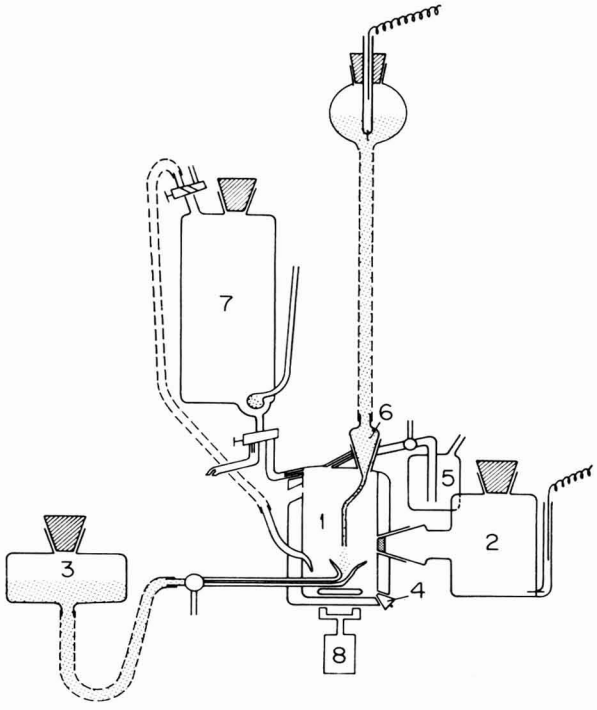


FIG. 1. Vessel for polarographic measurement: (1) polarographic vessel with mantle; (2) saturated mercury sulphate electrode; (3) levelling reservoir; (4) outlet stopper; (5) safety valve; (6) mercury-dropping electrode; (7) vessel for preparation of the reaction mixture; and (8) electromagnetic stirrer.

ments to be carried out not only under elimination of the gas phase, into which bromine might volatilise, but also at a constant temperature set by the thermostat. Also, there was a facility for studying the reaction course practically from the time t_0 onward by use of the side tubulation. The beaker-shaped mercury reservoir and the compensating mercury reservoir allowed a constant mercury surface area to be maintained in contact with the solution. After oxygen had been removed from a stock solution of already diluted acetic acid, either containing or lacking bromide ions, by a stream of nitrogen a part of the solution was transferred to the polarographic vessel and the i/E relation was measured. The vessel was then emptied and dried while being constantly washed with nitrogen gas which also was passed through the stock solution. After drying with a stream of hot air and thorough washing with nitrogen, bromine was added to the stock bottle containing the acetic-acid solution, the mixture was agitated and filled into the polarographic vessel so as to also fill the capillary leading to the safety valve; the taps were then closed and the polarogram recorded. In the case of reactions with short half-times $\left(T_{\frac{1}{2}} = \frac{[Br_2]_0}{2}\right)$ the i/t dependence was recorded at a given potential. With longer half-times, entire polarization curves were recorded and repeated after certain periods of time. There were experimental difficulties with using the rotating platinum electrode, since no suitable manner of closing and sealing the polarographic vessel could be found. This would have eliminated, at least in part, volatilisation of bromine. The saturated mercury sulfate electrode (Fig. 1) was fitted with a Type G-3 porous sinter-glass plate carrying a layer of agar with potassium sulfate. A calomel electrode was not used, as attempts to eliminate the diffusion of chloride ions into the solution studied were unsuccessful.

Chronopotentiometric Investigation of Solutions of Bromine in Acetic Acid

Into 10 ml studied solution in a thermostated vessel, a pair of electrodes was immersed (platinum and SCE with ground stopper) connected to the instrument Polarecord 261-E, used as electronic voltmeter together with a recorder registering 10-2000 mV on the full scale. The record, 75 cm long, was glued to form a loop one revolution of which lasted 18 or 6 minutes. No applied current passed through the solution, and the bromine concentration decrease was caused by a self-induced reaction. For long

reaction half-times (high concentration of bromine or acetic acid or the presence of bromide ions present at the start of the reaction) the chronopotentiometric record was not made.

Conductivity Measurements of Bromine Solutions in Acetic Acid

For the measurement, a tempered conductivity cell was filled with a 4 ml volumetric bromine solution, the vessel was closed, and the measurement carried out with the CDM-2d Radiometer instrument, using a Type CDC-104 cell with blackened platinum, or Type PP-1042 cell with bright platinum electrodes. The measurement was continuous, conductivity values (in units of mho) were read in defined intervals of time.

Spectrophotometric Measurements of Bromine Solutions in Acetic Acid

A cell of 1.00 cm layer thickness, fitted either with ground lid or teflon stopper was filled with a fresh volumetric bromine solution. The cells with ground lid were used to measure extinction values at constant wavelength with the Spekol spectrophotometer. Cells with teflon stoppers were used for the automatically repeated spectrum recordings in the 190-450 nm range carried out with the Unicam 800-SP spectrophotometer, the facilities of which allowed a time interval to be selected for the repeated measurements, automatic cell exchange, and operation at a pre-set temperature by the use of a thermostat. The recording of the spectral interval mentioned required a time of two minutes. The comparison cell was filled with acetic acid of the respective concentration.

Gas Chromatographic Investigation of Bromine Solutions in Acetic Acid

For every measurement, 1 ml of the gas phase was taken from the reaction mixture in the test tubes with rubber stoppers after the given time intervals. Each test tube served for one measurement. Measurements were carried out: (a) with the Carlo Erba instrument with flame-ionisation detector; and (b) with the Carlo Erba instrument with heat-conductivity detection. The optimum conditions were the following for the two cases: (a) column 80 cm long, 4 mm in diameter, filled with 9% silicone oil on Celite, measurement at 23°C, carrier gas-flow rate (N_2) 35 ml/minute; and (b) column 40 cm long, 4 mm in diameter, filled with active carbon (Supersorbon Hrušov) activated at 240°C in a stream of nitrogen, measurement at 70°C, carrier gas-flow rate (N_2) 10 ml/minute. The areas below the elution curve were measured by means of the elec-

tronic decadic integrator (Carlo Erba) connected to an automatic recording instrument (Kinzle).

Coulometric Bromine Generation

Bromine coulometrically generated from bromide ions, in acetic acid solutions of the respective concentrations, was used for comparisons with solutions to which elementary bromine had been added. A Coulometer made by the Metrohm company was used for the generation experiments; its separate cathode space was filled with 35% acetic-acid solution. The anodic space was filled with acetic acid of the respective concentration, which contained dissolved bromide ions. The liquid levels in the cathodic and anodic spaces were at the same height. Generation was continued at constant current for a predetermined time and the solution obtained was then divided for individual measurements.

A selected part of the results is summarized in the following tables in order to illustrate the changes taking place in the solutions.

Bromine was prepared and studied in solutions containing 100, 90, 80, 70, 60, 50, 40, 35, 30, 25, 18, and 10% acetic acid. The underlined concentrations were selected from these; after them, Tables (8-9) are shown for cases where bromine was generated coulometrically. The tables are arranged in such a way that the first column shows the serial number of the experiment, after this follows the temperature at which the reaction took place and a column of other data, amplifying the conditions given in the caption of the table. The following columns include values of the initial bromine and bromide ion concentrations determined volumetrically, and the $T_{\frac{1}{2}}$ value determined from the bromine concentration decrease by titration. Included are, moreover, molar extinction coefficient values (ε) and $T_{\frac{1}{2}}$ values obtained from the decrease of the maximum extinction corresponding to bromine, molar concentrations of bromide ions, and bromoacetic acid and $T_{\frac{1}{2}}$ values obtained from the bromine concentration decrease determined polarographically, values obtained by chonopotentiometric measurement ($E^{f}_{equiv.}$) corresponding to $\frac{\tau}{4}$ (τ-transition time), equivalent conductivity values (λ_c) at the start of the measurement, and the concentration ratio of gaseous products at the end of the experiment.

The above results will serve as basis for conclusions concerning the system studied, which will be presented in the next part of this study (Communication II) in the form of a discussion.

TABLE 1

REACTION OF BROMINE IN 90% ACETIC ACID

Exp. No.	Temp. (° C)	Remark	Volumetrically			Polarographically			$E^f_{equiv.}$ (mV)
			(Br_2) 10^{-4}	(Br^-) 10^{-2}	$T_{\frac{1}{2}}$ (minutes)	(Br^-) 10^{-4}	($CH_2BrCOOH$) 10^{-5}	$T_{\frac{1}{2}}$ (minutes)	
1	20	a	2.1	—	360	2.1	1.5	300	—
2	20	a	3.7	2	equiv.	—	—	—	—
3	60	a	3.4	—	60	3.2	—	—	—
4	60	a	4.0	2	60	60	—	47	—

			Gas chromatographically				Spectrophotometrically	
			λ_c	CH_4	CH_3Br	$CH_3COO\text{-}CH_3$	ε	$T_{\frac{1}{2}}$ (minutes)
1	20	a	20.0	1	—	1	357	> 6 hours
2	20	a	—	2	1	—	432	equiv.
3	60	a	11.3	2	1	10	328	60
4	60	a	—	—	—	—	525	62

a. Acetic acid bubbled with nitrogen.

TABLE 2

REACTION OF BROMINE IN 70% ACETIC ACID

Exp. No.	Temp. (° C)	Remark	Volumetrically			Polarographically			$E^f_{equiv.}$ (mV)
			(Br_2) 10^{-4}	(Br^-) 10^{-2}	$T_{\frac{1}{2}}$ (minutes)	(Br^-) 10^{-4}	(x^b) 10^{-5}	$T_{\frac{1}{2}}$ (minutes)	
1	20	a	5.7	1	95	3.4	—	> 250	—
2	20	a	10.5	2	> 280	1.80	—	> 240	—
3	60	a	6.7	—	17	3.3	—	—	830
4	60	a	3.5	2	25	—	—	—	—

Exp. No.	Temp. (° C)	Remark	Gas chromatographically				Spectrophotometrically	
			λ_c	CH_4	CH_3Br	$CH_3COO\text{-}CH_3$	ε	$T_{\frac{1}{2}}$ (minutes)
1	20	a	1.98	—	—	—	220	> 6 hours
2	20	a	4.13	1	1	140	290	> 6 hours
3	60	a	4.12	1	6	160	157	20
4	60	a	—	4	4	205	429	30

a. Acetic acid bubbled with nitrogen.

b. $CH_2BrCOOH$.

TABLE 3

REACTION OF BROMINE IN 50% ACETIC ACID

Exp. No.	Temp. (° C)	Remark	Volumetrically			Spectrophotometrically			
			$(Br_2) \times 10^{-4}$	$(Br^-) \times 10^{-2}$	$T_{\frac{1}{2}}$ (minutes)	ε	$T_{\frac{1}{2}}$ (minutes)	$E^f_{equiv.}$ (mV)	λ_c
1	20	b	4.25	—	—	55	32	830	0.84
2	20	b	11.0	—	65	139	77	795	0.99
3	20	b	11.0	—	65	136	75	—	—
4	20	b	21.0	—	—	70	105	—	0.88
5	20	b	90.3	—	—	57	130	—	0.90
6	20	b	180.0	—	—	79	13 days	880	0.96
7	20	b	450.0	—	—	80	constant	—	1.20

b. Acetic acid not bubbled with nitrogen; extinction values were read at 400 nm.

TABLE 4

REACTION OF BROMINE IN 35% ACETIC ACID

Exp. No.	Temp. (° C)	Remark	Volumetrically			Polarographically			$E^f_{equiv.}$ (mV)
			(Br_2) $\times 10^{-4}$	(Br^-) $\times 10^{-2}$	$T_{\frac{1}{2}}$ (minutes)	(Br^-) $\times 10^{-4}$	(x^f) $\times 10^{-5}$	$T_{\frac{1}{2}}$ (minutes)	
1	20	a	2	—	12	2	—	15	890
2	20	a	8	—	25	7.5	—	30	870
3	20	a	12	—	—	—	—	—	865
4	20	a	20	—	60	18	—	40	—
5	20	a	1.5	2	20	—	8	30	—
6	20	b	16.6	0.045		—	—	—	—
7	20	b	26.5	0.25		—	—	—	—
8	20	b	26.8	0.35		—	—	—	—
9	20	b	13.7	10		—	—	—	—
10	20	b	37.2	100		—	—	—	—
11	15	b	4.0	—	—	—	—	—	860
12	30	b	1.35	—	—	—	—	—	870
13	40	b	5.9	—	—	—	—	—	850
14	60	a	17	—	3	1.5		6	860
15	70	b	29	—	—	—	—	—	860
16	60	a	22.5	2	10	—	10	13	—
17	20	c	2	—	15	1.8	—	—	890
18	20	d	29	—	75	26	—	65	—
19	20	e	1.8	—	140	1.2	—	150	—
20	20	b	7.5	—	21	2.8	35	20	875

Exp. No.	Temp. (°C)	Remark	λ_c	Gas chromatographically			Spectrophotometrically		τ (minutes)
				CH_4	CH_3Br	CH_3COO-CH_3	ε	$T_{\frac{1}{2}}$ (minutes)	
1	20	a	—	—	—	—	380	—	
2	20	a	0.21	2	1	8	150	25	
3	20	a	0.19	5	1	40	108	27	
4	20	a	0.22	22	6	64	188	80	
5	20	a	0.30	3	1	3	400	34	
6	20	b	0.19	—	—	—	(47)	28	
7	20	b	0.24	—	—	—	(81.5)	45	
8	20	b	0.25	—	—	—	(73.5)	62	
9	20	b	0.92	—	—	—	(320)	125	
10	20	b	—	—	—	—	(282)	8 hours	
11	15	b	0.20	—	—	—			150
12	30	b	0.25	—	—	—	(190)	21	48
13	40	b	0.25	—	—	—	—		36
14	60	a	0.32	4	3	10	209	6	33
15	70	b	0.34	1	300	92	—		9
16	60	a	—	3	2	4	285	15	
17	20	c	0.27	2	2	1	150	15	
18	20	d	—	1	10	—	152	15	
19	20	e	1.3	3	1	—	390	120	
20	20	b	0.18	3	1	—	134	25	

a. Acetic acid bubbled with nitrogen.

b. Acetic acid nonbubbled with nitrogen; extinction values were read at 400 nm.

c. 0.2 g $NaClO_4$ added.

d. Exposed to u.v. light in quartz cuvettes.

e. Nondistilled acetic acid.

f. $CH_2BrCOOH$.

TABLE 5

REACTION OF BROMINE IN 35% ACETIC ACID

Exp. No.	Temp. (° C)	Remark	Volumetrically			Polarographically				Spectrophotometrically	
			(Br_2) $\times 10^{-4}$	(Br^-) $\times 10^{-2}$	$T_{\frac{1}{2}}$ (minutes)	(Br^-) $\times 10^{-4}$	(x^g) $\times 10^{-5}$	$T_{\frac{1}{2}}$ (minutes)	$E^f_{rov.}$ (mV)	ε	$T_{\frac{1}{2}}$ (minutes)
21	20	[a]	1.5	—	10	1.4	—	—	870	—	10
22	20	[b]	5.0	—	5	4.6	—	5	860	260	5
23	20	[c]	1.0	—	—	—	—	—	880	(70)	15
24	20	[d]	10.0	—	—	—	—	—	840	(97)	65
25	20	[e]	4.5	—	—	—	—	—	—	(112)	8 hours
26	20	[f]	4.0	—	—	—	—	—	—	(100)	30 hours

[a] Acetic acid nonbubbled with nitrogen, sodium acetate added, pH = 4.5.

[b] Acetic acid bubbled with nitrogen, sodium acetate added, pH = 3.1.

[c] Acetic acid nonbubbled with nitrogen, sodium acetate added, pH = 2.5.

[d] Acetic acid nonbubbled with nitrogen, sodium acetate added, pH = 2.25.

[e] Glacial acetic acid diluted with H_2SO_4, of pH = 1.6, pH = 0.9.

[f] Ten M H_2SO_4 with 23 g sodium acetate, pH $\simeq -4$.

[g] $CH_2BrCOOH$.

TABLE 6

REACTION OF BROMINE IN 18% ACETIC ACID

Exp. No.	Temp. (° C)	Remark	Volumetrically			Polarographically			$E^f_{equiv.}$ (mV)
			(Br_2) $\times 10^{-4}$	(Br^-) $\times 10^{-2}$	$T_{\frac{1}{2}}$ (minutes)	(Br^-) $\times 10^{-4}$	(x^c) $\times 10^{-5}$	$T_{\frac{1}{2}}$ (minutes)	
1	20	b	1.0	—	—	—	—	—	868
2	20	a	1.3	—	—	1.0	5.0	—	900
3	20	a	2.5	—	15	2.0	—	23	870
4	20	b	14.7	—	—	—	—	—	—
5	20	b	43	—	—	—	—	—	—
6	20	a	1.7	2.0	28	1.2	—	25	—
7	20	b	5.9	—	—	—	—	—	—
8	60	a	2.8	—	15	1.3	—	12	900
9	60	a	6.3	2	15	30	800	8	—

Exp. No.	Temp. (° C)	Remark	Gas chromatographically			Spectrophotometrically	
			λ_c	CH_4	CH_3Br	$CH_3COO\text{-}CH_3$	ε	$T_{\frac{1}{2}}$ (minutes)
1	20	b	0.48	—	—	—	—	25
2	20	a	—	1	1	—	385	—
3	20	a	—	2	1	4	280	45
4	20	b	0.51	—	—	—	(92)	45
5	20	b	0.51	—	—	—		3 days
6	20	a	—	2	1	4	413	45
7	20	b	—	—	—	—	—	140
8	60	a	0.66	1	20	6	286	15
9	60	a	—	2	1	20	206	8

a. Acetic acid bubbled with nitrogen.

b. Acetic acid nonbubbled with nitrogen, extinction values were read at 400 nm.

c. $CH_2BrCOOH$.

TABLE 7

REACTION OF BROMINE IN 10% ACETIC ACID

Exp. No.	Temp. (° C)	Remark	Volumetrically			Polarographically			$E^f_{equiv.}$ (minutes)
			(Br_2) $\times 10^{-4}$	(Br^-) $\times 10^{-2}$	$T_{\frac{1}{2}}$ (minutes)	(Br^-) $\times 10^{-4}$	(x^c) $\times 10^{-5}$	$T_{\frac{1}{2}}$ (minutes)	
1	20	a	1.7	—	45	2.0	—	130	—
2	20	b	48	—	—	—	—	—	—
3	20	a	2.0	2.0	105	—	—	7 hours	—
4	60	a	15.5	—	6 hours	12	—	35	—
5	60	a	10.0	2.0	4.5 hours	7.7	20	45	—

				Gas chromatographically			Spectrophotometrically	
			λ_c	CH_4	CH_3Br	$CH_3COO\text{-}CH_3$	ε	$T_{\frac{1}{2}}$ (minutes)
1	20	a	0.78	2	1	6	411	165
2	20	b	0.84	—	—	—	(39)	> 10 hours
3	20	a	1.32	1	1	3	405	7 hours
4	60	a	1.37	1	80	3	143	> 3 hours
5	60	a	—	1	2	1	270	120

a. Acetic acid bubbled with nitrogen.

b. Acetic acid nonbubbled with nitrogen, extinction values were read at 400 nm.

c. $CH_2BrCOOH$.

TABLE 8

REACTION OF GENERATED BROMINE IN 35% ACETIC ACID

Exp. No.	Temp. (° C)	F (coulomb)	t (minutes)	$(Br_2) \times 10^{-4}$	(Br^-) 10^{-2}	$T_{\frac{1}{2}}$ (minutes)	τ^a (minutes)
1	20	0.097	16.17	5.2	5.95	3.8	16
2	20	0.19	10.5	9.8	5.95	9.5	40
3	20	0.960	16.0	50.2	5.95	32	131
4	20	1.905	10.5	98.5	5.95	77	320
5	20	2.79	15.5	145	5.95	95	380
6	20	9.35	15.6	480	5.95	125	—
7	20	18.7	31.1	970	5.95	180	—
8	20	0.1905	10.5	9.85	0.046	2.5	7
9	20	0.1905	10.5	9.85	0.41	3.0	—
10	20	0.1905	10.5	9.85	4.00	—	10
11	20	0.1905	10.5	9.85	40.0	—	14

[a] τ time equals time max. $\Delta E/min$.

TABLE 9

REACTION OF GENERATED BROMINE IN ACETIC ACID

Exp. No.	Temp. (° C)	AcOH %	F (coulomb)	$T_{\frac{1}{2}}$ (minutes)	τ (minutes)
1	20	10	0.960	7 hours	—
2	20	18	0.963	140	230
3	20	25	0.962	65	160
4	20	30	0.960	70	170
5	20	35	0.960	58	130
6	20	40	0.962	130	180
7	20	50	0.961	140	200
8	20	60	0.961	240	—
9	20	70	0.961	380	—
10	20	80	0.961	24 hours	—
11	20	90	0.962	90 hours	—

Note.—Constant bromine amount generated from 5.95×10^{-2} M-KBr solution. Generation time 16 minutes. τ min = time max. $\Delta E/t$.

SUMMARY

The behavior of bromine in acetic acid, as a dependence on its concentration, temperature, and light, with regards to the stability of the above element in the medium used and to the products formed, was studied. In this first part of the study, experiments with the use of various techniques of measurements (volumetric methods, potentiometry, polarography, chronopotentiometry, conductivity, spectro-

photometry, and gas chromatography), are described. The discussion and scheme of the reaction is presented in the second part of this study.

REFERENCES

1. ANBAR, M., AND GINSBURG, D., Organic hypohalites. *Chem. Rev.* **54**, 925-958 (1954).
2. ANBAR, M., AND REIN, R., The isotopic exchange between hypohalites and halide ions. I. The Exchange between hypobromous acid bromide ion. *J. Am. Chem. Soc.* **81**, 1813-1815 (1959).
3. BELL, R. P., AND GELLES, E., The halogen cations in aqueous solutions. *J. Chem. Soc.*, 2734-2740 (1951).
4. BENNETTO, H. P., FEAKINS, D., AND TURNER, D. J., Studies in ion solvation in non-aqueous solvents and their aqueous mixtures. Part V. The halogen acids in 10, 20, 40 and 60% (W/W) acetic acid-water mixtures at 25°. *J. Chem. Soc (A)*, 1211-1216 (1966).
5. BRADFIELD, A. E., JONES, B., AND ORTON, K. J. P., The halogenation of phenolic ethers and anilides. Part I. The bromination of ethers in 50% acetid acid. *J. Chem. Soc.*, 2810-2817 (1929).
6. BRAY, W. C., The hydrolysis of iodine and of bromine. *J. Am. Chem. Soc.* **32**, 932-938 (1910).
7. BRAY, W. C., AND CONNOLY, E. L., The hydrolysis of iodine and of bromine. A correction. *J. Am. Chem. Soc.* **33**, 1485-1487 (1911).
8. CONANT, J. B., AND WERNER, T. H., The determination of the strength of weak bases and pseudo bases in glacial acetic acid solutions. *J. Am. Chem. Soc.* **52**, 4436-4448 (1930).
9. ČÍHALÍK, J., "Potentiometry," Publishing House of the Czechoslovak Academy of Sciences, Prague, 1961. (in Czech).
10. ČÍHALÍK, J., AND ŠIMEK, J., Polarography in anhydrous acetic acid I. Introductory communication. *Chem. Listy* **51**, 1283-1288 (1957). (in Czech).
11. HAGISAWA, H., A study, by means of the glass electrode, of the hydrolysis of bromine. *Bull. Inst. Phys. Chem. Res. (Tokyo)* **21**, 593-596 (1942).
12. HALL, N. F., AND VOGE, H. H., Electrical conductivities of mixtures of sulfuric acid, acetic acid and water. *J. Am. Chem. Soc.* **55**, 239-246 (1933).
13. HARNED, H. S., AND EHLERS, R. W., The dissociation constants of acetic acid from 0 to 35° centigrade. *J. Am. Chem. Soc.* **54**, 1350-1357 (1932).
14. HEGERTY, A. F., AND SCOTT, F. L., The kinetics of bromination of hydrazones. *J. Chem. Soc. (B)* **1966**, 672-675
15. HUTCHINSON, A. W., AND CHANDLEE, G. C., Activity coefficients of sulfuric acid in anhydrous acetic acid. *J. Am. Chem. Soc.* **53**, 2881-2888 (1931).
16. INGBERMAN, A. K., The functionality of phenols by bromination. *Anal. Chem.* **30**, 1003-1004 (1958).
17. JAKOWKIN, A. A., Zur theorie der Lösungen. IV. Dissociation und Polymerisation. *Z. Physik. Chemie* **70**, 182-192 (1909).
18. JANDER, G., AND FEIT, H., Über die sich durch die Gegenwart verschiedener Elektrolyte ändernde Flüchtigkeit des Broms aus wässrigen Lösungen und über die bromometrische Analyse. *Z. Anorg. Allgem. Chem.* **44**, 305-312 (1924).

19. JONES, J. W., The determination of solubility coefficients by aspiration. *J. Am. Chem. Soc.* **99**, 392-404 (1911).

20. KAUFMANN, H. P., AND HANSEN-SCHMIDT, E., Bromometrische Versuche. *Arch. Pharm.* **263**, 32-50 (1925).

21. KENDALL, J., AND GROSS, P. M., Compound formation and specific conductivity in solutions of the types acid:ester, acid:ketone and acid:acid. *J. Am. Chem. Soc.* **43**, 1426-1439 (1921).

22. LEWIN, M., The composition of hypobromite solutions at various pH values and bromine concentrations. *Bull. Res. Council Israel* **2**, 101-104 (1952); *CA* **47**, 4779a (1953).

23. LUCHINSKIJ, G. P., Phase equilibria in the system containing halides and free halogenes. III. Tensimetric investigation of the system H_2O-Br_2-KBr. *J. Gen. Chem.* (U.S.S.R.) **9**, 708-713 (1939).

24. MANCHOT, W., AND OBERHAUSER, F., Bromometrische Bestimmungen. *Z. Anorg. Allgem. Chem.* **139**, 40-50 (1924).

25. MEYER, R. J., "Gmelins Handbuch der anorganischen Chemie 7," 8th ed., Verlag Chemie G.m.b.H., Berlin, 1931.

26. RIGBI, Z., A tetrahedral representation of the relationship between four phases. *Bull. Res. Council Israel,* **2**, 99-100 (1952).

27. ROBERTSON, P. W., CLARE, N. T., MCNAUGHT, K. J., AND PAUL, G. W., The kinetics of bromine addition to olefinic compounds. *J. Chem. Soc.,* 335-343 (1937).

28. SCAIFE, D. F., TYRRELL, J. V., Equilibrium constants for the reaction between bromine and bromide ions at 5°, 25°, and 35° in aqueous medium of constant ionic strength and acidity. *J. Chem. Soc.* (1958) 386-392.

29. TOMÍČEK, O., AND HEYROVSKÝ, A., Titration in non-aqueous solutions IV. Titration in oxidoreduction medium of glacial acetic acid. *Chem. Listy* **44**, 245-253 (1950) (in Czech).

30. VULTERIN, J., AND ZÝKA, Hydrazine sulphate as volumetric reagent (Hydrazinometry) I. Determination of bromate. *Chem. Listy* **48**, 1745-1753 (1954) (in Czech).

31. WALKER, I. K., AND ROBERTSON, P. W., The kinetics of bromine addition to olefinic compounds. Part II. The homogenous mechanism. *J. Chem. Soc.,* (1939) 1515-1518.

32. WILLIAMS, M. D., The addition of halogenes to unsaturated acids and esters Part IV. The rate of addition of bromine to cinnamic acid in acetic acid. *J. Chem. Soc.* (1932) 979-984.

MICROCHEMICAL JOURNAL **12**, 491-511 (1967)

II. Study of the Reactivity of Bromine in the System Acetic Acid–Water

JIŘÍ ŠEVČÍK AND JAROSLAV ZÝKA

Department of Analytical Chemistry, Charles University, Prague, Czechoslovakia

Received April 3, 1967

INTRODUCTION

In the first part of this study, Communication I, (*45*) we have given a detailed description of experiments in which the most variegated methods have been used to study the loss of bromine in acetic-acid solutions of various concentrations, as well as the reaction products thus formed. The present communication forms a continuation of Communication I and discusses the course of the reaction; this paper also proposes a definite reaction scheme.

DISCUSSION OF RESULTS

The bromine concentration, studied by volumetric means as well as by the other methods mentioned, exhibited a lasting decreasing tendency. The rate at which this concentration decrease proceeded depended on a number of factors—in particular the acetic acid and bromine concentrations, temperature, etc.

In order to be able to exclude the fact that the bromine concentration decrease might have been caused by reduction by means of impurities accidentally present in the reacting components, attention was first given to the purity of the individual reaction components.

The probability of the presence of reducing substances in the bromine was small, since Meyer (*39*) mentions only small amounts of chlorine, and chloride and bromide ions as possible impurities. Coulometric generation of bromine was used as well as elementary bromine, and the relations of bromine loss observed were the same in both cases.

The acetic-acid solutions used were prepared from glacial acetic acid purified in several ways. The acetic acid was either of reagent grade purity, or purified by distillation (*13, 23*), redistillation, redistillation with

additions of chromium oxide (*24, 26*) or it was allowed to stand with chromium oxide for three hours and redistilled with an addition of acetic acid anhydride (*29*). In all the kinds of acetic acid, purified by different methods, the bromine concentration decrease is the same. Similarly, the saturation of the acetic acid solution with nitrogen gas did not influence the bromine loss.

The water used was either distilled or bidistilled (in the presence of

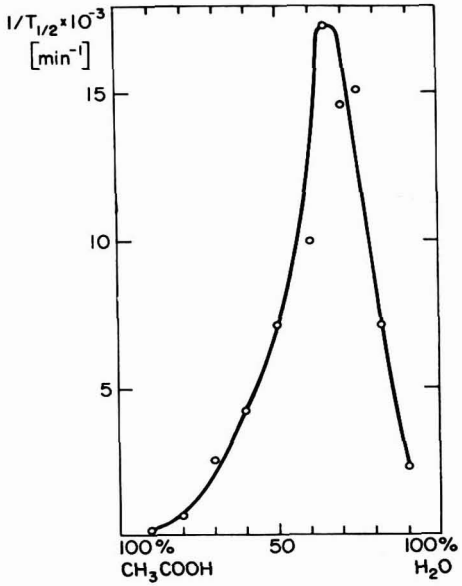


FIG. 1. Dependence of the half-time of the reaction of 2×10^{-3}N Br_2 with various concentrations of acetic acid, on the composition of the solution at 20°C.

$KMnO_4$) and did not exhibit any reducing properties. It follows, therefore, that the bromine concentration decrease is not caused by any impurity of the initial substances, and the loss may thus be ascribed to a reaction between bromine and the aqueous acetic acid solution.

As follows from the tables given in Communication I (*45*), the rate of decrease of a given bromine concentration in relation to the acetic acid concentration may be expressed by means of a curve, as shown in Fig. 1. The figure shows, that the shortest half-time of the assumed reaction $(T_{1/2} = \frac{(Br_2)_0}{2})$ corresponds to approximately 35% CH_3COOH, while in the direction toward the pure reaction components of the binary mix-

ture, the reaction half-time becomes longer (e.g. in 90% CH_3COOH). The bromine concentration decrease is observable only in periods of about one-half hour.

To be able to answer the question of whether the change of acidity of the solution, caused by dilution of the acetic acid, is a reason for the altering rate of decrease of the bromine concentration in relation to the acetic-acid concentration, attention was given to possible acidity changes in dependence on the dilution of the acetic acid.

Table 1 gives pH values calculated by means of the dissociation constant $K_{CH_3COOH} = 1.86 \times 10^{-5}$ and values of the H_0 function from data

TABLE 1

DEPENDENCE OF SOME CONSTANTS OF ACETIC ACID ON ITS CONCENTRATION

CH_3COOH		Acidity		
%	mol/l	pH	H_0	pK'_{CH_3COOH}
90	15	1.78	0.62	2.25
70	11.6	1.84	1.60	3.26
50	8.35	1.91	1.95	4.22
35	5.85	2.00	2.09	5.63
18	3.00	2.13	2.17	5.15
10	1.67	2.26	2.24	4.95
6	1.0			4.86
0.6	0.1			4.76

given by Wiberg (*48*). Solutions of the acidity function H_0 in the system $HBr - CH_3COOH - H_2O$ are cited by Schwarzenbach and Stensby and Zajac and Nowiski (*41*, *49*).

It follows from the values given, that the changes, in the pH as well as H_0 scales, are small in the range of shortest half-time reactions, and exhibit no great differences even for solutions more dilute than 35% acetic acid. In the second range of long half-time reactions—more concentrated acetic-acid solutions—and especially over 70%, the differences found are greater.

It may be concluded, from the results obtained, that the most rapid bromine concentration change around 35% CH_3COOH is not caused by a change of the acidity of the solution due to dilution of the acetic acid.

Table 5 of Communication I (*45*) lists reaction half-times in dependence on the varying pH of the solution due to different additions of sodium acetate. To be able to decide whether this is a process conditioned by the pH variation, or whether this great difference of the rate of bromine

concentration decrease is caused by a change of the acetate anion concentration, or possibly by a change of the ionic strength of the solution, experiments have been carried out with phosphate-buffer solution; the ionic strength of the solution being kept constant by means of sodium perchlorate.

Additions of sulfuric acid to the acetic-acid solution were also tried, although sulfuric acid is not a strong acid in acetic acid (*12*); yet it causes a concentration increase of ionized $CH_3COOH_2^+$ forms (*5, 44*) corresponding to $pK_{AcH_2}^+ = -6, 12$ (*44*) and thus it causes a decrease of the acetate anion concentration. This addition caused the reaction half-time to be prolonged (Table 5, No. 26) (*45*).

Therefore, it may be stated that the active component of the system is the overall acetate-anion concentration which is subject to the influence of the hydrogen-ion concentration.

Since the prolonged $T_{1/2}$ values of solutions less concentrated than 35% CH_3COOH could not be explained from the above experiments, attention had to be given to other questions concerning the use of acetic acid as solvent, i.e. the form of acetic acid in the solution, the ionic strength of the solvent, and solvatation of the system.

Up to the present, the opinion has been maintained (*10, 31*), that in the concentrated state, acetic acid is present in the form of the dimer, polymers being mentioned by Batuev (*3*) only. A study by other authors (*43*) has been published at the same time as Campbell and as Gieskes (*10*), in which measurements of the nonzero dipole moment of acetic acid confirms some of the earlier results (*25, 40*), by stating that in the concentrated state the concern is not with the cyclic nonpolar form of acetic acid, but with a mixture with the linear chain form which has a certain dipole moment.

The dielectric constant of the system mentioned has also been measured in the present study; for the concentration range of 0-20% H_2O, the same dependence of the dielectric constant on composition of the mixture has been obtained as in Campbell and Gieskes (*10*).

For more strongly diluted acetic acid solutions, the dielectric constant attains values far greater than 100, and in our arrangement (universal Type OH-301 Dielectrometer, Szabó-Nagy system made by Radelkis, operating on a frequency of 3 MC and permitting DC measurements in the 1-100 range) it could not be measured.

As is to be seen from Fig. 2, a high polarity corresponds to the medium

acetic acid concentration range: this polarity may be due to solvation, ionization (*32, 37*) or dissociation.

The possible variation of the value of K'_{CH_3COOH} was studied by means of conductivity data. From the Oswald dilution law follows the following relation for the dissociation constant K'_{CH_3COOH} $K' = \frac{c\,\lambda_c^2}{1.225 \times 10^5 - 350\,\lambda_c}$ from which we see the dependence of the dissociation constant on the value

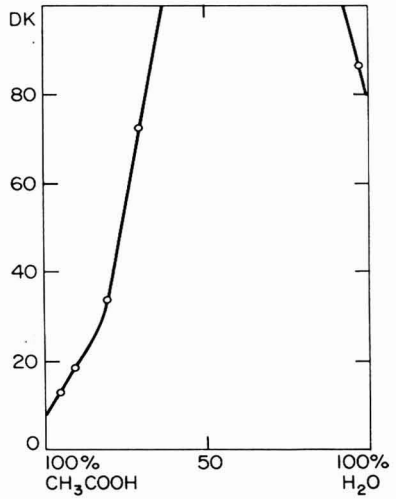


FIG. 2. Variation of the dielectric constant of the system acetic acid-water in dependence on composition at 20°C.

of λ^c. The curve shown in Fig. 3 can be constructed from data given in Tables 1-7 (Communication I (*45*)), using data given by Eichelberger and La Mer (*18*).

It is to be seen from the course of pK'_{CH_3COOH} values in dependence on the composition of the acetic acid-water system, that the dissociation constant does not attain its greatest value at 18% CH_3COOH—a concentration to which the greatest conductivity value corresponds (*18*), but that a comparison of the $1/T_{\frac{1}{2}}$ values from Fig. 1 with pK'_{CH_3COOH} values from Fig. 3 allows a linear dependence to be obtained, and thus the change of the rate of bromine concentration decrease may be related to the change of the apparent dissociation constant of acetic acid. The great increase of the dielectric constant of the system, however, cannot be ascribed only to a change of the K'_{CH_3COOH} values.

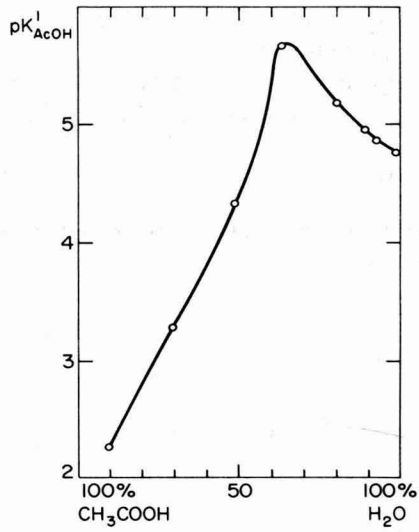


FIG. 3. Dependence of the pK'_{CH_3COOH} value on the composition of the bindery mixture CH_3COOH-H_2O calculated from conductivity data.

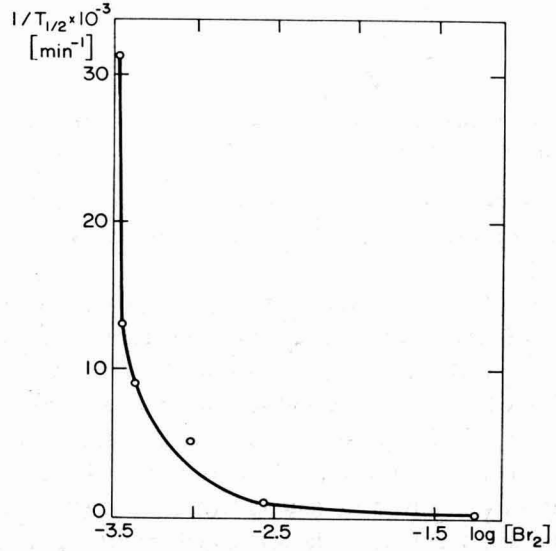


FIG. 4. Dependence of the gradually increasing reaction half-time on the rising initial bromine concentration in 50% CH_3COOH.

From the work of some authors, dealing with the solvatation effect of water, follows (*3, 48*) that the number of solvatation molecules is greatest in a medium of about 35% acetic acid—a fact which confirms our observations of the lowest stability of bromine precisely in acetic acid of the above concentration.

As already stated, there is a decrease of the bromine concentration in the system studied, in such a manner that the half-time of the reaction becomes longer with the rising bromine concentration (Fig. 4).

However, even if the same ratio of molar concentrations of the reacting components is maintained, the reaction half-time is not the same, e.g. with a ratio of $Br_2:CH_3COOH = 1:2 \times 10^4$ in 35% CH_3COOH the half-time is 25 minutes, while in 100% CH_3COOH there is no observable bromine concentration decrease.

When considering the reasons for the loss of bromine, it was necessary to admit the possibility of its disproportionality which is often mentioned in literature.

The hydrolysis of bromine to hypobromite with the equilibrium constant $K_h = 2.4 \times 10^{-8}$ (*8, 9*), 5.8×10^{-9} (*34*), 4.2×10^{-9} (*22*) and the dissociation of hypobromite with the equilibrium constant $K = 2 \times 10^{-9}$ (*34*) and 2×10^{-11} (*46*) were assumed for aqueous solutions as well as the solvatation form $HBrO \cdot H_2O$ supposed by Jakowkin (*27*).

For aqueous solutions also, the equilibria (*36*) originating from bromide and hypobromite ions may be considered. It is evident that with the equilibria mentioned (*36*), the equilibrium constants $K_{Br_3^-}$ and $K_{Br_5^-}$ would compete in the presence of bromide ions.

Bromine is more soluble in a mixture of acetic acid and water than in water alone, and the equilibrium constant of tribromide ions depends, beside temperature and ionic strength of the solution (*21*), also on the composition of the acetic acid-water system (*28*); it shifts in favor of the formation of Br_3^- in solutions with increasing acetic acid concentrations.

Beside the equilibria and manners of disproportionation mentioned, we must also consider the ionization of bromine in the solution (*4*) and the formation of Br^+ ions, or their hydrated form, H_2O^+Br.

In all the cases of bromine disproportionation mentioned, the concentration of the oxidising component remains constant. In the case of the problem studied in the present investigation this, however, was not the case. Decreasing hydrazine sulphate consumption and spectrophotometric measurements, in the 190-450 nm range, have shown that a hydrolytic

reaction is out of consideration (no extinction peak was found on the extinction curve at 325 nm).

The possibility of bromine concentration loss by volatilisation was negligible (*38*) as the solution was, in some cases, stored in vessels in which there was no phase boundary.

Based on the experiments and the above-mentioned possibilities of changes of the molecular form of bromine, it may be assumed that after bromine is added to an acetic acid solution, ionization of bromine takes place first, followed by the formation of tribromide ions: (1) $Br_2 + H_2O \rightarrow H_2O^+Br + Br^-$; and (2) $Br_2 + Br^- \rightleftharpoons Br_3^-$.

It follows from the above-mentioned scheme, which holds true for all acetic acid concentrations, that the presence of tribromide ions immediately at the beginning of the reaction will influence the course of this reaction. Therefore, measurements have been carried out in which bromide ions were dissolved in the solution before the addition of elementary bromine. In this case, the order of the reactions (1) and (2) will be reversed, and the Br^+ concentration will be lower than in the case when bromide ions are absent. The prolonged reaction half-time also corresponds to this assumption, as seen from Table 4, No. 6-10 (Communication I (*45*)).

During the experiments, a dependence on the order in which bromine and bromide ions are added was also observed. As stated by Anbar and Ginsberg (*1*), the following reversible reaction takes place: (3) $CH_3COOBr + Br^- \rightleftharpoons Br_2 + CH_3COO^-$. Thus, the addition of bromide ions to a solution of acetic acid and bromine will cause a new portion of molecular bromine to be set free. This may be observed from the increased extinction coefficient and prolonged reaction half-time (see Tables 1 and 2).

TABLE 2

DEPENDENCE OF THE CONSTANT k'_1 ON THE CONCENTRATION OF ACETIC ACID

AcOH %	k'_1 (min^{-1})	
	20°C	60°C
90	1.23×10^{-3}	4.17×10^{-3}
70	1.77×10^{-3}	3.85×10^{-2}
50	6.1×10^{-3}	—
35	2.7×10^{-2}	1.00×10^{-1}
18	4.86×10^{-3}	2.00×10^{-1}
10	3.41×10^{-3}	9.1×10^{-2}

The fact has been confirmed experimentally that bromine cannot be formed from bromide ions after illumination in analogy to iodine (*16*).

Bromine also was added to solutions by means of coulometric generation from bromide ions. However, it must be kept in mind that the conditions are not identical with the conditions of experiments in which bromine was added from a burette.

The difference is that bromide ions convert first into atomic and not molecular bromine; the two bromine forms differ in reactivity. Also, under the assumption that atomic bromine would first react to the molecular form, which would later react according to the reaction scheme (equations 1 and 2), we, even in this, cannot speak of equal conditions. The free molecular bromine concentration will rise, due to the equilibrium constant $K_{Br_3^-}$, and this will cause a rise of the concentration of Br^+ formed by ionization of Br_2. Since the establishment of the $K_{Br_3^-}$ equilibrium is practically immediate, it was found when bromine had been added to a solution of acetic acid with dissolved bromide ions and the extinction peak at 266 nm was measured, that the extinction value arrives immediately at the maximum value, and does not increase with time in the way which would correspond to a reaction taking place in time. Thus the bromine concentration rise is given by the rate of ionization and reaction of Br^+ with the system.

The results of experiments with bromine obtained coulometrically thus serve to confirm the veracity of the phenomenon (not to compare individual measured values).

The reaction scheme proposed was investigated as follows:

(a) The sum of oxidizing components was studied by volumetric means (titration with 1×10^{-3} M $N_2H_4 \cdot H_2SO_4$). The potentiometric curves, the inflexion potential of which was around 620 mV in the absence of bromide ions and around 520 mV in the presence of bromide ions, showed no anomalies, and the potential change in the point of equivalence was 450-500 mV/0.1 ml.

(b) The spectrophotometric method made it possible to distinguish the two oxidation forms, Br_2 and Br_3^-, present in the solution. Bromine in the form of Br_2 has an extinction peak at 390 nm with $\varepsilon = 220$ (*11*), while for Br_3^- the extinction peak is at 266 nm (*2, 20*) and $\varepsilon = 3.46 \times 10^4$ (*2*).

When elementary bromide is added to an acetic-acid solution, a peak appears on the extinction curve at 390 nm only, and decreases with time

while a new one starts to appear at 266 nm. While the bromine concentration decreases continuously, the tribromide ion concentration achieves maximum values in the time t_{max} (Fig. 5) after which a decrease follows.

An isosbestic point may be observed on the extinction curves up to the time t_{max}, after which the extinction curve decreases symmetrically, as seen in Fig. 6.

When bromide ions have been added to the solution before bromine was

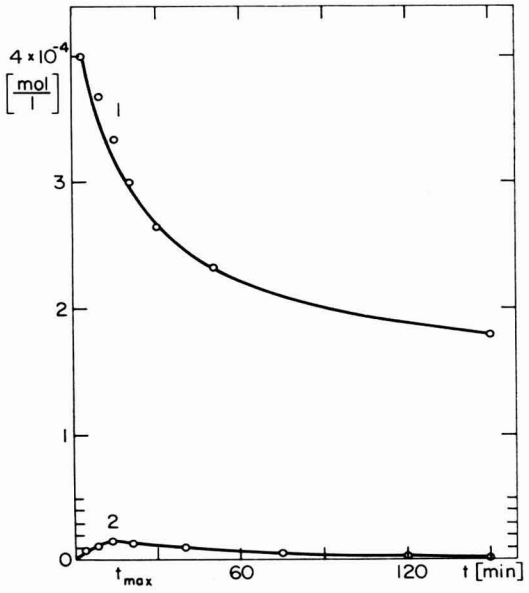


FIG. 5. Course of molar concentrations of Br_2 (curve 1) and Br_3^- (curve 2) in 35% CH_3COOH at 20°C in dependence on time.

added, two peaks appear on the extinction curve, the first one at 390 nm ~ Br_2, the second at 266 nm ~ Br_3^-. During the course of time the entire extinction curve starts to decrease symmetrically.

The molar extinction coefficient, calculated from the initial Br_2 and Br_3^- concentrations, using $K_{Br_3^-} = 0.022$ (*28*) attains values lower than the published 2.45×10^4 (cm^2/millimole), nonetheless, it is so high that the extinction scale was insufficient for more concentrated solutions.

(c) The concentration rise of bromide ions was studied volumetrically, using potentiometric indication of the point of equivalence. The potential change in the point of equivalence was around 90 mV/0.1 ml 10^{-3}M $N_2H_4 \cdot H_2SO_4$.

(d) A polarographic technique was also used to study the bromide ion concentration. (Polarography in anhydrous acetic acid is studied, for example, by Botta-Conesa, *et al.*, and Covington, *et al.* (*7, 15*). Bromide ions was manifested by an anodic wave, while of the cathodic limiting current, it is difficult to decide unambigously whether it corresponds to bromine in the form of Br_2 or to some other oxidizing form or possibly a sum of oxidizing components. As will be mentioned, there is a partial justification of the assumption that this is the limiting current of bromine. The peak on the polarization curve also cannot be explained in any

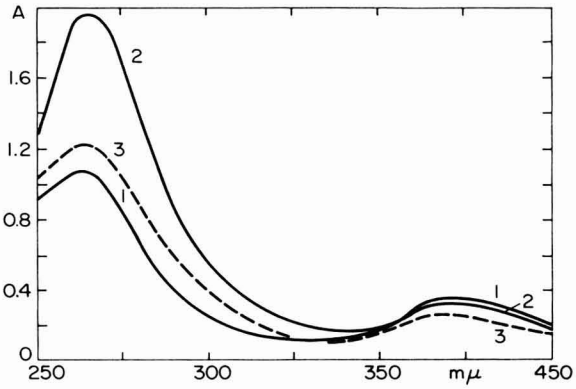


FIG. 6. Course of the extinction curve of 2×10^{-3}N Br_2 in 35% CH_3COOH at 20°C in dependence on time from the beginning of the reaction: curve 1 = after 1 minute; curve 2 = after 5 minutes; and curve 3 = after 150 minutes.

detail. Blažek (*6*) mentions its existence, stating that it is located on the oxygen wave and is of nonturbulent character. Efforts at removing it were unsuccessful.

After bromine is added to an acetic acid solution, a cathodic wave appears with $E_{\frac{1}{2}} = -0.33$ V, the limiting current of which is deformed by the peak already mentioned. With the course of time an anodo-cathodic wave forms, the half-wave potential of which does not vary with the growing anodic and decreasing cathodic part. Together with this phenomenon the peak starts to decrease. In solutions in which bromine has disappeared totally, the polarization curve shows only an anodic wave of $E_{\frac{1}{2}} = -0.33$ V, there is no maximum and the cathodic limiting current is zero (Fig. 7).

When the i/t relation was studied at a potential of -0.25 V, there was, at first, only a slight current increase, and after a certain time a

bend appeared on the curve, with a more rapid change of the current intensity.

In case bromide ions are present in the acetic-acid solution, a high anodic current may be observed on the polarization curve. It is proportional to the initial bromide ion concentration in a range from 10^{-5} to 10^{-3} M, not increasing observably any more after the addition of bromine (the bromide ion concentration is substantially greater than the bromine

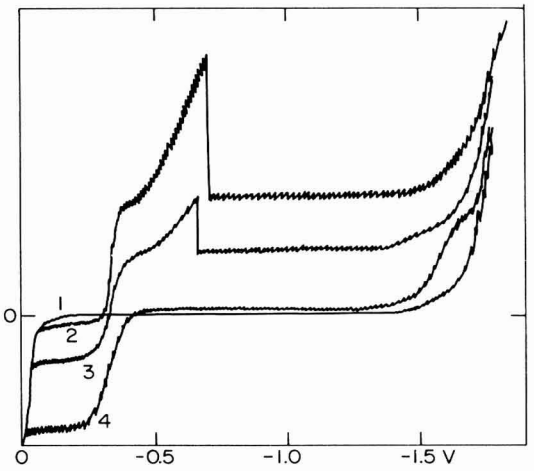


FIG. 7. Polarization curves of 2×10^{-4}N Br_2 in 35% CH_3COOH at 20°C. Range 0-2 V, 200 mV/minutes, 50 mV/abs, sensitivity 1/100, and drop time 2.1 seconds: curve 1 = supporting electrolyte; curve 2 = 7 minutes after bromine addition; curve 3 = 20 minutes after bromine addition; and curve 4 = 180 minutes after bromine addition.

concentration). In the cathodic part of the polarization curve a peak does not appear in this case, and the limiting current is substantially lower than in the absence of bromide ions. It may thus be assumed, that the cathodic-limiting current corresponds to the molecular form of bromine, given in the case by the equilibrium constant $K_{Br_3^-}$.

(e) The concentration ratio Br_2:Br^- was measured by the chronopotentiometric technique (E = f/t) but, differing from Botta-Conesa *et al.*, (*7*), without the use of an operating electrode. The assessment was carried out according to Lingane (*37*). The diffusion coefficient was not calculated.

After the addition of bromine to the acetic acid solution, bromide ions started to form according to equation 1, and therefore the potential of

the system Br_2:Br^- was measured. With the decreasing bromine concentration the potential of the cell measured decreases, and after bromine had disappeared from the solution in the τ, there are bromide ions only in the solution (Fig. 8). The value of $E^f_{eq} \approx E_{\frac{1}{4}}$ of the given system was determined by means of data from Lingane (*37*) from the time $t = \tau/4$.

In the cases where bromide ions were present in the acetic acid solution before the addition of bromine, the chronopotentiometric measurement

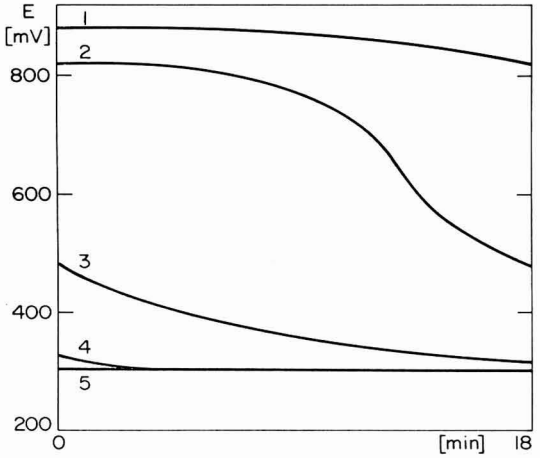


FIG. 8. Course of the potential of a reaction mixture of 5×10^{-4}N Br_2 in 35% CH_3COOH at 20°C in dependence on time: 1-0-18, 2-18-36, 3-36-54, 4-54-72, 5-72-90 minutes.

was used to compare the time in which bromine disappeared; but it was used only for low concentrations. At higher concentrations the reaction half-times are so large, that bromine disappearance is complete only after several hours or an even longer period of time.

As we see from Fig. 5, equilibrium is not established after attaining the time t_{max}, and other reactions must therefore be assumed.

A number of authors believe Br^+ to be the active component in bromation reactions, and based on the above considerations and assumptions, this is also expected to hold true for the reaction with acetic acid. The overall scheme expressing the course of the reaction of bromine with acetic acid may be formulated in the following manner with regard to the products formed. The radical form of the reactions must also be taken into account:

$$CH_3COO^- + Br^+ \rightarrow CH_3COOBr$$
$$CH_3COOBr + CH_3COOH_2^+ \rightarrow CH_3COOHBr^+ + CH_3COOH$$
$$CH_3COOHBr^+ \rightarrow CH_3COOBr + H^+$$
$$CH_3COOH + Br^+ \rightarrow CH_3COOHBr^+$$

$$CH_3COOBr \rightarrow CH_3COO\cdot + Br\cdot$$
$$CH_3COO\cdot \rightarrow CH_3\cdot + CO_2$$
$$CH_3\cdot + CH_3COOBr \rightarrow CH_3Br + CH_3COO\cdot$$
$$CH_3\cdot + Br\cdot \rightarrow CH_3Br$$

$$CH_3COOBr + Br_2 \rightarrow CH_2BrCOOBr + HBr$$
$$CH_2BrCOOBr + CH_3COOH \rightarrow CH_2BrCOOH + CH_3COOBr$$
$$CH_2BrCOOH + H_2O \rightarrow CH_2OHCOOH + HBr$$
$$CH_3Br + H_2O \rightarrow CH_3OH + HBr$$
$$CH_3COOH + CH_3OH \rightarrow CH_3COOCH_3 + H_2O$$

For the cleavage of bromine we may consider the following reaction

$$Br_2 \xrightarrow{h\nu} Br\cdot + Br\cdot$$

To confirm the hypothesis mentioned, experiments have been carried out to measure ESR spectra, but no measurable pulses were obtained. However, by means of these measurements the radical mechanism cannot be excluded, since if the radical concentration is lower than 10^{-5}M the signal cannot be recorded with the instrument used (ESR Spectrometer made in Japan).

Some authors have proposed conversions of the substances participating in the reaction scheme, with a radical as possible intermediate.

$$CH_3COOH \rightarrow CH_3COOBr \rightarrow CH_3COO\cdot \quad (42)$$
$$CH_3COO^- \rightarrow CH_3COO\cdot \rightarrow CH_3 \rightarrow CH_3^+ \quad (41)$$
$$2\ CH_3COO\cdot \rightarrow CH_3COOCH_3 + CO_2 \quad (19, 30, 35)$$

The experimental results also show that both mechanisms are probably involved.

To prove the reaction mechanism, several reaction products (carbon dioxide, methane, methylbromide, and methyl acetate) were studied by gas chromatographic means: heat conductivity detection was used for carbon dioxide and methane; flame ionization detection was used for methane, methylbromide, and methyl acetate.

It was proved that after addition of bromine to the acetic acid solution the elution wave corresponding to methane and methyl bromide appears in dependence on time. At the start of the reaction the ratio CH_4:CH_3BR > 1, and it became lower than one during the course of the reaction. While the ratio changes, the elution wave of methyl acetate starts to appear. The chromatogram of the reaction mixture gas phase is shown in Fig. 9.

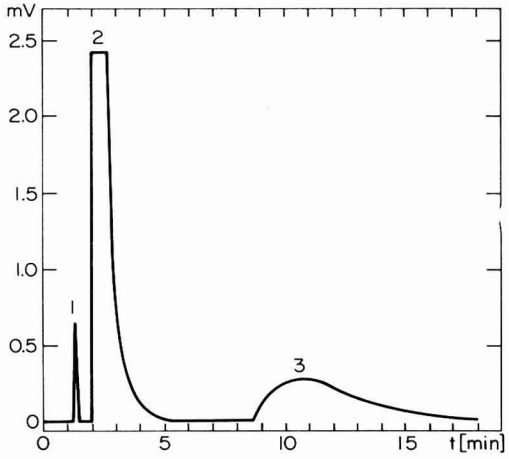


FIG. 9. Chromatogram of the gaseous phase of a reaction mixture of 5×10^{-3}N Br_2 with 35% CH_3COOH, reacting at 60°C 310 minutes after the beginning of the reaction: $1 = CH_4$, $2 = CH_3Br$, $3 = CH_3COOCH_3$.

A quantitative assessment of the gas-chromatographic data, i.e. determination of molar concentrations of the individual products was impossible because the system, due to the large number of components, is too complicated. The concentrations mentioned are concentrations in the gas phase which is in equilibrium with the liquid phase; therefore, the concentrations only illustrate the relative amounts of the individual products.

When bromide ions were present in the solution already before the addition of bromine, there were no great differences in the products formed.

The polarographic technique was used to study bromoacetic acid according to Leška and Čapla (*33*). Since the bromoacetic acid wave appears only in the region of very negative potentials, it is difficult to

evaluate. Especially at low concentrations only, a deformation of the polarization curve can be observed. The wave itself cannot be evaluated. For small bromoacetic acid concentrations a derivative circuit was used; this permitted at least a qualitative proof of the formation of bromoacetic acid in the reaction mixture in question.

Figure 10 shows the course of concentrations of Br_2, Br^-, and $CH_2BrCOOH$ determined polarographically in dependence on time. For

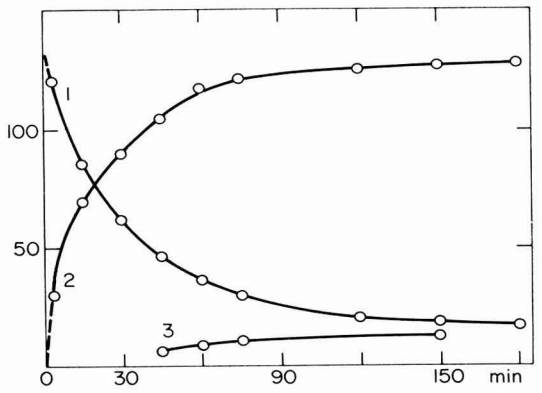


FIG. 10. Course of the limiting current of 8×10^{-4}N Br_2 and 35% CH_3COOH in dependence on time: 1 = at -1.10 V(Br_2), 2 = at -0.25 V(Br_3^-), 3 = at -1.60 V($CH_2BrCOOH$).

the concentration of bromoacetic acid, however, hydrolysis to glycolic acid must be taken into account.

Beside the polarographic technique, an attempt was made to measure IR spectra (with the recording spectrophotometer Perkin-Elmer). Although silica cells of different thickness (0.5-6 cm), measurement in the liquid and gas phases, different solvents etc. were tested, no satisfactory result was achieved. The concentrations of acetic acid and water are at least the thousandfold of the concentrations of products formed, so that only a continuous band of the maximum extinction value was obtained without the possibility of distinguishing the individual functional groups.

According to the shape of the Br_3^- concentration decrease the reaction of bromine with acetic acid may be divided into three concentration groups: 100-80%, 80-60%, and 20-10%, 60-20% CH_3COOH.

In solutions containing more than 90% CH_3COOH the decrease of

the tribromide ion equilibrium concentration was so slow that for measurable times a reaction scheme expressed by equations 1 and 2 may be assumed.

In the second group of acetic acid concentrations there is a more rapid and prolonged bromine concentration decrease so that with shorter periods of observation, the formation of a pseudoequilibrium may be observed. Among the reaction products, Br_3^-, Br^-, $CH_2BrCOOH$, CH_3Br, CH_4, CH_3COOCH_3, and CO_2 can be proved and, therefore, the reaction scheme proposed holds for these cases.

In the third group of acetic acid concentrations a continuous decrease down to a zero concentration of bromine as well as of tribromide ions may be observed in measurable times (with formation of the products mentioned in the reaction scheme).

The possibility of following the concentration changes of Br_2, Br^-, and Br_3^- from the reaction scheme has allowed the kinetic assessment of reactions expressed by equations 1 and 2.

The solution has been obtained by means of the analog computer MEDA 40 TA, solving the following system of differential equations:

$$\frac{d[Br_2]}{dt} = -k_1[Br_2] - k_2[Br_2][Br^-] + k_3[Br_3^-]$$

$$\frac{d[Br^-]}{dt} = k_1[Br_2] - k_2[Br_2][Br^-] + k_3[Br_3^-]$$

$$\frac{d[Br_3^-]}{dt} = k_2[Br_2][Br^-] - k_3[Br_3^-]$$

The calculation[1] has confirmed that the ionization of bromine is the controlling reaction of the system since the rate constants k_2 and k_3 (describing the rate of formation and decomposition of the tribromide ions) are far more rapid than mentioned above.

[1] We thank Ing. O. Schmidt, of the Department of Automation, Institute of Chemical Technology, Prague, for designing the program for solving the differential equations.

With respect to the initial condition (no bromide ions present at the beginning of the reaction), it has been assumed that the initial bromide concentration decrease will be controlled by the rate constant of the monomolecular reaction, and by constructing a tangent to the bromine loss curve (Fig. 5) in the initial phase the approximate value of the

constant k'_1 has been obtained, which in dependence on the acetic acid concentration achieves values given in Table 2.

By substituting the values of k'_1 in the program proposed, a value of the rate constant k_1 comparable in orders of magnitude has been obtained, and after finding the conditions describing the course of the Br_2 and Br_3^- concentrations in dependence on time, the values of k_1, k_2 and k_3 have been calculated for 35% CH_3COOH and 20°C they achieve the following values:

$$k_1 = 2{,}68 \times 10^{-2}\ [\text{min}^{-1}]$$
$$k_2 = 7.0 \times 10^{5}\ [\text{min}^{-1}\,\text{mol}^{-1}]$$
$$k_3 = 2.0 \times 10^{3}\ [\text{min}^{-1}]$$

SUMMARY

The experimental results of the study of the reactivity of bromine in the acetic acid-water system (described in Part I of this study) are discussed in the present communication from the point of view of the reaction products formed, and a scheme of the respective reactions is proposed.

REFERENCES

1. Anbar, M., and Ginsberg, D., Organic hypohalites. *Chem. Rev.* **54**, 925-958 (1954).
2. Barber, H., Spectrophotometric studies of bromine complexes. *Univ. Microfilm* (Ann Arbor, Mich.), L.C.Card No. Mic 60-534, 139 pp. CA **54**, 11794b (1960).
3. Batuev, M. I., Evidence of cyclic dimeric association of fatty acids in the Raman spectra of the hydoxyl groups. *Doklady Akad. Nauk S.S.S.R.* **59**, 1117-1120 (1948). (in Russian).
4. Bell, R. P., and Gelles, E., The halogen cations in aqueous solutions. *J. Chem. Soc.* 2734-2740 (1951).
5. Bennetto, H. P., Feakins, D., and Turner, D. J., Studies in ion solvation in non-aqueous solvents and their ageous mixtures. Part V. The Halogen acids in 10, 20, 40, and 60% (W/W) acetic acid-water mixtures at 25°C. *J. Chem. Soc. (A)*, 1211-1216 (1960).
6. Blažek, A., Polarometric determination of some unsaturated organic compounds. *In* "Proceedings of the I. International Polarographic Congress in Prague, Part III." pp. 555-565 (1952).
7. Botta-Conesa, M. C., Molina, R., and Trémilion, R., Étude polarographic de la formation du calomel dans l'acide acétique anhydre. *Bull. Soc. Chim. France* 2097-2098 (1965).
8. Bray, W. C., The hydrolysis of iodine and bromine. *J. Am. Chem. Soc.* **32**, 932-938 (1910).
9. Bray, W. C., and Connoly, E. L., The hydrolysis of iodine and of bromine. A correction. *J. Am. Chem. Soc.* **33**, 1485-1487 (1911).

10. CAMPBELL, A. N., AND GIESKES, J. M. T. M., The dietectric behavior of the system: acetic acid-chloroform-water at 25°C. *Can. J. Chem.* **42**, 1379-1387 (1964).

11. CHILD, C. L., AND WALKER, O. J., The continuous absorption spectrum of bromine in solutions. *Trans. Farady Soc.* **34**, 1506-1515 (1938).

12. CONANT, J. B., AND HALL, N. F., A study of superacid solutions II. A chemical investigation of the hydrogen-ion activity of acetic acid solutions. *J. Am. Chem. Soc.* **49**, 3062-3070 (1927).

13. CONANT, J. B., AND WERNER, T. H., The determination of the strength of weak bases and pseudo bases in glacial acetic acid solutions. *J. Am. Chem. Soc.* **52**, 4436-4450 (1930).

14. COREY, E. J., AND CASANOVA, J., JR., The formation of the carbonium ions by oxidative decarboxylation of carboxylic acids with lead tetraacetate. *J. Am. Chem. Soc.* **85**, 165-169 (1963).

15. COVINGTON, A. K., TALUKDAR, P. K., AND THIRSK, H. R., Standard potential of the mercury/mercurous acetate electrode at 25° C. *Trans. Faraday Soc.* **60**, 412-416 (1964).

16. DAINTON, F. S., AND SILLIS, S. A., The rates of some reactions of hydrogen atoms in water at 25° C. *Proc. Chem. Soc.*, 223-224 (1962).

17. DAVIS, D. G., AND EVERHART, M. E., Chronopotentiometry of the bromide-bromine couple at platinum and carbon paste electrodes. *Anal. Chem.* **36**, 38-40 (1964).

18. EICHELBERGER, W. C., AND LA MER, V. K., The analytical determination and conductance of sulfuric acid in anhydrous acetic acid. *J. Am. Chem. Soc.* **55**, 3635-3638 (1933).

19. FRY, A., TOLBERT, B. M., AND CALVIN, M., Mechanism of the decomposition of acetyl peroxide in acetic-2-C^{14} acid. *Trans. Faraday Soc.* **49**, 1444-1451 (1953).

20. GALAL-GORCHEV, H., AND MORRIS, J. C., Formation and stability of bromamide, bromimide, and nitrogen tribromide in aqueous solution. *Inorg. Chem.* **4**, 899-905 (1965).

21. GRIFFITH, R. O., MCKEOWN, A., AND WINN, A. G., The bromine-bromide-tribromide equilibrium. *Trans. Faraday Soc.* **28**, 101-107 (1932).

22. HAGISAWA, H., A study, by means of the glass electrode, of the hydrolysis of bromine. *Bull. Inst. Phys. Chem. Res.* **21**, 593-596 (1942).

23. HALL, N. F., AND VOGE, H. H., Electrical conductivities of mixtures of sulfuric acid, acetic acid and water. *J. Am. Chem. Soc.* **55**, 239-246 (1933).

24. HARNED, H. S., AND EHLERS, R. W., The Dissociation constant of acetic acid from 0 to 35° centigrade. *J. Am. Chem. Soc.* **54**, 1350-1357 (1932).

25. HARRIS, J. T., JR., AND HOBBS, M. E., A study of the association of some organic acids by infrared absorption measurements. *J. Am. Chem. Soc.* **76**, 1419-1422 (1954).

26. HUTCHINSON, A. W., AND CHANDLEE, G. C., Activity coefficients of sulfuric acid in anhydrous acetic acid. *J. Am. Chem. Soc.* **53**, 2881-2888 (1931).

27. JAKOWKIN, A. A., Zur Theorie der Lösungen. *Z. Physik. Chem.* **70**, 158-197 (1909).

28. JONES, J. W., The determination of solubility coefficients by aspiration. *J. Chem. Soc.* **99**, 392-404 (1911).
29. KENDALL, J., AND GROSS, P. M., Compound formation and specific conductivity in solutions of the types acid:ester, acid:ketone and acid:acid. *J. Am. Chem. Soc.* **44**, 1426-1439 (1921).
30. KHARASH, M. S., FRIEDLANDER, H. N., AND URRY, W. H., Reactions of atoms and free radicals in solutions. XXVI. Comparison of the reactions of lead tetraacetate and acetyl peroxide with acetic acid and isopropyl ether. *J. Org. Chem.* **16**, 533-542 (1951).
31. KILPI, S., AND LINDEL, E., Zur Dielektrizitätskonstante der wasserarmen Essigsäure-Wassergemische. *Suomen Kem.* **37B**, 65-66 (1964).
32. KOLTHOFF, I. M., AND BRUCKENSTEIN, S., Acid-base equilibria in glacial acetic acid. I. Spectrophotometric determination of acid and base strengths and of some dissociation constants. *J. Am. Chem. Soc.* **78**, 1-9 (1956); Acid-base equilibria in glacial acetic acid. II. Spectrophotometric determination of the ionization and dissociation constants of p'p'-dimethylaminobenzene and pyridine. The abnormal effect of water on dicator bases. *J. Am. Chem. Soc.* **78**, 10-15 (1956).
33. LEŠKA, J., AND ČAPLA, M., A kinetic study of the mechanism of bromoacetic acid and α-bromopropionic acid reactions with some basic reactants. *Chem. Zvesti* **19**, 339-352 (1965) (in Czech).
34. LEWIN, M., The composition of hypobromite solutions at various pH values and bromide concentrations. *Bull. Res. Council Israel* **2**, 101-104 (1952); *CA* **47**, 4779 (1953).
35. LEWY, M., AND CZWARE, M., The kinetics of decomposition of acetyl peroxide. III. The reactions of radicals produced in the decomposition. *J. Am. Chem. Soc.* **76**, 5981-5985 (1954).
36. LIEBHAFSKY, H. A., The equilibrium constant of the bromine hydrolysis and its variation with temperature. *J. Am. Chem. Soc.* **56**, 1500-1505 (1934).
37. LINGANE, J. J., Analytical aspects of chronopotentiometry. *Analyst* **91**, 1-9 (1966).
38. MANCHOT, W., AND OBERHAUSER, F., Bromometrische Bestimmungen. *Z. Anorg. Allgem. Chem.* **139**, 40-50 (1924).
39. MEYER, R. J., "Gmelins Handbuch der anorganischen Chemie," 7th and 8th Eds. Verlag Chemie G.m.b.H., Berlin, 1931.
40. POHL, H. A., HOBBS, M. E., AND GROSS, P. M., Electric polarization of carboxylic acids in dilute solutions of nonpolar solvents. I. The relation of electric polarization to the association of carboxylic acids in hydrocarbon solvents. *J. Chem. Phys.* **9**, 408-414 (1941).
41. SCHWARZENBACH, G., AND STENSBY, P., Die Acidität der schweren Halogenwasserstoffe und der Perchlorsäure in Essigsäure als Lösungsmittel. *Helv. Chim. Acta* **42**, 2342-2355 (1959).
42. SEEWANN-ALBERT, H., AND KAHOVEC, L., Halides of acetic, propionic, and toluic acids. *Acta Phys. Austriatica* **1**, 352-358 (1948).
43. SRIRAMAN, S., AND SHANMUGASUNDARAN, V., The diamagnetic study of the nature of association in acetic acid. *Bull. Chem. Soc. Japan* **37**, 395-398 (1964).

44. SUMIE HOSHINO, HOSUYA, H., AND NAGAKURA, S., Ultraviolet, infrared, and Raman spectra of protonated carboxylic acids. *Can. J. Chem.* **44**, 1961-1965 (1966).

45. ŠEVČÍK, J., AND ZÝKA, J., I. Study of the reactivity of bromine in the system acetic acid-water. *Microchem. J.* **12**, 00-00 (1967).

46. ŠKRABAL, A., Über die Stärke der unterhalogenigen Säuren. *Z. Elektrochem.* **48**, 314-327 (1942).

47. WALKER, I. K., AND ROBERTSON, P. W., The kinetics of bromine addition to olefinic compounds. Part II. The homogenous mechanism. *J. Chem. Soc.,* 1515-1518 (1939).

48. WIBERG, K. B., "Physical Organic Chemistry," John Wiley & Sons, Inc., New York, 1964.

49. ZAJAC, W. W., JR., AND NOWISKI, R. B., The acidity function, H_0, of hydrogen bromide in acetic acid-water mixtures. *J. Phys. Chem.* **69**, 2649-2652 (1965).

MICROCHEMICAL JOURNAL **12**, 512-524 (1967)

Centimilligram Determination of Organic Nitrogen with Sealed Tube Combustion

KEIICHIRO HOZUMI AND KOUICHIRO UMEMOTO

Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan

Received April 10, 1967

INTRODUCTION

A method for decimilligram determination of nitrogen in organic compounds involving sealed-tube combustion and measurement of volume of nitrogen collected over 50% KOH solution has been successively developed with higher accuracy and reliability for routine analytical works (*1*, *3*, *4*, *7*, *8*). Recently one of the present authors introduced a new technique for measuring the collected nitrogen over the KOH solution by means of a tenfold expansion of the nitrogen under a reduced pressure (*2*). The method of its calculation was successively revised by further investigations concerning the nitrogen meniscus over the KOH solution that was reversed against the meniscus of water which had replaced the expanded nitrogen, the capillary rise of the KOH solution in the combustion tube, and the wetness of the inside wall of the same tube (*5*). Extremely accurate results with several standard organic compounds ranging 5-40% nitrogen contents were thereby obtained with a standard deviation of 0.05%.

A further application of the new technique has been extended to a smaller sample size of 30-80 μg by investigations on the above three correction factors which respond to thinner combustion tubes.

APPARATUS AND REAGENTS

Electrolytic Oxygen Generator

An electrolytic cell with an internal volume of 500 ml is filled with 6 *M* sulfuric acid and is energized by a direct current power supplier (*5*). These are integrated in a compact hausing as illustrated in Fig. 1.

Electric Furnace

A cylindrical furnace with a length of 50 cm and an inside diameter of 3 cm is controlled at 700°C by a thermoregulator.

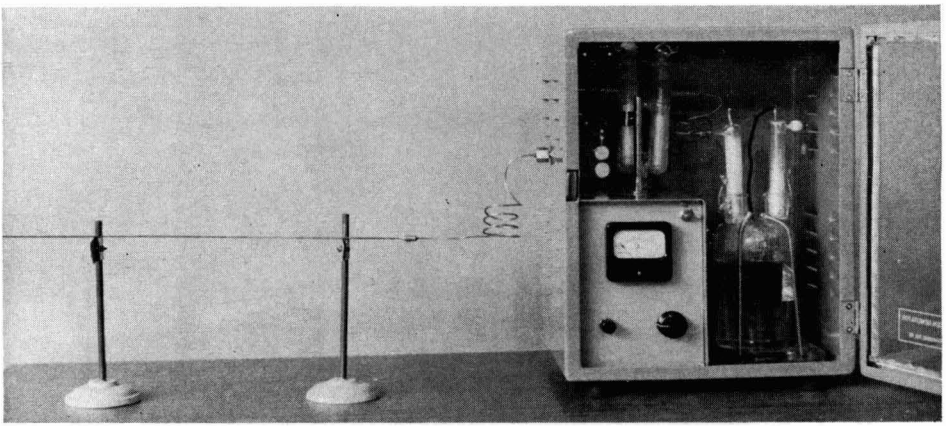


FIG. 1. Electrolytic oxygen generator. For preparing the sealed combustion tubes, 1.5 A is supplied to obtain a pure oxygen flow of 4-5 ml/minute, but otherwise 0.2 A is supplied all the time preventing inward diffusion of air.

Gas Expansion Arrangement

A borosilicate glass cylinder with a mercury manometer where the nitrogen collected over the 50% KOH solution is expanded under the reduced pressure has been reported previously (*2*). As the volume of nitrogen is measured at much lower pressure at the centimilligram analysis than at the decimilligram analysis, the manometer tube and the mercury should be carefully cleaned to insure natural convexes of the menisci.

Measurement of the manometric pressure and the length of the expanded nitrogen in the combustion tube are carried out by a cathetometer which is so called a reading microscope with either vertical and horizontal spans of 30 cm.

Titrator

A Metrohm's 5 ml piston buret delivers water through a stainless steel capillary tube replacing the volume of expanded nitrogen in the combustion tube which has been fixed along a caliper rule as illustrated in Fig. 2.

Combustion Tube

A Heatron-P tube with inside diameter of 2-3 mm has been used for the material of combustion tubes which have extremely small expansion coefficient of 6×10^{-7} cm/cm°C. The softening point is said to be 950°C, but the safety limit for operation with the above diameter might

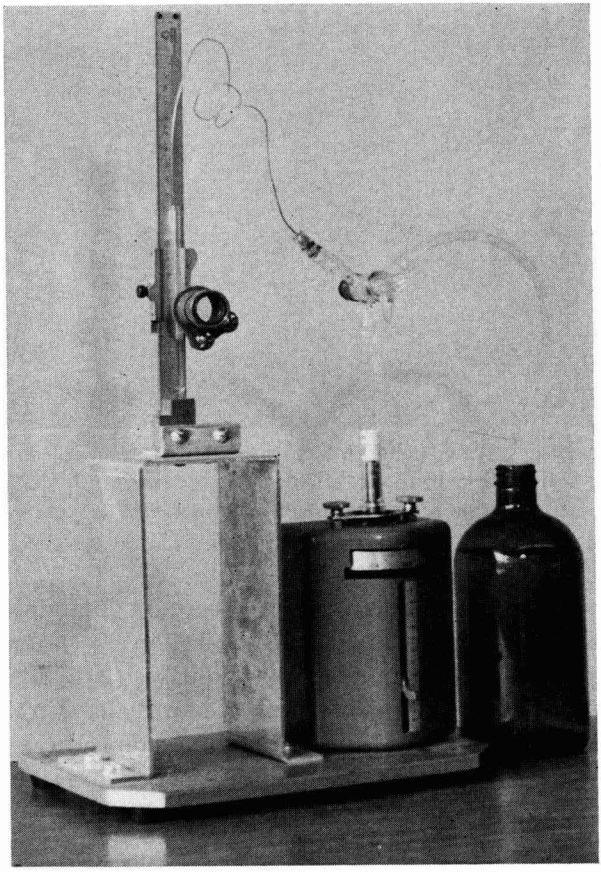


FIG. 2. The caliper rule has been somewhat modified with a magnification lens mounted on the sliding jaw so that the end point of the titration is accurately indicated with the minimum of parallax.

be around 750°C. The material is economically available from Fuji Film Co. Ltd., Tokyo. Supremax and Pyrex 1720 are, of course, suggested for local conveniences.

The glass tubes are cut into 33 cm long and heated in the electric furnace at 700°C for 1.5 hours. After cooling they are washed by distilled water and dried in an oven. Every tube is sharply drawn at the point of 5 cm from one end with a thin hydrogen flame to form a fine tip as illustrated in Fig. 3 A.

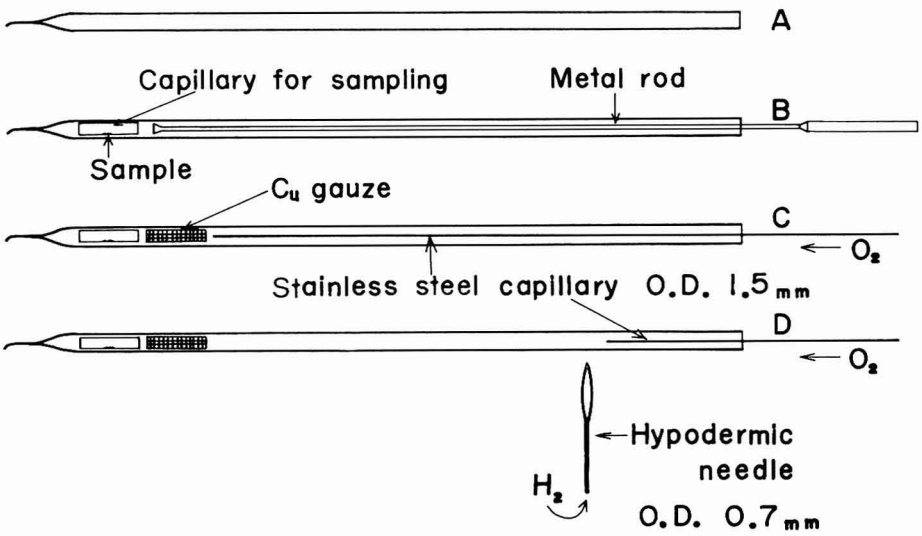


FIG. 3. Sealing of charged combustion tube.

Copper Wire Gauze

A copper wire gauze of 50 mesh is cut into small pieces of 15 × 5 mm^2 which are then dipped in a hot acetic acid for a few minutes. The pieces are washed in distilled water and dried in an oven. Every piece is picked up by forceps and passed through a flame of gas burner where some surface oxidation takes place.

50% KOH Solution

The so-called 50% KOH solution is prepared by dissolving 500 g of fresh KOH pellets into 500 ml of distilled water (*10, 11*). The actual concentration of KOH should be assured to be 41-42% by measuring the specific gravity of the solution.

METHOD OF ANALYSIS

A Heatron-P tube with an inside diameter of 3.5-4.0 mm, which has been previously suggested as the combustion tube for decimilligram determination, is drawn to be a capillary tube with an inside diameter of approximately 1.5 mm. The tube is cut into small pieces of 15 mm long which have average weight of 50 mg. One of the capillary tubes is ac-

curately weighed by an ultramicrobalance. Some numbers of particles of organic sample are sticked at a tip end of a platinum needle and transported into the capillary tube. Light touching on the inside wall of the capillary tube with the tip of the needle leaves necessary amount of the sample ranging 30-80 μg. Care must be taken to introduce the sample to the middle part of the capillary tube as possible to avoid any loss of the sample from open ends of the capillary tube during weighing and afterwards charging it into the combustion tube.

The capillary tube is then introduced into the combustion tube and is carefully pushed until the tip end by a metal rod as illustrated in Fig. 3. A piece of the copper gauze is further inserted and the charged combustion tube is drawn over a stainless steel capillary through which the electrolytic oxygen flows out. The last trace of air in the combustion tube is purged during 8 minutes of the oxygen flow of 4-5 ml/minute. The combustion tube is then partly drawn out of the stainless steel capillary and sealed by a thin hydrogen flame as suggested in Fig. 3.

Several sealed tubes thus prepared are heated in the electric furnace at 700°C for 1.5 hours. The tubes are taken out when the furnace has been cooled to 500°C after having been turned off. Every combustion tube is gently pushed down into the KOH solution reservoir to break the tip end with the minimum force. The KOH solution gradually goes up in the combustion tube absorbing carbon dioxide and water to collect nitrogen at the top of the combustion tube. The broken tip is then further pressed down to the bottom of the reservoir in order to have wider opening.

All the tubes are fixed on a holding plate by scotch tape and transferred into the gas expansion arrangement. The arrangement is evacuated by a water pump to 40-50 mm Hg and it is tightly closed by a stopcock.

After 15 minutes of standing, the length (L) mm from the top of every tube to the N_2-KOH solution meniscus in the tube is measured by the cathetometer within an accuracy of $\pm$ 0.1 mm and, at the same time, the height of the meniscus H mm from the level of KOH solution in the reservoir is registered with an accuracy of $\pm$ 1 mm as illustrated in Fig. 4. The manometric pressure P mm Hg is also measured within an accuracy of $\pm$ 0.1 mm Hg.

After these measurements, the combustion tubes are taken out of the arrangement and a part of 5 cm from the broken tip of every tube is cut away. Washing and drying of the combustion tubes are processed as previously described (*2*). One of the combustion tubes is fixed along

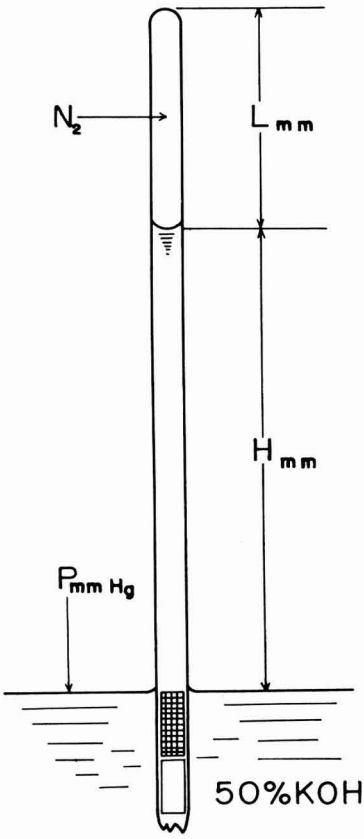


FIG. 4. Measurement of expanded nitrogen.

the caliper rule locating the sealed end exactly at the zero level. The sliding jaw is so adjusted that the reading will coincide to the length of (L) mm. Titration is then proceeded with the 5 ml piston buret through the stainless steel capillary tube (as illustrated in Fig. 2) until the meniscus reaches to the level preliminarily indicated by the sliding jaw.

Calculation

Calculation of the nitrogen content in the sample involves two steps as follows. First, the actual volume of nitrogen ($V = \mu l$ collected over the KOH solution) is estimated at the normal pressure and secondly the weight percentage of the nitrogen in the sample is calculated.

$$V = (V' - v) \times \frac{P \times F_p - (H - h) \times 0.105 - P_v}{760} \times F_v$$

$$\%N = (V - \text{blank value}) \times \frac{F_n}{S} \times 100$$

In the first step, V′ μl is the reading of Metrohm's piston buret used for the titration and v is a correction for reversed meniscus which has been differed from that of nitrogen over the KOH solution. The correction value is functional to the variation of the inside diameter of the combustion tube as tabulated in Table 1.

TABLE 1
CORRECTION FOR VOLUME OF TITRANT DUE TO REVERSED MENISCUS OF WATER AT END POINT

I. D. of Combustion tube mm	2.0	2.2	2.4	2.6	2.8	3.0
Volume μl	2.8	3.2	3.7	4.2	4.9	5.9

The manometric pressure P mm Hg must be also corrected with the variation of room temperature as shown in Table 2 in order to reduce the density of mercury at 0°C.

TABLE 2
CORRECTION FACTOR FOR MANOMETER READING

Temp., °C	15-20	20-25	25-30
F_p	0.997	0.996	0.995

The KOH solution level in the combustion tube H mm is subtracted by natural capillary rise h mm according to the inside diameter of the combustion tube as listed in Table 3. A conversion factor of 0.105 is multiplied to transfer the unit to mm Hg.

TABLE 3
CAPILLARY RISE OF 50% KOH SOLUTION IN COMBUSTION TUBE

I. D. of Combustion tube mm	2.0	2.2	2.4	2.6	2.8	3.0
h mm	14	12	11	10	9	9

The vapor pressure over 50% KOH solution is given in Table 4 which has been interpolated from Milner's observation (*9*).

Wetness of the inside wall of the combustion tube with KOH solution during the measurement of expanded nitrogen causes narrower diameter

TABLE 4
VAPOR PRESSURE OVER 50% KOH SOLUTION

Temp., °C	P_v mm Hg	Temp., °C	P_v mm Hg
15	2.9	23	5.3
16	3.1	24	5.7
17	3.4	25	6.1
18	3.7	26	6.5
19	4.0	27	7.0
20	4.3	28	7.4
21	4.6	29	7.9
22	4.9	30	8.4

of the nitrogen column which can be cancelled by applying a correction factor F_v shown in Table 5.

The blank value in the second step of the calculation is sometimes evaluated by burning several pure organic compounds which have no nitrogen as benzoic acid. The mean value should be normally 0.1-0.05 μl. The percentage composition is then calculated with the conversion factor F_n μg/μl at the given temperature and the sample weight S μg.

TABLE 5
CORRECTION FACTOR FOR WETNESS ON INSIDE WALL OF COMBUSTION TUBE

I. D. of combustion tube mm	2.0-2.5	2.5-3.0
F_v	0.992	0.993

A series of analyses with different types of standard organic samples (available from Kishida Chemicals Co. Ltd., Osaka) was carried out and the results are listed in Table 6. A standard deviation of 0.17% which may be compared to the ordinary Dumas microdetermination has been attained. Approximately 10 analyses are normally carried out in a day if the second group of 5 analyses will be started during the combustion of the first group of 5 analyses.

DISCUSSION

Electrolytic Oxygen

The electrolysis of hydrogen peroxide with alternation current previously described (*2, 6*) has been recently unusable because of high blank value resulted from EDTA added to most of the commercial products. The conventional method of electrolysis with sulfuric acid using direct current was therefore preferred for the minimum of blank value (*5*).

TABLE 6

CENTIMILLIGRAM DETERMINATION OF NITROGEN WITH A SERIES OF SEVERAL STANDARD ORGANIC SAMPLES

Substance	Wt. of sample (S μg)	Buret reading (V′ μl)	I.D. of combustion tube (D mm)	Manometer reading (P mm Hg)	Height of KOH solution (H mm)	Temperature (°C)	N_2 volume at 760 mm Hg (V^a μl)	Nitrogen[b] % Found	Nitrogen[b] % Error
p-Bromoacetanilide	56.40	120	2.3	38.3	124	23.0	3.14	6.42	− 0.12
	49.00	113	2.5	38.3	135	23.0	2.75	6.47	− 0.07
$N = 6.54\%$	61.05	137	3.0	38.3	129	23.0	3.39	6.40	− 0.14
Hippuric acid	41.90	120	2.8	38.3	133	23.0	2.91	8.01	+ 0.19
	49.00	140	3.0	38.3	134	23.0	3.38	7.96	+ 0.14
$N = 7.82\%$	54.35	162	3.0	38.3	141	23.0	3.78	8.02	+ 0.20
Acetanilide	77.50	200	2.4	44.7	119	22.7	7.09	10.56	+ 0.20
	74.20	192	2.4	44.7	120	22.7	6.78	10.55	+ 0.19
$N = 10.36\%$	36.10	104	2.7	44.7	137	22.7	3.29	10.52	+ 0.16
Fluoroacetamide	82.65	341	2.9	44.7	97	22.7	13.14	18.35	+ 0.17
	38.25	184	2.8	44.7	137	22.7	5.99	18.08	− 0.10
$N = 18.18\%$	43.60	210	2.6	44.7	136	22.7	6.95	18.40	+ 0.22
Acetone-2.4-dinitrophenylhydrazone	59.95	311	2.5	45.2	93	23.2	12.36	23.76	+ 0.24
	40.15	233	2.9	45.2	119	23.2	8.27	23.74	+ 0.22
$N = 23.52\%$	39.65	227	2.8	45.2	116	23.2	8.17	23.75	+ 0.23
Thiourea	55.30	424	2.9	45.2	76	23.2	17.78	37.06	+ 0.26
	45.35	382	3.0	45.2	102	23.2	14.57	37.03	+ 0.23
$N = 36.80\%$	41.40	338	2.9	45.2	98	23.2	13.11	36.50	− 0.30

[a] Average blank value of 0.07 μl was subtracted from the volume of nitrogen at 760 mm Hg.

[b] Standard deviation = ± 0.17%.

Titration

The volume of water delivered from the piston buret is larger than the volume of the expanded nitrogen due to the formation of reversed meniscus at the end point of the titration. The difference of these volume, v μl, was empirically determined at different sizes of inside diameters of combustion tubes.

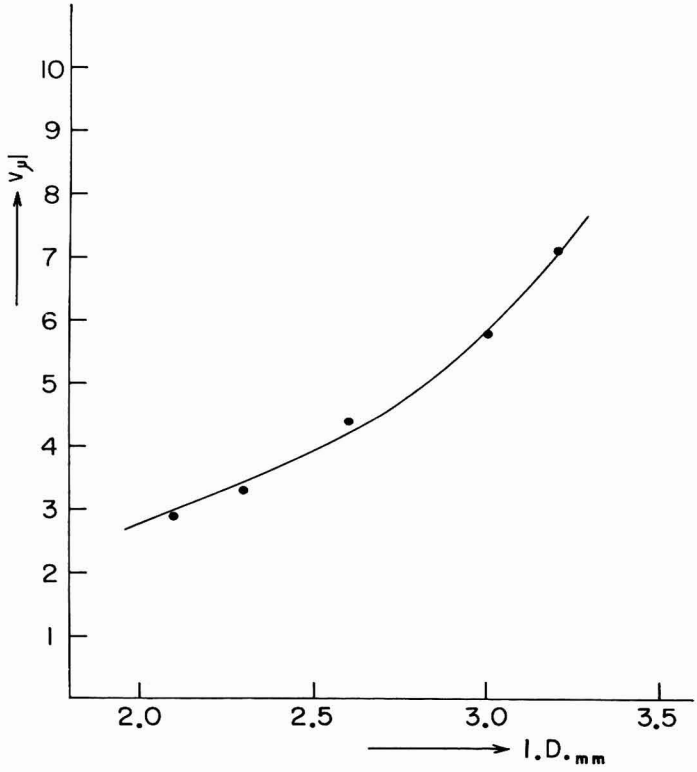


FIG. 5. Volume of space between reversed menisci formed by mercury and water.

Mercury was charged to the every tube, its meniscus was precisely registered, and the volume of the mercury was estimated by weight. The titration with water was then proceeded until the meniscus coincided at the registered level. The differences of the volumes of mercury and water are plotted against the inside diameter of the combustion tube as illustrated in Fig. 5. The data given in Table 1 have been derived from interpolations in this figure.

Capillary Rise

The natural capillary rise of 50% KOH solution is also functional to the inside diameter of the combustion tube. The actual values were measured with different sizes of inside diameters and are plotted in Fig. 6 from which the data in Table 3 have been derived.

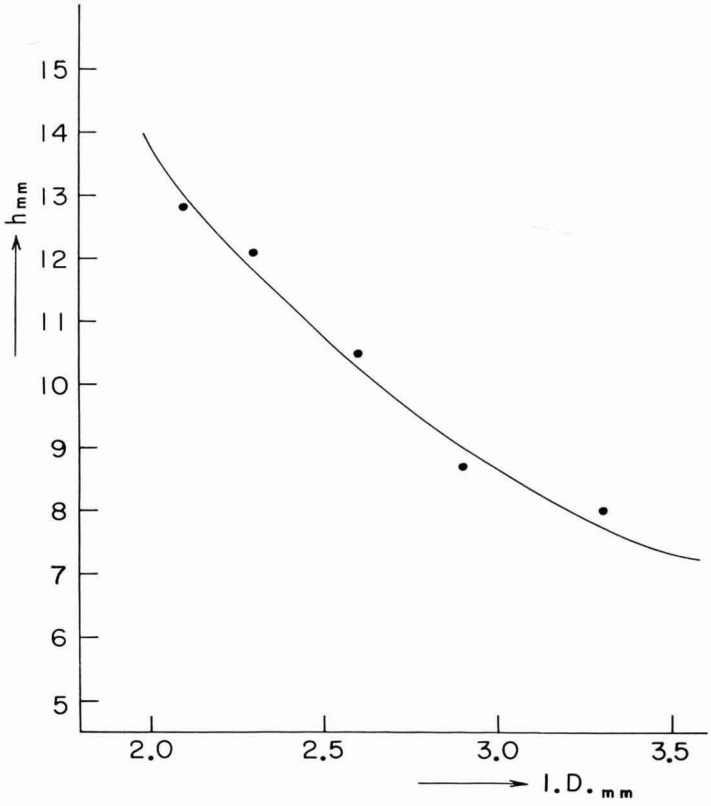


FIG. 6. Capillary rise of 50% KOH solution in combustion tube.

Wetness on inside wall

The volume of 50% KOH solution film on inside wall of the combustion tube was measured empirically. A combustion tube of 10 cm long with both ends opened was precisely weighed by a microbalance and was clamped in upright position. A small amount of the KOH solution was then introduced from the top of the tube until the solution flowed down from the lower end. Hanging drops were sometimes wiped away by a

filter paper. After 15 minutes of standing, the tube was weighed and the volume of the KOH solution film was estimated. The measurement was repeated with different sizes of inside diameters. Absorption of moisture during the standing time was prevented as possible by carrying out the operation at 10°C in dry season. The volume ratios of the film to the internal volumes of the different sizes of combustion tube are plotted in Fig. 7 which has given the correction factors of Table 5.

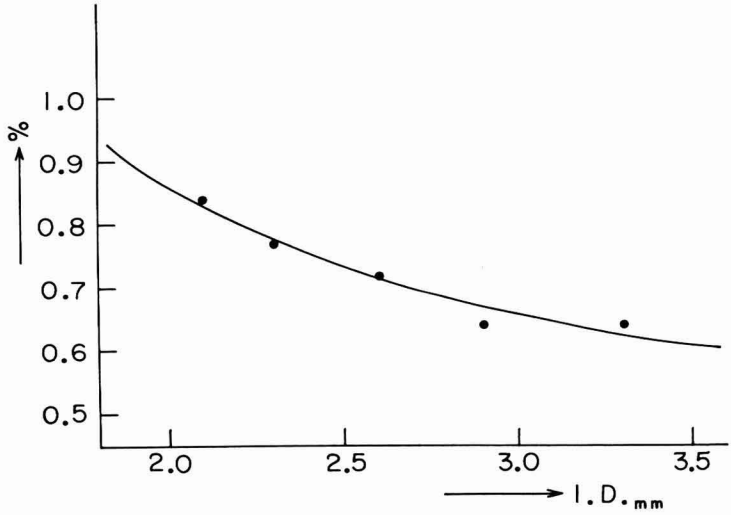


FIG. 7. Volume ratio of 50% KOH solution film on inside wall of combustion tube.

SUMMARY

The new technique involving the sealed tube combustion and the measurement of nitrogen collected over 50% KOH solution under reduced pressure has been extended to the centimilligram determination of organic nitrogen. The volume of water delivered from a piston buret to replace the volume of nitrogen was corrected by measuring the difference of the two volumes which had been functional to the inside diameter of the combustion tube. The natural capillary rise of the KOH solution in the combustion tube and the volume adhering to the inside wall of the same tube were also precisely estimated to have better approach to the actual volume of nitrogen. A standard deviation of 0.17% was obtained with different types of organic samples ranging from 30-80 μg.

REFERENCES

1. HOZUMI, K., Decimilligram determination of nitrogen with a simple sealed tube combustion method. *Anal. Chem.* **35**, 666-669 (1963).
2. HOZUMI, K., Improved method for decimilligram determination of organic nitrogen with sealed tube combustion. *Anal. Chem.* **38**, 641-644 (1966).

3. HOZUMI, K., AND KIRSTEN, W. J., New method for the ultramicrodetermination of nitrogen. *Anal. Chem.* **34**, 434-435 (1962).
4. HOZUMI, K., AND KIRSTEN, W. J., Simultaneous determination of carbon, hydrogen, and nitrogen at the decimilligram level. *Anal. Chem.* **35**, 1522-1527 (1963).
5. HOZUMI, K., AND UMEMOTO, K., Decimilligram determination of organic nitrogen with sealed tube combustion. *Japan Analyst* **16**, 800-807 (1967).
6. KIRSTEN, W. J., "Comprehensive Analytical Chemistry, The Dumas Determination of Nitrogen," Vol. A-11, 467 pp, Elsevier Amsterdam, 1960.
7. KIRSTEN, W. J., AND HOZUMI, K., Gas-volumetric ultramicro- and microdetermination of nitrogen and other elements in organic compounds. *Mikrochim. Acta* 777-786 (1962).
8. KIRSTEN, W. J., AND HOZUMI, K., Decimilligram determination of nitrogen. *Microchem. J.* **6**, 591-599 (1962).
9. MILNER, R. T., AND SHERMAN, M. S., Organic microanalysis I. Nitrogen by Dumas method. *Ind. Eng. Chem.* **8**, 331-332 (1936).
10. PREGL-ROTH, "Quantitative Organische Mikroanalyse," 7th ed. 92 pp, Springer, Vienna, 1958.
11. STEYERMARK, AL, "Quantitative Organic Microanalysis," 2nd ed., 152 pp, Academic Press, New York, 1961.

MICROCHEMICAL JOURNAL **12**, 525-533 (1967)

Rapid Distillation Separation of Microgram Quantities of Fluoride[1]

HISASHI KUBOTA

Analytical Chemistry Division, Oak Ridge National Laboratory
Oak Ridge, Tennessee 37830

Received May 8, 1967

INTRODUCTION

The literature on fluoride separations comprises a lengthy list, but the trends can be classed into three main groups. The first and historically the oldest is the separation as volatile silicon tetrafluoride (*3*). This method requires strict observance of anhydrous conditions to prevent the hydrolysis of the silicon tetrafluoride and its subsequent loss. It is not surprising, therefore, that the Willard-Winter distillation (*5*), in which fluoride is steam distilled as fluosilicic acid, won wide acceptance quickly. The more recent innovation is pyrohydrolytic separation (*4*) which takes advantage of the thermodynamics that favors the release of fluoride in the form of hydrofluoric acid from solid samples in a moisture laden atmosphere at 1000-1200°C. There are other techniques like ion-exchange separation that can be used for special separations (*2*), but the three above mentioned methods will separate fluoride from most interfering ions.

One of the problems in the processing of transuranium elements is the removal of fluoride from process solutions once the desired operation with fluoride is completed. The residual fluoride has to be determined by analysis. The solution to be analyzed is usually a fairly concentrated actinide solution containing various impurities and corrosion products like iron, aluminum, or zirconium. They usually contain high concentration of nitric or hydrochloric acid. One pertinent item here is that the solutions are radioactive, and the analysis has to be conducted in a hot cell. The procedure that has been used up to the present is the pyrolytic separation followed by a SPADNS-Zr spectrophotometric determination (*1*).

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

Considerable difficulty has been experienced carrying out the many required operations called for by the pyrohydrolytic procedure in a hot cell. Thus, a search was begun for a more simple and yet reliable separation, and a modification of the Willard-Winter separation was developed which gives good recovery of microgram quantities of fluoride from solutions containing cations which form very stable fluoride complexes and large quantities of volatile mineral acids.

The separated fluoride is determined spectrophotometrically with the alizarin-complexone reagent. The procedure developed by Yamamura, Wade, and Sikes (*6*), with lanthanum in place of cerium, was used. As will be discussed later, one of the reasons prompting the choice of this reagent was to take advantage of the property of alizarin-complexone to tolerate limited amounts of sulfate ions.

EXPERIMENTAL

Reagents

The directions given in reference (*6*) for preparing the alizarin-complexone reagent were followed faithfully except that stock lanthanum solution containing 3.616 g $La(NO_3)_3 \cdot 6H_2O$/500 ml was used in place of cerium nitrate. The 1:1 ratio solution is used in this work.

A 1:1 H_2SO_4-H_2O solution was prepared by pouring in 500 ml of concentrated H_2SO_4 slowly into 500 ml of water while the mixture was stirred.

Apparatus

The still assembly is shown in Fig. 1. The fluoride is distilled from a 100-ml r.b. flask with a 24/40 standard taper joint. The stillhead is a Kjeldahl spray trap with a 24/40 male standard taper joint to fit into the boiling flask and a sidearm terminated with a 12/5 ball joint to make connection to the condenser. This spray trap is covered with a thin coat of asbestos tape wound with about 2 feet of 32 gage nichrome wire. The windings are spaced evenly about an inch apart. The length of the wire should not be much over 2 feet since too long a length will result in insufficient heating capacity. The winding is covered with asbestos tape, and the entire cover is coated with Sauereisen (Sauereisen Cements Co., Pittsburgh, Pa.) dried, and baked at 150°C. Provision should be made for a thermocouple lead or a thermometer within this coating to allow monitoring the jacket temperature. The leads from this heating element pass through a variac which controls the jacket temperature.

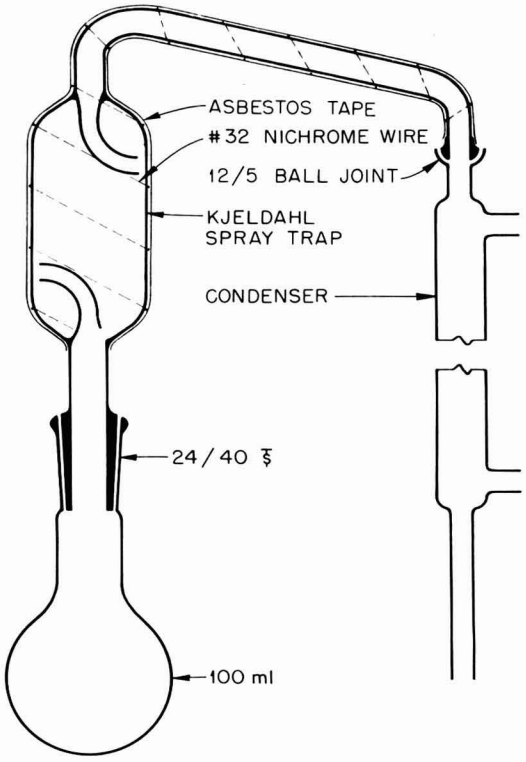


FIG. 1. Still assembly. ORNL-DWG. No. 67-3207.

Procedure

The current to the still head is turned on, and the variac is adjusted so that the jacket temperature is 170°C. Fifty ml of the 1:1 sulfuric acid is placed in the boiling flask along with the sample (up to 5 ml). Enough water is added to make the total volume of sample plus water equal 5 ml. Two silica boiling chips are put in, and the flask is attached to the still-head. The distribution is started and terminated when 20 ml of distillate is collected. The distillate is neutralized to the phenolphthalein endpoint with 0.1 N sodium hydroxide and is transferred to a 50-ml volumetric flask. Fifteen ml of the fluoride reagent is added, the volume made up to 50 ml, and the solution is mixed well and allowed to stand for an hour. The absorbance is read at 617 mμ vs. a reference with no fluoride.

EXPERIMENTAL RESULTS

The results of fluoride analysis in distillates of standard fluoride solutions carried out by several analysts are shown as the solid line in Fig. 2. Each point represents the average of eight separate determinations. These

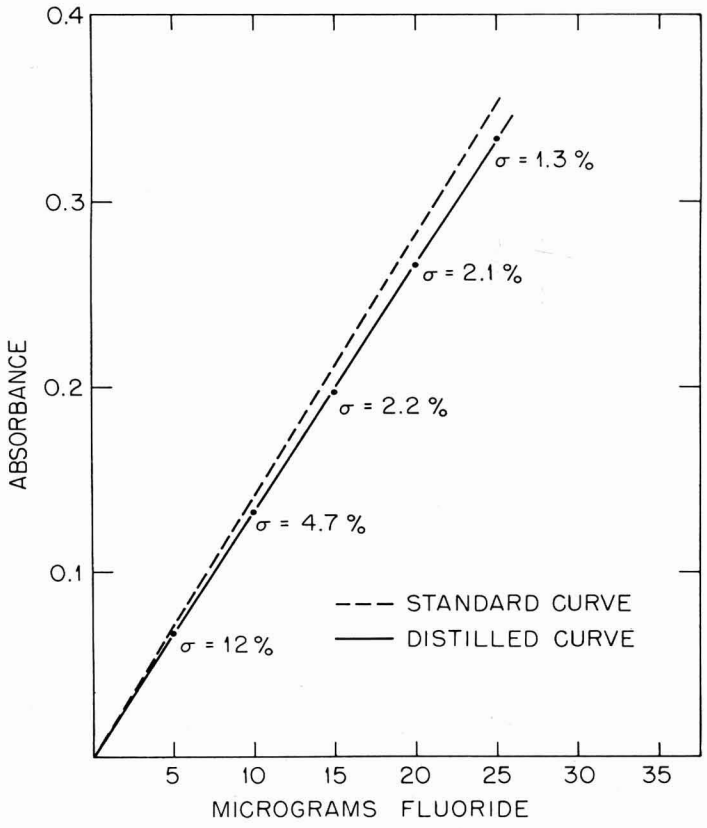


FIG. 2. Analysis of fluoride solution. ORNL-DWG. No. 67-3206.

points line up rather remarkably in a linear manner. The corresponding standard deviation is noted besides each point. The reproducibility at the upper levels is very good, while that at the lower level leaves something to be desired. The dashed line gives the curve for undistilled fluoride made directly from standard fluoride solution. The distilled values come up to about 95% of the undistilled.

DISCUSSION

This distillation procedure was devised to separate fluoride simply and rapidly, and it took into consideration the limitations of hot cell operations. It was decided to eliminate the stream of steam or moisture-saturated gas that is used in most distillation procedures and utilize only

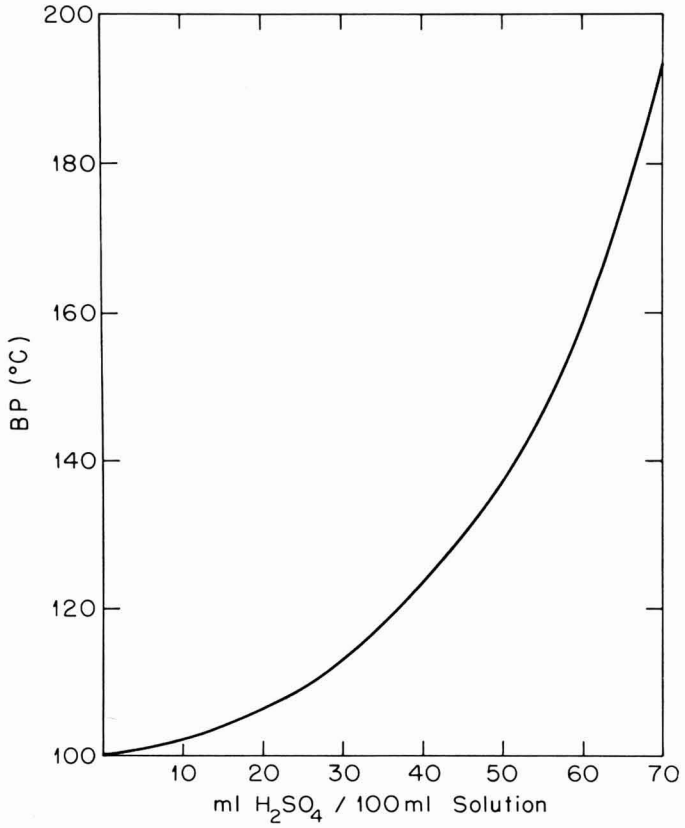


FIG. 3. Boiling point curve of H_2O-H_2SO_4 solutions. ORNL-DWG. No. 67-3205.

the vapor from the distilling solution to transport the fluoride. First of all, the distillate volume was arbitrarily restricted to 20 ml. The spectrophotometric procedure calls for the addition of 15 ml of color reagent, and the volume of the final solution is 50 ml. Thus, 20 ml of distillate gives a leeway of 15 ml to carry out neutralization and transfer operations. Sulfuric acid was chosen to liberate fluoride in preference to phosphoric

acid because the alizarin-complexone is more tolerant to sulfate. The next step was to determine what solution makeup would bring about the quantitative liberation of fluoride in the allotted volume.

The boiling point curve of sulfuric acid solutions in water is shown in Fig. 3. During the determination of the boiling point relation, small

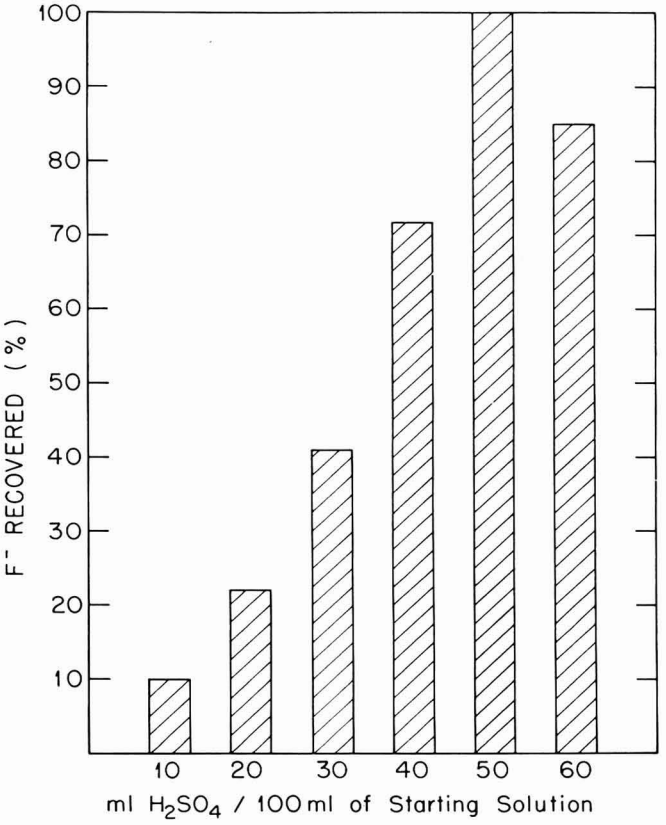


FIG. 4. Fluoride recovered at various H_2SO_4 concentrations. ORNL-DWG. No. 67-3204.

amounts of acid were found to distill over from about 160°C. Starting with 50 ml of sulfuric acid solution and 5 ml of water containing 25 μg of fluoride, distillations were made at various dilutions of acid and 20 ml distillate volumes were collected. These solutions were analyzed for fluoride, and the relative values are shown in Fig. 4. It is seen that complete recovery was obtained at 50:50 acid to water ratio. This solution

makeup was adopted for the method. It is conceivable that other ratios and distillate volumes also will give quantitative recovery.

Reference to the boiling point curve of sulfuric acid-water solutions shows that a solution with the indicated starting composition will start boiling at about 135°C, and the boiling temperature will be about 190°C when 20 ml of distillate is collected. The boiling point of the acid solution is the temperature controlling mechanism here and eliminates the need for special temperature controlling devices. The distillation of 25 μg fluoride is complete in just about 15 ml of distillate. This method calls for 20 ml of distillate to make allowances for slight variations in solution makeup and sample addition.

Yamamura *et al.* (*6*) showed that the presence of 0.10 mmole of sulfate in the final color solution will depress the absorbancy of about 5%. Larger quantities cause sufficient bleaching of the color to constitute a definite interference. Their observation is consistent with the results seen here in that the distilled values read roughly 5% below the undistilled values as shown in Fig. 2. The distillate contains some sulfuric acid as is evident from the amount of base required to neutralize the distillate. This emphasizes the importance of strict observation of solution makeup and volume as well as consistency in the volume of distillate collected. It also indicates that a standard curve should be constructed using distillates of standard fluoride solution, and this curve should be used for the analysis of fluoride solutions which need to be distilled. The poor reproducibility at the 5 μg level is due partially to the effect of sulfate in that slight variations in the sulfate content affect the absorbancy to a relatively greater degree here than with the more concentrated solutions.

One of the drawbacks of the Willard-Winter distillation is the large volume of distillate required to complete the carry-over of the fluoride. It was suspected that this effect is due partially to the fluosilicic acid refluxing at the stillhead where the temperature is closer to the boiling point of water than the solution itself. As a result, provision was made for heating the spray trap to minimize refluxing. By this setup, up to 500 μg can be distilled with a recovery of better than 95% in 20-ml distillate volume. The same degree of recovery can be attained with 5 mg of fluoride from 100 ml of solution and 40 ml distillate volume.

The Yamamura paper also lists a number of ions that will interfere with the alizarin-complexone fluoride determination. To check the possibility of these interferences distilling over with the fluoride, the maximum

amount of each of the listed ions that could be contained in 1 ml of solution was put through the distillation scheme. With the exception of sulfate, there was no interference. The halides and nitrate will distill but not interfere, while borate and phosphate will not distill. One ml of 40° sodium silicate solution allowed 60% recovery. No hindrance was observed with 10 mg of silicate. The transuranium elements and the metals used in their fabrication like aluminum, zirconium, and iron also do not distill. No interference from elements which form volatile oxyfluorides like molybdenum and niobium has been observed. The spray trap arrangement has proved very effective in preventing any entrainment of solution in the vapor stream.

Distillation and Determination of 1-5 μg *of Fluoride*

The procedure described above can be scaled down to provide a more precise determination of fluoride up to 5 μg. A 25-ml flask is used, and the stillhead is modified to accommodate this smaller flask. Ten ml of the 1:1 sulfuric acid and 1 ml of sample comprise the distilling mixture. Four ml of distillate is collected, 3 ml of reagent is added, and the volume is made up to 10 ml. The absorbancy curve for 1-5 μg fluoride (in 10 ml volume) coincides with the curve for 1-25 μg curve (in 50-ml volume). The relative standard deviation obtained for six replicate determinations was 14% at 1 μg, 10% at 2.5 μg, and 4% at 5 μg. This scaled down version is rather difficult to use in the hot cell because of the need to work with smaller glassware; however, it is an excellent procedure for determining fluoride at this low level on the benchtop.

Radiation Stability

This analysis was designed for use in hot cells where the radiation intensity can go up to several hundred rads/hour; consequently, the stability of the alizarin-complexone chromophore to the effects of cobalt-60 radiation was determined. It was found that there is nearly a 1% loss in absorbancy/1000 rads absorbed dose, and the rate of bleaching is linear at least to 50,000 rads. This order of radiation stability seems adequate in view of the radiation intensities that are now being encountered in the hot cells.

REFERENCES

1. Bellack, E., and Schouboe, P. J., Rapid photometric determination of fluoride in water. *Anal. Chem.* **30**, 2032-2034 (1958).

2. KELSO, F. S., MATHEWS, J. M., AND KRAMER, H. P., Ion exchange method for determination of fluoride in potable waters. *Anal. Chem.* **35**, 577-579 (1964).
3. REYNOLDS, D. S., AND JACOB, K. D., Method for determination of fluorine in phosphate rock and phosphatic slugs. *Ind. Eng. Chem., Anal. Ed.* **3**, 366-370 (1931).
4. WARF, J. C., CLINE, W. D., AND TEVEBAUGH, R. D., Pyrohydrolysis in the determination of fluoride and other halides. *Anal. Chem.* **23**, 342-346 (1954).
5. WILLARD, H. H., AND WINTER, O. B., Volumetric method for determination of fluorine. *Ind. Eng. Chem., Anal. Ed.* **5**, 7-10 (1933).
6. YAMAMURA, S. S., WADE, M. A., AND SIKES, J. H., Direct spectrophotometric fluoride determination. *Anal. Chem.* **34**, 1308-1312 (1962).

MICROCHEMICAL JOURNAL **12**, 534-541 (1967)

Spectrophotometric Determination of Nitrogen Dioxide in Nitroglycerin

MAE I. FAUTH AND ANNIE C. RICHARDSON

Analytical Applications Branch, General Research Division, Research & Development Department, Naval Ordnance Station, Indian Head, Maryland 20640

Received May 12, 1967

INTRODUCTION

This investigation was undertaken to attempt to find an improved method for determining the stability of nitroglycerin. The present method involves the use of the Abel or potassium-iodide test. As required by the specification (*3*), this test involves heating three samples of 2 ml each in test tubes placed in a bath maintained at 82.2°C. Strips of standard potassium iodide-starch paper are suspended in the tube in the space above the liquid. Test papers are wet with a 50% solution (v/v) of glycerin in water. This test is both hazardous and time-consuming and requires that large quantities of newly manufactured nitroglycerin be kept in holding tanks pending completion of the laboratory tests.

The series of reactions involved in the potassium iodide test is as follows (*1*): (1.) $RONO_2 \rightarrow RO\cdot + NO_2$; (2.) $H_2O + 2NO_2 \rightarrow HNO_3 + HNO_2$; and (3.) $2NO_2^- + 2I^- + 4H^+ \rightarrow I_2 + 2H_2O + 2NO$.

Since moisture is required to produce the nitrite ion, which actually reacts with the potassium-iodide test paper, the effect of moisture both on the test paper and in the sample must be considered. The specification test (MIL-N246B) requires that the test paper be wet with a 1:1 glycerin-water solution.

Reagents and test papers were evaluated in order to find a suitable analytical method for monitoring Plant nitroglycerin. Optimum factors sought were the following: (1.) colorimetric test which might be readily correlated with the present potassium iodide test; (2.) time not to exceed 15 minutes; and (3.) temperature not to exceed 50°C.

MATERIALS AND METHODS

Four reagents and their analogous test papers were investigated. These are:

1. Starch-potassium iodide.
2. Griess' reagent (1-naphthylamine and sulfanilic acid).
3. 1-Naphthylamine oxalate and sulfanilic acid.
4. N-(1-naphthyl)-ethylenediamine dihydrochloride, (Saltzman's reagent).

All four produce color changes which could be the basis for colorimetric or spectrophotometric analysis.

The use of celite-silicic acid mixtures for separating the impurities in plant nitroglycerin by column chromatography was investigated. Results indicated that total impurities (excluding water) were less than 0.1% in acceptable plant samples.

Apparatus

The nitroglycerin was weighed into 25-ml flasks equipped with cork stoppers and maintained in a constant temperature bath. The absorbance was measured with the Bausch and Lomb Spectronic 505 with the use of 1 cm cells.

Reagents

Starch-Potassium Iodide—1.05 g of dried recrystallized potassium iodide were dissolved in 250 ml of distilled water. Then 3.17 g of washed and dried starch were treated with enough water to make a thick paste, transferred to a beaker, and enough boiling water added to make 200 ml. The solution is then boiled for ten minutes, cooled, added to the 250 ml potassium iodide solution and made up to 500 ml volume.

The Griess' reagent was prepared by the method of Feigl (*1*); 1-naphthylaminoxalate was prepared as given by F. L. Hahn (*2*) and *n*-(1-naphthyl)-ethylenediamine dihydrochloride reagent was prepared by the method of Saltzman (*4*).

Preparation of Reagent Papers

Since all reagents are sensitive to the atmosphere, the papers should be prepared in a closed container. Number 41 Whatman filter paper was

saturated with the reagent in a Petri dish for five minutes. Another piece of filter paper was used to remove the excess reagent. A vacuum desiccator containing silica gel was used to dry the paper overnight. The paper was then cut into desired strips and stored in a brown screw-cap container.

All reagents were prepared daily; however, refrigeration may be used to keep them unreactive for periods not exceeding 24 hours.

Procedure

All tests were carried out with the nitroglycerin in direct contact with the reagent or paper. Samples were placed in closed containers and kept at constant temperature. The first definite color produced was recorded as positive. Reactions were recorded at 5-minute intervals up to 30 minutes.

TABLE 1
ABSORBANCE MAXIMA FOR COLOR TESTS

Reagent	Maxima, mμ
Potassium iodide-starch	585
Griess' reagent	490-500 and 328
1-Naphthylamine oxalate	533 and 347
N-(1-naphthyl)-ethylenediamine dihydrochloride	550

At room temperature, samples containing reagents were shaken gently for 30 seconds to insure proper mixing and allowed to stand for an additional $1\frac{1}{2}$ minutes. The absorbance was measured on the Bausch and Lomb Spectronic 505 at the wavelength maxima shown in Table 1.

The concentration was determined from a previously constructed curve of absorbance vs. concentration of NO_2. Sodium nitrite was used as the standard. Details of the method were given by Saltzman (*4*).

For laboratory work, it was found that 1 g samples of nitroglycerin and 10 ml of reagent produced a detectable color after 2 minutes using Saltzman's reagent. All samples of nitroglycerin used for measurements met plant specifications, therefore the color developed was a minimum because of the very low level of NO_2. Table 2 shows the effect of temperature and sample size on the absorbance for Saltzman's reagent. These values are presented to show these effects but are below the accuracy level of the instrument. The use of larger cells or sample sizes would result in greater absorbance. However, sample sizes were regulated because of safety requirements.

To obtain samples which would correspond to low-stability nitroglycerin, synthetic samples were prepared by adding known amounts of nitrogen dioxide to nitroglycerin. Weighed portions were then analyzed by adding 25 ml of N-(1-naphthyl)-ethylenediamine dihydrochloride and allowing a 15-minute development period. A stable red-violet color was produced, and the absorbance of nitrogen dioxide present was found by use of a calibration curve.

After the concentration of nitrogen dioxide in the nitroglycerin had been determined, the samples were tested at room temperature with dry potassium iodide paper. The minimum detectable amount of nitrogen

TABLE 2

EFFECT OF TEMPERATURE AND SAMPLE SIZE ON ABSORBANCE OF SALTZMAN'S REAGENT ON NITROGLYCERIN

Sample weight of nitroglycerin (g)	Temperature °C	Absorbance
0.5	25	0.005
0.5	35	0.013
0.5	40	0.015
0.5	50	0.017
1.0	25	0.013
1.0	35	0.022
1.0	40	0.028
1.0	50	0.029

dioxide was found to be 1.5 μl. Five minutes were required for development of a detectable color.

Samples of nitroglycerin up to 1 g were analyzed for the added nitrogen dioxide by the N-(1-naphthyl)-ethylenediamine reagent method. Griess' reagent and 1-naphthylamine oxalate reagent were not considered suitable for prolonged contact with the nitroglycerin as they appeared to react with the samples under these conditions.

RESULTS

The effect of temperature and sample size on the Saltzman's reagent-nitroglycerin system is shown in Table 2.

Variables which were investigated include time, temperature, size of nitroglycerin sample, and effect of moisture.

Tests of the reagent in contact with nitroglycerin were run for different amounts of nitroglycerin and reagent. In general, the time required

TABLE 3

MINIMUM TEMPERATURE REQUIRED FOR POSITIVE COLOR TEST WITH TEST PAPERS UP TO 30 MINUTES AND 70°C

Test paper	Nitroglycerin (weight g)	Time-temperature conditions for positive results		
		Dry paper	Glycerin water-wet paper	Water-wet paper
Starch-Potassium Iodide	.05	No reaction	No reaction	25 minutes, 50°
	.80	No reaction	No reaction	20 minutes, 55°
Griess' reagent	.05	30 minutes, 45°	30 minutes, 45°	30 minutes, 30°
	.80	15 minutes, 50°	25 minutes, 35°	30 minutes, 30°
1-Naphthylamine oxalate	.05	25 minutes, 35°	15 minutes, 30°	20 minutes, 30°
	.80	25 minutes, 40°	5 minutes, 30°	5 minutes, 30°
n-(1-naphthyl) ethylene-diamine dihydrochloride	.05	No reaction	30 minutes, 50°	20 minutes, 35°
	.80	20 minutes, 50°	10 minutes, 35°	20 minutes, 35°

TABLE 4

TEST RESULTS OF REAGENT IN CONTACT WITH NITROGLYCERIN UP TO 30 MINUTES AND 60°C

Reagent	Nitroglycerin (weight g)	Amount of reagent	Time-temperature conditions for positive results	Comments
Starch-potassium iodide	0.1	6 drops	30 minutes, 60°	Only very slight change noted
Starch-potassium iodide	0.8	0.8 cc	No reaction noted	
Griess' reagent	0.1	6 drops	30 minutes, 35°	Nitroglycerin became brown after 5 minutes at higher temperatures
Griess' reagent	0.8	0.8 cc	10 minutes, 30°	
1-Naphthylamine oxalate	0.1	6 drops	15 minutes, 30°	Nitroglycerin became brown after 10 minutes 40°C.
	0.8	0.8 cc	5 minutes, 30°	
n-(1-naphthyl)-ethylene diamine dihydrochloride	0.1	6 drops	5 minutes, 30°	No change noted in the nitroglycerin but became turbid after 10 minutes, 35°C.
	0.8	0.8 cc	5 minutes, 30°	

to produce a positive test at specified temperatures was less than with the test papers.

In order to standardize tests, the amounts of nitroglycerin taken as the sample, moisture content of the reagent or test paper, and temperature have to be specified. A summary of the conditions required for positive color tests to be obtained with the test papers is given in Table 3.

When the liquid reagents were in contact with the nitroglycerin, lower temperature and contact times gave positive results. These data are shown in Table 4.

DISCUSSION

The most suitable reagent appeared to be N-(1-naphthyl)-ethylenediamine dihydrochloride. This reagent prepared daily was capable of detecting 0.1 μl of nitrite ion.

A development time of 2 minutes gave an absorbance of 0.010 using 1 g samples of nitroglycerin and 10 ml of reagent. Extending the time from 2 to 5 minutes would increase color development. The length of the flow-tube would have to be sufficient to allow a minimum contact of sample and reagent of 2 minutes. Color development occurs at room temperature, but increased intensities may be obtained by use of temperatures up to 50°C. Moisture content of the nitroglycerin should be known approximately.

The use of this and several other reagents with a modified commercially available flow colorimeter is being evaluated for the on-stream analysis of nitroglycerin.

SUMMARY

Spectrophotometric methods for the determination of nitrogen dioxide in nitroglycerin under various conditions, that may be correlated with the present stability test, are described. The following reagents and test papers were evaluated: starch-potassium iodide, Griess' reagent, 1-naphthylamine oxalate, and sulfanilic acid, and N-(1-naphthyl)-ethylenediamine dihydrochloride (Saltzman's reagent). The method selected is based on the colorimetric determination of nitrogen dioxide using Saltzman's reagent. The system obeys Beer's Law and levels of nitrogen dioxide as low as 0.1 μliter are detectable.

ACKNOWLEDGMENT

This work was funded under the Foundational Research Program of the Director of Naval Laboratories.

REFERENCES

1. FEIGL, F., "Spot Tests in Inorganic Analysis," 5th ed., Elsevier p. 331-332 (1958).
2. HAHN, L. F., Tüpfelreaktion auf nitrit. *Mikrochemie,* **9**, 31-33 (1931).
3. Military Specification Nitroglycerin MIL-N-246B, 19 February 1962.
4. SALTZMAN, B. E., Colorimetric microdetermination of nitrogen dioxide in the atmosphere. *Anal. Chem.* **26**, 1949-1955 (1954).

MICROCHEMICAL JOURNAL **12**, 542-546 (1967)

New Titrimetric Microdetermination of Antipyrine

O. C. SAXENA

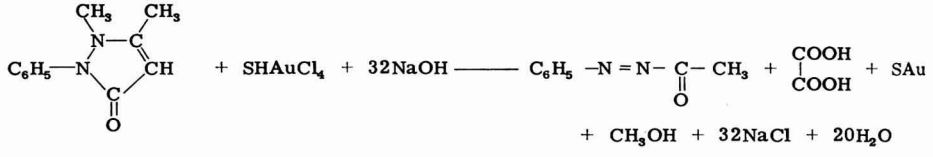
Chemical Laboratories, University of Allahabad, Allahabad, India

Received May 29, 1967

INTRODUCTION

Various methods have been described by different workers concerning the determination of antipyrine. It has been determined iodometrically (*2, 12*) by chrome-sulphuric acid in the presence of silver nitrate (*3*) by forming picrate (*5, 6, 17*) in an ice-cold solution of nitrous acid (*1, 23*), bromatometrically (*9, 13, 14, 15, 19, 21*), potentiometrically (*15*) with iodine monochloride (*7*), polarometrically (*10*), colorimetrically (*11*), spectrophotometrically (*24*), photometrically (*8*), amperometrically (*16*), titrimetrically in nonaqueous media in presence of color indicators (*20*), coulometrically (*22*), and complexometrically (*4*).

Present method deals with the determination of antipyrine by oxidation with an excess of gold chloride in the presence of an alkali. A known excess of gold chloride is added to a solution of antipyrine in the presence of a large excess of sodium hydroxide; it is then heated for about two hours on a hot plate (keeping in mind that the reaction mixture may not evaporate). Gold-chloride solution is reduced to metallic gold, which corresponds to the antipyrine oxidized. Probably the following reaction occurs between gold chloride and antipyrine in alkaline media:



Antipyrine

Antipyrine

In the above case the formation of *N*-acetyl diazobenzene and oxalic acid give positive tests of their presence.

Remaining gold chloride is estimated (*18*) by acidifying the solution

and titrating back the remaining excess of potassium ferrocyanide, which is added in known excess, against a standard solution of ceric sulphate using *N*-phenyl anthranilic acid as indicator.

It is observed that the results agree with those obtained by the standard method (*1*) and give concordant and precise values.

EXPERIMENTAL

Chemicals Employed

1. Gold chloride (Palmston's grade sample).
2. Antipyrine (E. Merck grade sample).
3. Sodium hydroxide (E. Merck grade sample).
4. Potassium ferrocyanide (ANALAR B.D.H. grade sample).
5. Sulfuric acid (ANALAR B.D.H. grade sample).
6. Ceric sulfate (Technical B.D.H. grade sample).
7. Sodium carbonate (ANALAR B.D.H. grade sample).
8. *N*-phenyl anthranilic acid (B.D.H. grade sample).
9. Ferrous ammonium sulfate (ANALAR B.D.H. grade sample).

Ceric sulfate (in 8N H_2SO_4) solution is standardized against a standard solution of ferrous ammonium sulfate (in 1N H_2SO_4) using *N*-phenyl anthranilic acid as indicator.

PROCEDURE

The reaction mixture, comprising a known solution of antipyrine, a known excess of standard solutions of gold chloride, sodium hydroxide, and distilled water, is put on a hot plate (keeping in mind that the reaction mixture may not evaporate) for two hours. In case the volume of the reaction mixture is reduced to about 7 or 8 ml, add 20 ml of distilled water. The beaker containing the reaction mixture must be covered with a beaker cover. After cooling at room temperature, the metallic gold precipitated, corresponding to the antipyrine oxidized, is filtered off and thoroughly washed with distilled water. The remaining gold chloride (unused) solution in the filtrate is titrated back by acidifying the solution and adding a known excess of potassium ferrocyanide (standard) solution. The remaining excess of potassium ferrocyanide is titrated against a standard solution of ceric sulfate (in 8N H_2SO_4) using *N*-phenyl anthranilic acid as indicator. Upon completion of the reaction, a brown-red color appears.

RESULTS

It has been observed that the equivalence of the reacting species is eight, hence the calculations have been made accordingly. The range in which antipyrine has been estimated varies from 0.0728 mg/liter to 0.2914 mg/liter (See Table 1).

TABLE 1
RANGE IN WHICH ANTIPYRINE HAS BEEN FOUND

Range and compounds used for oxidizing antipyrine							
$C_{11}H_{12}ON_2$ 0.019 M	NaOH 1.08 N	H_2O	$HAuCl_4$ 0.015 N	$K_4Fe(CN)_0$ 0.0385 N	$Ce(SO_4)_2$ 0.0025 N	$Ce(SO_4)_2$ 0.0025 N	Amount of antipyrine
ml	ml	ml	ml	ml	ml	ml	mg/liter
—	—	—	—	2	30.80	—	—
—	—	—	3	2	12.80	18.00	—
0.02	10	20	3	2	14.04	1.24	0.0728
0.04	10	20	3	2	15.30	2.50	0.1468
0.06	15	20	3	2	16.52	3.72	0.2185
0.08	15	20	3	2	17.76	4.96	0.2914

The method gives accurate results if antipyrine is present in traces. The peculiarity of this method is that under these present conditions only *N*-acetyl diazobenzene, oxalic acid, and methyl alcohol are formed. It has also been found that further oxidation is possible in different conditions and, in that case, the equivalence goes up to twelve. In the present conditions the reaction between gold chloride and antipyrine in alkaline medium takes place in the ratio of 3:8.

SUMMARY

New titrimetric microdetermination of antipyrine has been described by oxidizing with gold chloride in alkaline medium. Oxidation of antipyrine has been effected by means of an excess of gold chloride in the presence of a known excess of sodium hydroxide. The remaining excess of gold chloride is titrated back by acidifying and adding a known excess of potassium ferrocyanide against a standard solution of ceric sulfate with the use of *N*-phenyl anthranilic acid as indicator. The range in which antipyrine has been estimated varies from 0.0728 mg to 0.2914 mg.

ACKNOWLEDGMENT

The author is grateful to Dr. Bal Krishna, Dr. M. P. Singh, and Dr. M. N. Srivastava for their kind interest, and to the C.S.I.R. (Government of India) for providing financial assistance.

REFERENCES

1. BOCKNIUHL, M., Oxidative cleavage in the antipyrine series. *Med. Chem. Abhandl. Med. Chem. Forschungsstatten. I. G. Farbenind.* **3**, 294-309 (1936).
2. BORLOZ, A., Critical study of the methods of analysis of antipyrine and pyramidone. *Helv. Chim. Acta* **10**, 543-8 (1927).
3. CORDEBARD, H., AND MICHL, V., Volumetric method for the determination of organic material completely oxidizable by chrome-sulphuric acid in the presence of silver nitrate. *Bull. Soc. Chim.* **43**, 97-106 (1928).
4. DICK, J., AND RISTICI, J., New specific complexometric determination of antipyrine. *Acad. Rep. Populare Romine, Baza Cercetari Stiint. Timisoara, Studii Cercetari Stiinte Chim.* **9**, No. 3-4, 269-72 (1962).
5. DÓZSA, A., The analysis of antipyrine and some of its derivatives. *Magy. Gyógyszerésztud. Társaság Értesitöge.* **9**, 476-506 (1953).
6. ERICKSON, S., Determination of antipyrine and pyramidone. *Svensk Farm. Tids.* **34**, 1-7 (1930).
7. GENGRINOVICH, A. I., The use of HCl solution of iodine monochloride in volumetric determination. II. Volumetric determination of antipyrine. *Farm. Zhur.* **13**, 15-17 (1940).
8. HAHN, M., KOLŠECK, J., AND PERPAR, M., The photometric determination of antipyrine with p-dimethyl aminobenzaldehyde. *Z. Anal. Chem.* **151**, 104-8 (1956).
9. HOFSTETTER, R., AND MUHLEMANN, H., Determination of antipyrine. *Pharm. Helv.* **38**, 218-23 (1963).
10. KALVODA, R., AND ZÝKA, J., Application of polarometric titrations in pharmaceutical analysis. I. Mercurimetric estimation of antipyrine. *Chem. Listy.* **46**, 56 (1952).
11. KINOSHITA, YAYOE AND SHIGETAKA MORIYAMA, Colorimetric determination of antipyrine. *Bull. Nazoya City Univ. Pharm.* No. **2**, 28-30 (1954).
12. KOLTHOFF, I. M., Iodometric determination of antipyrine. *Pharm. Weekblad* **60**, 194-9 (1923).
13. LASZLOVASZKY, J., Utility of bromatometric redox indicators. *Ann. Univ. Sci. Budapest Rolando Eotvos Nominatae, Sect. Chim.* **2**, 519-25 (1960).
14. MADIS, V., Bromatometric determination of antipyrine. *Osterr. Chem-Ztg.* **42**, 290-3 (1939).
15. MARICQ, LOUIS, Potentiometric titrations of some compounds which can be precipitated by potassium iodomercurate. *Bull. Soc. Chim. Belg.* **2**, 361-70 (1931).
16. OGAWA, TADAHIKO, Amperometric determination of small amount of alkaloids. *Nippon Kagaku Zasshi.* **77**, 535-7 (1956).
17. RAE, J., A volumetric process for the estimation of phenazone. *Pharm. J.* **121**, 575 (1929).
18. SAXENA, O. C., A new volumetric method for the estimation of gold. *Indian. J. Appl. Chem.* No. **1**, 1074 (1966).
19. SCHULEK, E., AND KOVACS, J., The bromatometric determination of antipyrine. *Ber. Ungar. Pharm. Ges.* **16**, 344-7 (1940).

20. TADENSZ, JASIŃSKI AND IRENA PAWÉLCZAK, Titration of antipyrine in non-aqueous media in presence of color indicators. *Acta. Polon. Pharm.* **14**, 91-3 (1959).
21. TOMIČEK, O., KUČERA, R., AND ZÝKA, J., A further bromatometric determination of some drugs. *Českoslov. Farm.* **1**, 244-52 (1952).
22. WTADYSLAWA BARBARA SYKUT, Coulometric microdetermination of antipyrine. *Acta Polon. Pharm.* **16**, 21-4 (1959).
23. YAMAGISHI, M., YAKOO, M., AND INONE, S., Application of Azotometry. XV. Determination of antipyrine. *Yakugaku Zasshi.* **78**, 87-90 (1958).
24. ZÝKA, J., Bromine in glacial acetic acid as analytical solution. Determination of a few pharmaceutically important compounds. *Pharmazie* **9**, 812-24 (1954).

MICROCHEMICAL JOURNAL 12, 547-554 (1967)

Methods for the Isolation and Characterization of Constituents of Natural Products

V. Separation of 2,6-Dinitrophenylhydrazone Pyruvamides into Classes and Resolution of the Individual Members

D. P. SCHWARTZ AND C. R. BREWINGTON

Dairy Products Laboratory
Eastern Utilization Research and Development Division
Agricultural Research Service, U. S. Department of Agriculture
Washington, D. C. 20250

Received June 9, 1967

INTRODUCTION

In part IV of this series, preparation of a homologous series of 2,6-dinitrophenylhydrazone pyruvamides of *n*-aliphatic primary amines and a partial series of symmetrical secondary aliphatic amines was described (*3*). A brief discussion of the potential utility of pyruvyl chloride 2,6-dinitrophenylhydrazone for derivatizing amines in lipids was presented. This entailed the mild conditions under which the derivatives are formed, and properties which they possess enable them to be isolated directly from lipids in a lipid-free condition. It was also mentioned that the pyruvamides could be separated into classes, that is, into primary and secondary amine derivatives. This paper describes the method for performing the class separation both quantitatively by column adsorption chromatography, and qualitatively by thin-layer adsorption chromatography. The rapid resolution of an homologous series of members of both classes by thin-layer partition chromatography is also described.

APPARATUS AND MATERIALS[1]

Magnesium oxide (catalog no. 2477) suitable for chromatographic use was obtained from the J. T. Baker Co., Phillipsburg, New Jersey. The

[1] Reference to certain products or companies does not imply an endorsement by the Department over others not mentioned.

powder had an adsorption index (Food and Drug yellow no. 4) of 12-13 and was used without further treatment. Celite 545 and Analytical Grade Celite were products of the Johns-Manville Co., Baltimore, Maryland. Chloroform, benzene and methanol were ACS grade; diethylamine (Baker) was redistilled; hexane was the high purity grade obtained from the Phillips Petroleum Co., Bartlesville, Oklahoma; heptane was a product of Eastman Kodak Co., Rochester, New York; silica gel G was obtained from Brinkmann Instruments, Westbury, New York.

The thin-layer chromatographic equipment was the same as previously described (*1*). A borosilicate glass column with a coarse fritted glass disc was employed for the column chromatography. The dimensions were 2.2 cm i.d. by 29 cm measured from the top of the column to the disc.

EXPERIMENTAL

Separation of 2,6-dinitrophenylhydrazone pyruvamides of primary and secondary amines into classes by column adsorption chromatography. One g of MgO and 9 g of Celite 545 are slurried in about 50 ml of $CHCl_3$ and the slurry poured through a long-stemmed funnel into the column. The slurry is packed under moderate air pressure until a few ml of $CHCl_3$ remain above the bed. The sides of the column are carefully washed down with a few ml of $CHCl_3$ and the washings are permitted to drain. The derivatives are quantitatively transferred to the column using a minimum of $CHCl_3$. After the last of the solution has just drained, the sides of the column are washed with a few ml of $CHCl_3$ and when this has percolated into the bed, a small wad of glass wool is placed just above the bed and development of the chromatogram is begun with 75 ml of $CHCl_3$. The secondary amine derivatives move off readily with this solvent as a greenish band. The primary amine derivatives remain near the top of the column as a violet band and are eluted with 10% methanol in $CHCl_3$.

Separation of 2,6-dinitrophenylhydrazone pyruvamides of primary and secondary amines into classes by thin-layer adsorption chromatography. Thirty g of silica gel G are slurried with 60 ml distilled water and spread over five 8 $\times$ 8 inch plates in the usual manner. The plates are left to dry overnight at room temperature and then activated for 1 hour at 100°C prior to use. The derivatives are spotted from benzene solution and the plate is developed for about 30 minutes with diethylamine: heptane (1:1). The secondary amines move as greenish spots, the primary amine derivatives as violet spots. Evaporation of the diethylamine from the finished plate gives spots with the original yellow color.

Separation of homologous series of primary and secondary pyruvamides by normal thin-layer partition chromatography. The methods for preparing the normal thin-layer partition plates is identical to that described for separating homologous series of 2,6-dinitrophenylhydrazone derivatives of pyruvic acid esters (*1*), except that 65 ml of absolute ethanol are used instead of 60 ml when preparing the slurry.

The derivatives are spotted from benzene solution and developed (in an equilibrated tank lined with filter paper) with hexane:benzene (7:3) saturated with polyethylene glycol 400. At the end of the development, the plate is removed, inspected and placed in a tank containing cotton wetted with diethylamine. This procedure produces violet spots which can be detected much more readily than the original yellow spots.

Separation of Primary Amine Derivatives by Reversed-Phase Thin-Layer Partition Chromatography. The reversed-phase thin-layer plates are prepared as previously described (*1*). The derivatives are spotted from

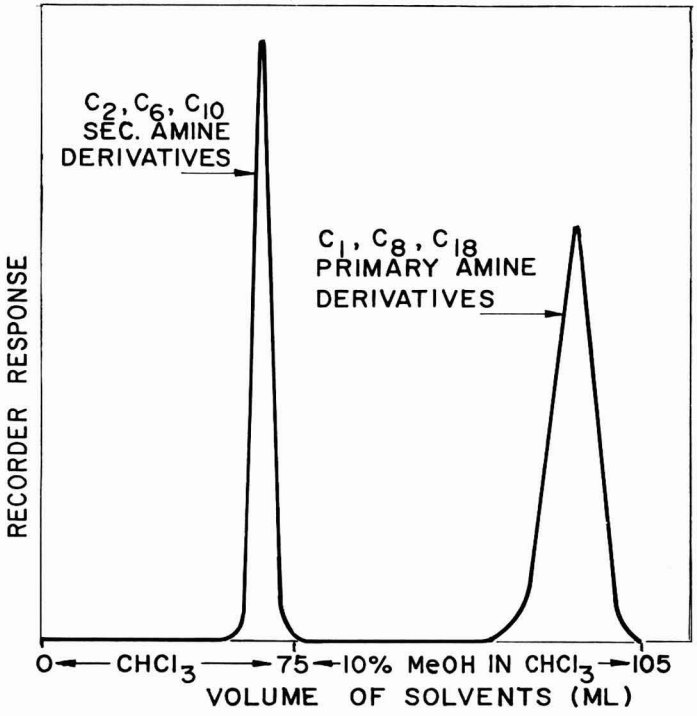


FIG. 1. Class separation of primary from secondary pyruvamide 2,6-dinitrophenylhydrazones by column absorption chromatography on magnesia-celite.

benzene solution at the origin of the plate. The top inch of support is scraped from the plate and the plate is placed scraped-side down in an equilibrated tank lined with filter paper. At the end of this time the plate is inverted and developed for approximately 1 hour in the solvent system acetonitrile:water (3:1) not previously saturated with the stationary phase (Nujol).

RESULTS AND DISCUSSION

Figure 1 shows the separation of primary and secondary amine derivatives achieved by adsorption chromatography on the magnesia-celite column. Approximately 0.2 μmole (each of the C_2, C_6 and C_{10}) secondary amine derivatives and 0.2 μmole (each of the C_1, C_8 and C_{18}) primary amine derivatives were selected for study since preliminary work indicated that the C_{10} secondary and C_{18} primary amine derivatives would be the most difficult pair to separate of the amine derivatives prepared in this laboratory. A 98.2% recovery of the secondary amine class and a 100.6%

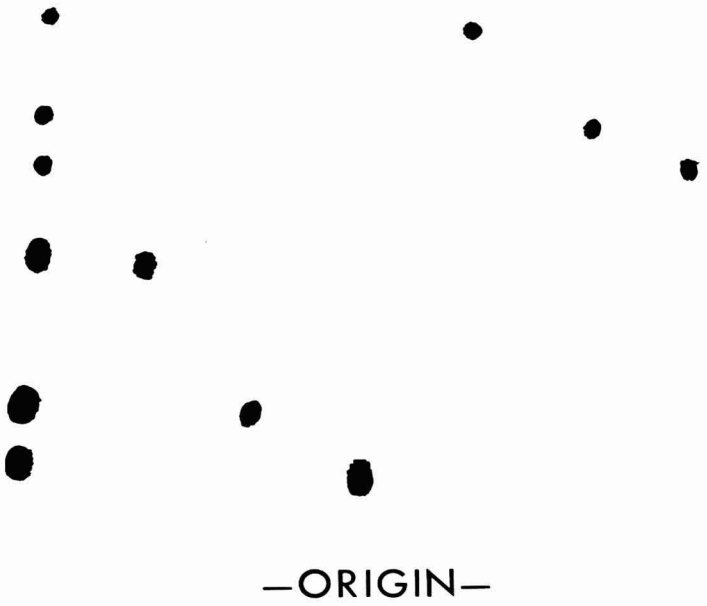


FIG. 2. Class separation of primary from secondary pyruvamide 2,6-dinitrophenylhydrazones by thin-layer adsorption chromatography on Silica Gel. G. Solvent system—diethylamine:heptane (1:1). Left to right: mixture of C_{18}, C_8, C_1 primary amine and C_{10}, C_6, C_2 secondary amine derivatives; then C_{18}, C_8, C_1 primary amine and C_{10}, C_6, C_2 secondary amine derivatives.

recovery of the primary amine class was achieved. The two classes are easily separated and readily distinguished by the differences in color of the derivatives on the adsorbent. The entire chromatographic separation takes approximately 30 minutes to complete. The data in Fig. 1 were obtained by monitoring the effluent from the column continuously using a 0.2 ml flow-through cell in an Hitachi-Perkin Elmer Model 139 spectrophotometer and recording the readings with a Honeywell-Brown 5 mv recorder.

Separation of the primary and secondary amine classes by thin-layer adsorption chromatography on silica Gel G is shown in Fig. 2. As with column adsorption chromatography, the secondary amine derivatives are

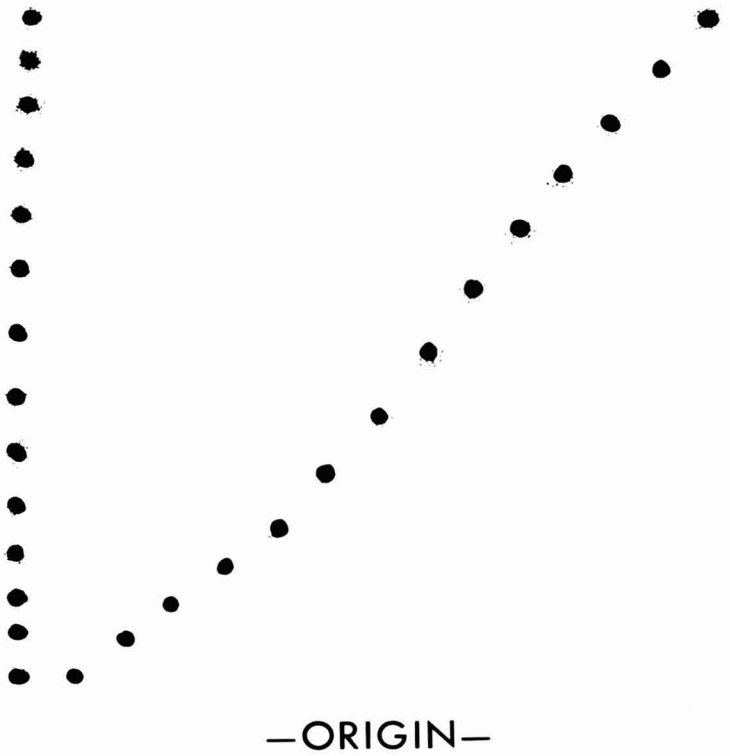


FIG. 3. Separation of primary amine pyruvamide 2,6-dinitrophenylhydrazones by normal partition chromatography. Support-Microcel T-38; stationary phase—polyethylene glycol 400; mobile phase—hexane:benzene (7:3) saturated with stationary phase. Diagonally from top to bottom C_{14} through C_1 derivatives. Column on left represents mixture of all 14 derivatives.

greenish and the primary amine derivatives are violet, even though a different adsorbent and solvent system are employed. The separation shown in Fig. 2 was achieved in about 30 minutes. The primary amine derivatives are detectable in the amount of 1.8×10^{-4} μmoles; the secondary amines in the amount of 5.4×10^{-4} μmoles.

Separation of the C_1 through C_{14} primary amine derivatives by normal thin-layer partition chromatography is depicted in Fig. 3. The C_{15} amine separates from the C_{14} but not from the C_{16} derivative and, therefore, amine derivatives above C_{14} are not included. It should be noted that the solvent system used to separate the amines is more polar than that used (*1*) to separate the alcohol derivatives. Approximately 1.5×10^{-4} μmoles of derivatives can be detected on the plate after exposure of the plate to diethylamine vapor.

Separation of the five secondary amine derivatives prepared in this laboratory is shown in Fig. 4. Although the secondary amine derivatives

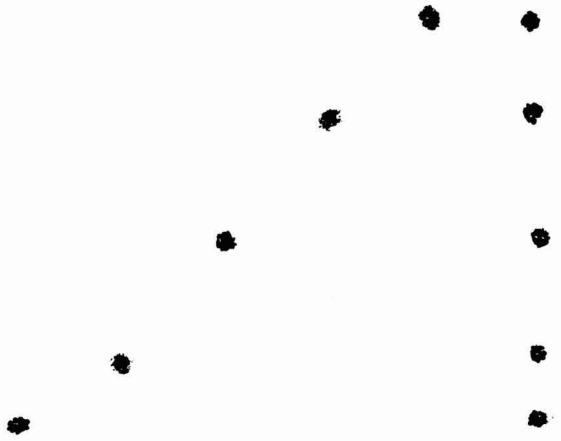


FIG. 4. Separation of secondary amine pyruvamide 2,6-dinitrophenylhydrazones by normal partition chromatography. Support-Microcel T-38; stationary phase—polyethylene glycol 400; mobile phase—hexane:benzene (7:3) saturated with stationary phase. Diagonally from top to bottom C_{10}, C_8, C_6, C_4, C_2 symmetrical secondary amine derivatives. Column on right represents mixture of all five amine derivatives.

higher than C_{10} were not prepared, it is likely that the C_{12} and C_{14} derivatives would also separate well if the primary amine separation is used as an analogy.

Figure 5 shows the separation of the higher primary amine derivatives by reversed-phase thin-layer chromatography. The C_{18} through the C_{11} derivatives are well separated. The C_{10} derivative separates from the C_{11} but does not separate cleanly from the C_9 and is, therefore, not shown. Approximately 2×10^{-4} μmoles can be detected after exposure of the plate to diethylamine vapor. No reversed-phase partition chromatography was carried out on the secondary amine derivatives since the C_{10} derivative was the longest chain derivative prepared.

Class separation of the amine derivatives should facilitate the analysis of complex mixtures of amines isolated from biological material. Moreover, since in the class separation of alcohol derivatives (described in part III of this series (*2*) no greenish bands are produced on the magnesia, the

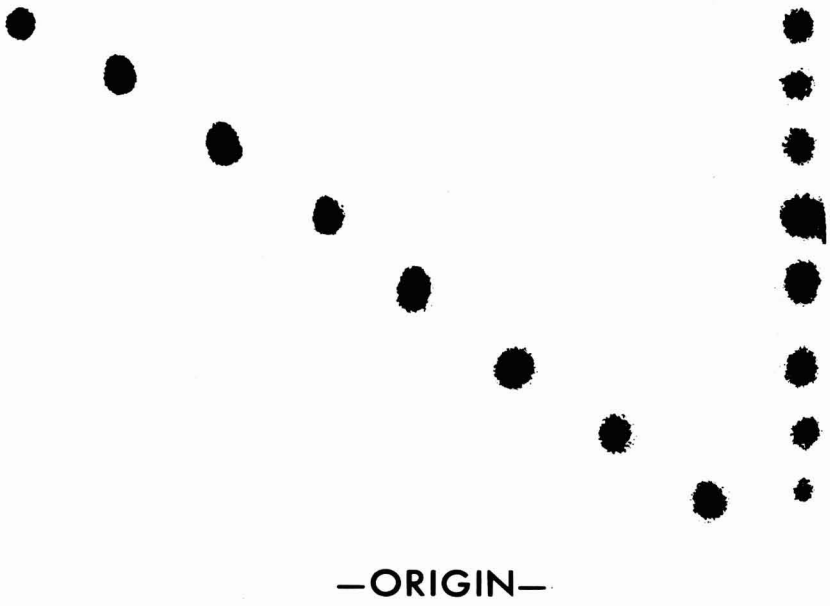


FIG. 5. Separation of primary amine 2,6-dinitrophenylhydrazones by reversed-phase partition chromatography. Support-Microcel T-38; stationary phase—mineral oil; mobile phase—acetonitrile:H_2O (3:1). Diagonally from top to bottom C_{11} through C_{18} primary amine derivatives. Column at right represents mixture of the 8 derivatives.

appearance of a greenish band in a mixture of unknown derivatives may indicate the presence of secondary amine derivatives. Work is in progress to attempt to class separate the alcohol derivatives from the amine derivatives.

SUMMARY

Class separation of the 2,6-dinitrophenylhydrazone derivatives of *n*-aliphatic primary and symmetrical secondary aliphatic amines is described. Both quantitative column adsorption and qualitative thin-layer adsorption chromatographic procedures are presented. In both procedures the classes are cleanly separated and they can also be distinguished readily by color; the secondary amine derivatives are green and the primary amine derivatives are violet during chromatography. Approximately 0.2 μmole of a derivative is easily seen in the column procedure whereas 1.8×10^{-4} μmole of a primary derivative and 5.4×10^{-4} μmole of a secondary amine derivative can be followed in the thin-layer procedure.

Methods for the separation of a homologous series of the primary amine derivatives and a partial series of secondary amine derivatives by thin-layer partition chromatography is also described. Approximately 1.5×10^{-4} μmoles of derivative can be utilized in this procedure. Reversed-phase separation of the higher primary amine derivatives by thin-layer chromatography is also presented.

REFERENCES

1. SCHWARTZ, D. P., AND BREWINGTON, C. R., Methods for the isolation and characterization of constituents of natural products. II. Separation of homologous series of esters of pyruvic acid 2,6-dinitrophenylhydrazone by thin-layer chromatography. *Microchem. J.* **12**, 1-6 (1967).
2. SCHWARTZ, D. P. BREWINGON, C. R. AND SHAMEY, JENNIE. Methods for the isolation and characterization of constituents of natural products. III. Separation of alcohol esters of pyruvic acid 2,6-dinitrophenylhydrazone by column and thin-layer chromatography. *Microchem J.*, **12**, 186-191 (1967).
3. SCHWARTZ, D. P., AND BREWINGTON, C. R. Methods for the isolation and characterization of constituents of natural products. IV. Amide derivatives of amines with pyruvyl chloride 2,6-dinitrophenylhydrazone. *Microchem. J.* **12**, 192-195 (1967).

MICROCHEMICAL JOURNAL 12, 555-567 (1967)

Pharmaceutical Applications of Internal Reflectance Spectroscopy

R. J. WARREN, I. B. EISDORFER, W. E. THOMPSON, AND J. E. ZAREMBO

Smith Kline & French Laboratories, Philadelphia, Pennsylvania

Received June 9, 1967

INTRODUCTION

One of the most interesting and useful new techniques for obtaining infrared spectral data of a variety of materials is internal reflectance spectroscopy (IRS; also known as Attenuated Total Reflectance, ATR).

The technique may be described briefly as follows. When a beam of radiation is internally reflected from the surface of a suitable transmitting medium, part of the radiation passes outside the surface of the transmitting medium before being returned to it by the process of reflection. When a sample is brought into contact with the surface of the transmitting medium, the radiation passing outside will be absorbed at those wavelengths where the sample material absorbs.

The radiation at those wavelengths where the sample does not absorb will be totally reflected. This reflection-absorption process takes place 35-50 times in a typical IRS plate and in this way an infrared spectrum is obtained. This infrared spectrum is comparable, but not always identical in every respect, to an ordinary transmission spectrum. It sometimes happens that absorption bands are distorted or slightly different in shape, but this problem may be circumvented by obtaining IRS spectra of standard samples of the material in question. The differences in IRS and transmission spectra are certainly not of an order to make identification impossible, but the fact that differences can and do exist should be mentioned.

IRS is a valuable analytical tool because absorption takes place only at the interface of sample and crystal, independent of sample thickness. This means that one can obtain an infrared spectrum of a material which would ordinarily be totally absorbing in the infrared. An added advantage is that little or no sample preparation is necessary. There is no need to

worry about the reproducibility of thin films since the radiation penetrates the sample only to the extent of a few microns. When spread out as a film on the surface of an IRS plate, samples present in minute quantities give intense spectra with no scale expansion required. Aqueous solutions may be run with no compensation for solvent absorption. These applications, as well as the illustrations to follow, will indicate the advantages and usefulness of IRS in pharmaceutical analysis.

EXPERIMENTAL

Spectra were recorded on a Perkin-Elmer Model 521 spectrophotometer equipped with a Model 12 double-beam internal reflectance attachment manufactured by the Wilks Scientific Corporation. A 2 $\times$ slit program was used. The samples were run in natural state on KRS-5 plates.

RESULTS AND DISCUSSION

Micro Samples

As has been described, internal reflectance spectroscopy is concerned only with the surface of the material to be examined. Since the beam of radiation penetrates only a few microns into the sample, the thickness of the sample as a whole is immaterial. Samples present in submilligram quantities, which normally would require a great deal of manipulation, special techniques, and precautions to obtain a transmission IR spectrum, are easily handled by IRS. Less than a milligram of material distributed as a film over the reflecting crystal is all that is needed for a good spectrum. Distribution of sample over crystal is accomplished by dissolving the sample in a volatile solvent such as chloroform and transferring the chloroform solution drop by drop to the IRS crystal or plate. The chloroform is evaporated to dryness leaving the sample spread out as a film on the plate, and an infrared spectrum is taken with the sample as is. This method is especially valuable where the material to be examined is not only present in very small amounts but must be recovered intact for subsequent testing. To recover the sample, it is washed from the plate with a suitable solvent. In addition to the ease of sample preparation, ease of sample recovery increases the value of the technique over conventional microtechniques of transmission spectroscopy. The latter techniques normally subject the sample to the high pressures and/or temperatures necessary in potassium bromide disc preparation and the use of beam-condensing systems.

Figure 1 illustrates the type of spectra one can expect from submilli-

gram quantities of material using the IRS technique. The spectra are of prochlorperazine. Spectrum A is a transmission IR spectrum; B is an IRS spectrum of a 64-μg sample. Figure 2 is an IRS spectrum of 32 μg of prochlorperazine; no scale expansion is required.

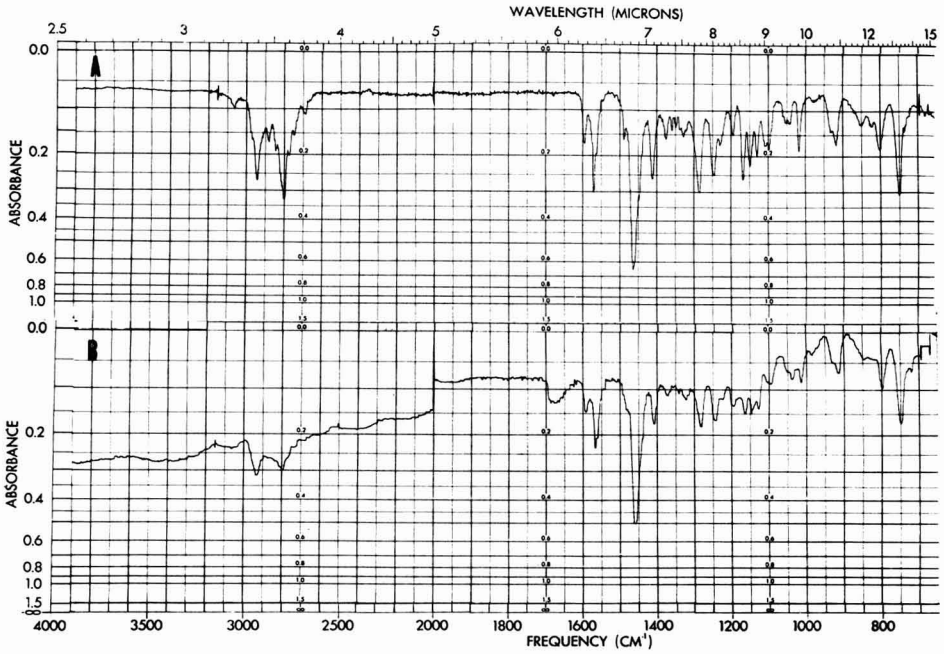


FIG. 1. Spectra of prochlorperazine: A. Transmission IR; B. IRS spectrum on 64-μg sample.

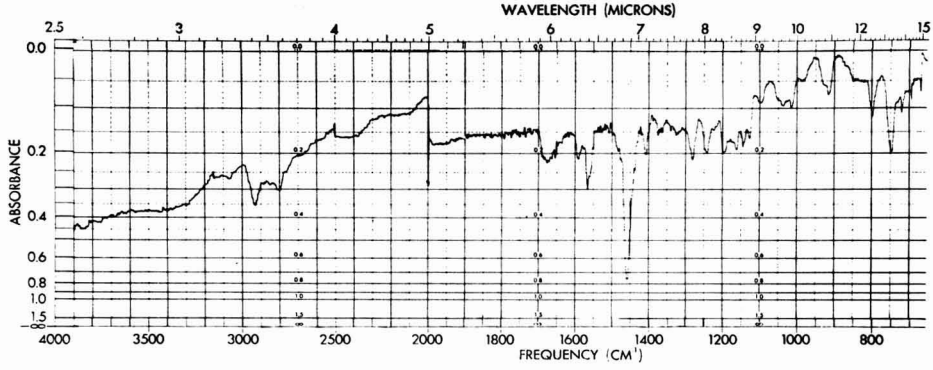


FIG. 2. Spectrum of prochlorperazine, 32-μg sample.

Gas-liquid chromatography (GLC) and thin-layer chromatography (TLC) fractions. Micro-samples such as those first illustrated are often derived from chromatography. Samples from GLC may be handled in several ways. If the fraction is collected as a tiny droplet of liquid it may be transferred to an IRS plate, and the IR spectrum is determined as described above.

Sometimes samples are not present in sufficient amounts to permit isolation and collection of condensates. In this situation, it is often possible to collect the sample directly on the IRS plate by holding the plate at the exit port of the chromatograph. Enough of the escaping gas fraction condenses on the plate to permit recording a spectrum. This is not surprising, if one remembers that IRS is measuring only the surface of the sample; the depth of sample is immaterial. A few micrograms should be enough for an identifiable spectrum.

In another method, the GLC fractions are collected directly in a suitably cooled volatile solvent such as chloroform. The resulting chloroform solution is transferred to the IRS plate and evaporated to dryness, which leaves the sample as a thin film on the plate.

Fractions or spots from thin-layer chromatograms present few, if any problems. After the thin-layer plate has been developed and zones of interest are marked, the spots or zones are removed and eluted with proper solvent either directly on to an IRS plate or to a suitable container if the volume of solution warrants. In our laboratories, a medicine dropper with a glass-wool plug is used for removal and elution of TLC spots. The spot or zone is removed from the TLC plate and transferred to the medicine dropper. A small amount of solvent is added to elute the sample and the solvent is allowed to collect either directly on the IRS plate or in a small vial. In either case the solution is evaporated to dryness on the IRS plate, and the spectrum is determined. The only necessary precaution is prewashing the thin-layer plate with the solvent to be used for elution. This removes any organic matter which could contaminate the chromatographic fraction during elution. It must be remembered when dealing with very small amounts of material that a contaminant, present in otherwise negligible quantity, assumes the proportions of a major component.

Figure 3 is the infrared spectrum of a TLC fraction. Spectrum A is the reference material. Spectrum B is the isolate from the chromatographic plate. In a study of the metabolites of chlorpromazine in human urine, a

material was isolated by TLC and identified by its IRS spectrum. The sample was then recovered and an ultraviolet spectrum obtained. From calculations based on a reference material, it was possible to determine the amount of metabolite present. The IRS spectrum of the metabolite shown in Fig. 3 was obtained from 102 μg of material.

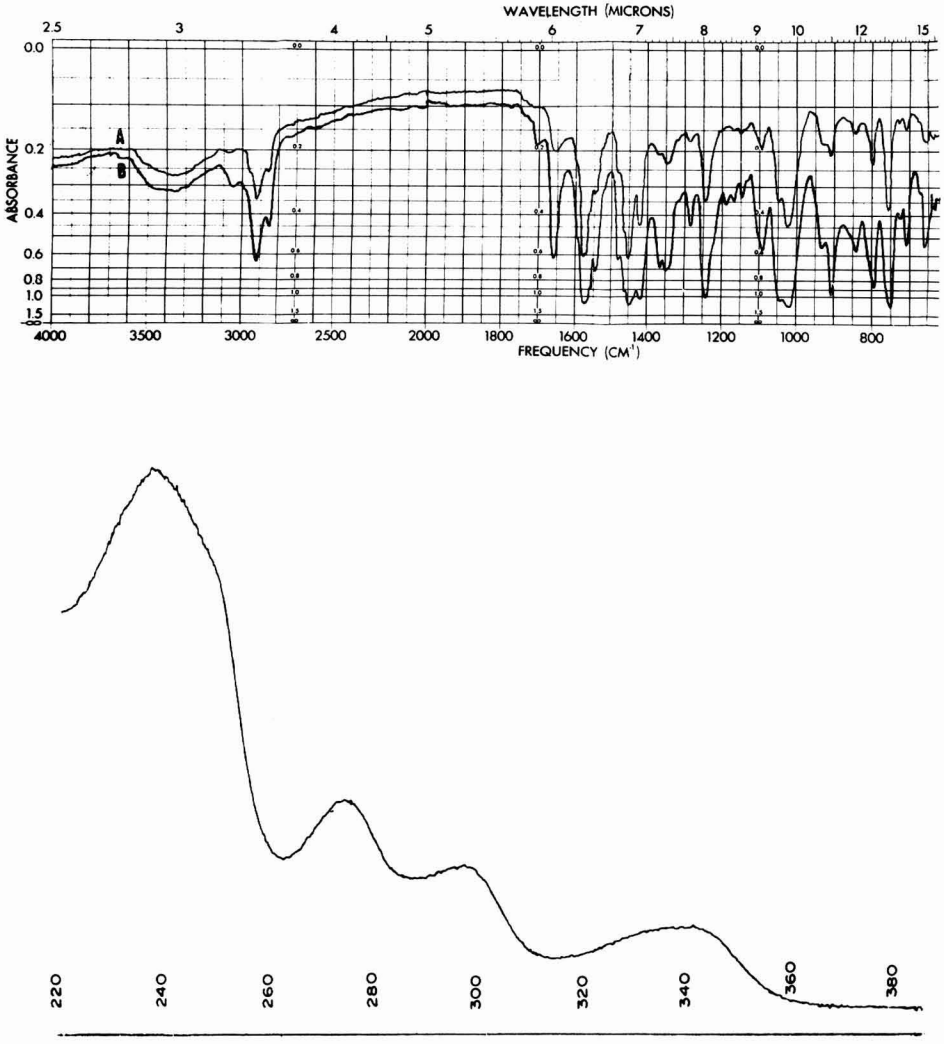


FIG. 3. UPPER. IR spectra: A. Reference; B. Metabolite, isolated by thin-layer chromatography. LOWER. UV spectrum: Metabolite.

Tablet Coatings

Infrared spectra on thin film coatings of tablets would be difficult if not impossible to obtain by conventional IR transmission techniques. It would involve removal of the coating and subsequent manipulation of the sample in order to obtain infrared analysis. The time required, as well as the possible alteration of the coating in the process, makes the procedure difficult. The IRS technique, on the other hand, allows one to obtain an infrared spectrum on a tablet coating with no sample preparation or manipulation whatsoever. The tablets are placed in a suitable holder and pressed against the IRS plate. The beam of radiation penetrates the tablet only as far as the coating and the resulting spectrum is that of the tablet coating. Thin film coatings are no problem since the beam samples only the surface of the tablet.

Figure 4 is the infrared spectrum of a commercially available tablet with a thin film coating. Spectrum A is that of the actual tablet coating. It appeared to be a mixture of carbowax and cellulose acid phthalate. A mixture of the two substances was prepared, and gave spectrum B, which proved conclusively that the coating consisted of such a mixture.

Further infrared analysis of subsequent mixtures of the components allows a quantitative determination of the exact proportions of the two components.

The IRS technique has potential use not only in identification of tablet coatings but also in evaluating the effects of long-term storage conditions on the coatings.

Wax Coatings

Infrared spectroscopy of waxes has been the subject of numerous investigations, and the results and spectra have been reported extensively in the literature. The infrared spectra of many waxes are also available commercially. The new and attractive feature of IRS infrared spectra is that the spectrum of a wax coating can be obtained without removal of the coating, and without any sample preparation other than placing the sample directly into the sample holder. The technique allows one to study the coating as is, under a variety of storage conditions. The effects of temperature, humidity, and the aging process, itself, can be followed in infrared spectra of the coating without altering the sample. The same sample can be withdrawn for examination, returned to storage, and withdrawn again for examination at a later date. Surface phenomena such as

color changes and fading at the surface of wax coating can be checked to determine whether the coating is or is not being affected.

IRS spectra of wax coatings can be taken without great time spent on sample preparation, as has been mentioned. This makes possible fast and convenient identification of materials such as are frequently of interest to law enforcement agencies. In the case of drugs, for example, determination of the exact nature of the outer surface of a tablet can save considerable time and effort in identification of the product itself. The

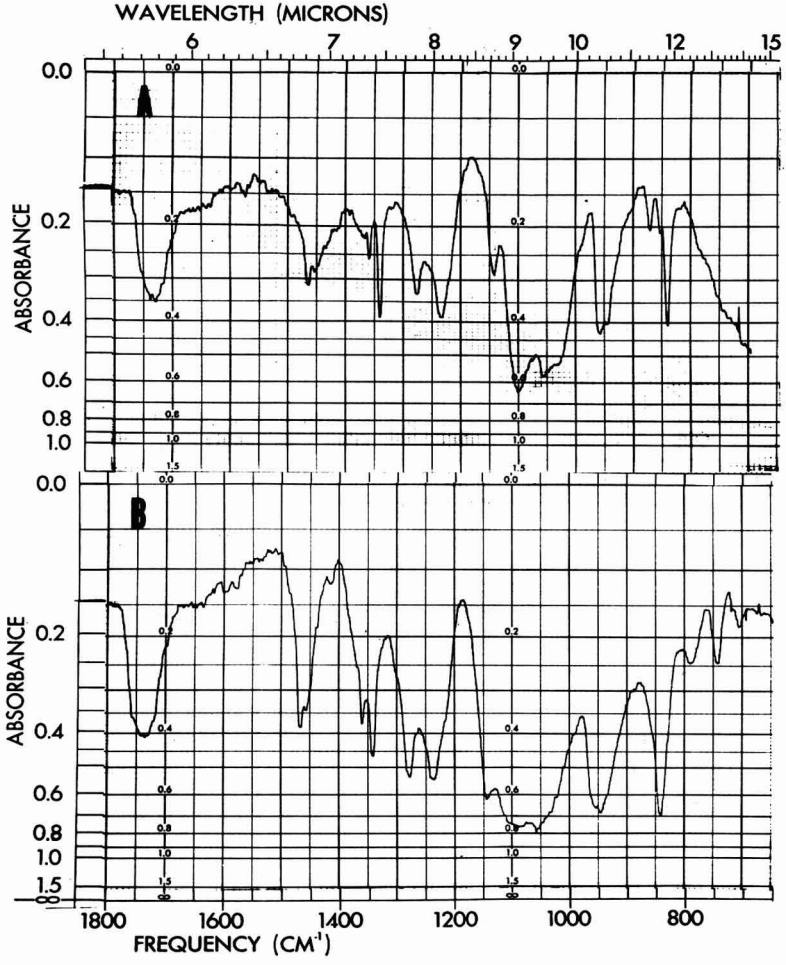


FIG. 4. Spectra: A. Tablet coating; B. Synthetic mix.

same type of information can be of assistance in identifying counterfeit drugs sold under the labels of ethical pharmaceutical companies. Figure 5 shows the IRS infrared spectra of three commercial product coatings. It is apparent that the spectra are unique and characteristic for the respective products. There would be no difficulty in identifying the product by its coating as a first step in the positive identification of the active ingredients.

Tissues

The difficulties involved in obtaining spectra of living tissues are well documented. Most, if not all, of the difficulties stem from the high water content of the tissues and the intense absorption of the water in 3.0 and 6.0-μ regions. This strong absorption masks important areas of the spectrum and necessitates use of time-consuming methods of sample preparation to circumvent the interference. Such techniques as homogenation of tissue, freeze-drying, mulling, and potassium bromide discs have been used to obtain spectra of sufficient quality to give meaningful data. All of these techniques require great time and may result in loss or alteration of the sample during preparation. The use of IRS reduces sample handling and preparation to a minimum. Since only the surface of the tissue is sampled by the beam of radiation, the ratio of water to solids is reduced and the usually intense absorption of water in the 3.0 and 6.0-μ regions is correspondingly reduced.

Tissue specimens for IRS infrared examinations are prepared by slicing the tissue so that it fits the IRS sample holder. The tissue is placed against the IRS plate or prism and the spectrum is run. This method provides spectra of good quality in a very short time. The IRS spectra thus obtained can be compared directly with transmission spectra of standards, since IRS and transmission spectra are the same for all practical purposes. Figure 6 shows the IRS infrared spectrum of liver tissue. Spectra of heart, muscle, and lung tissue are similar.

The technique has potential use in comparison of normal and diseased tissue, and in the detection of drugs or their metabolites in living tissue.

Vehicles

The IRS technique permits one to obtain spectra of vehicles used in creams and ointments without any prior sample preparation. The operation consists of placing a smear of the ointment or cream on the IRS

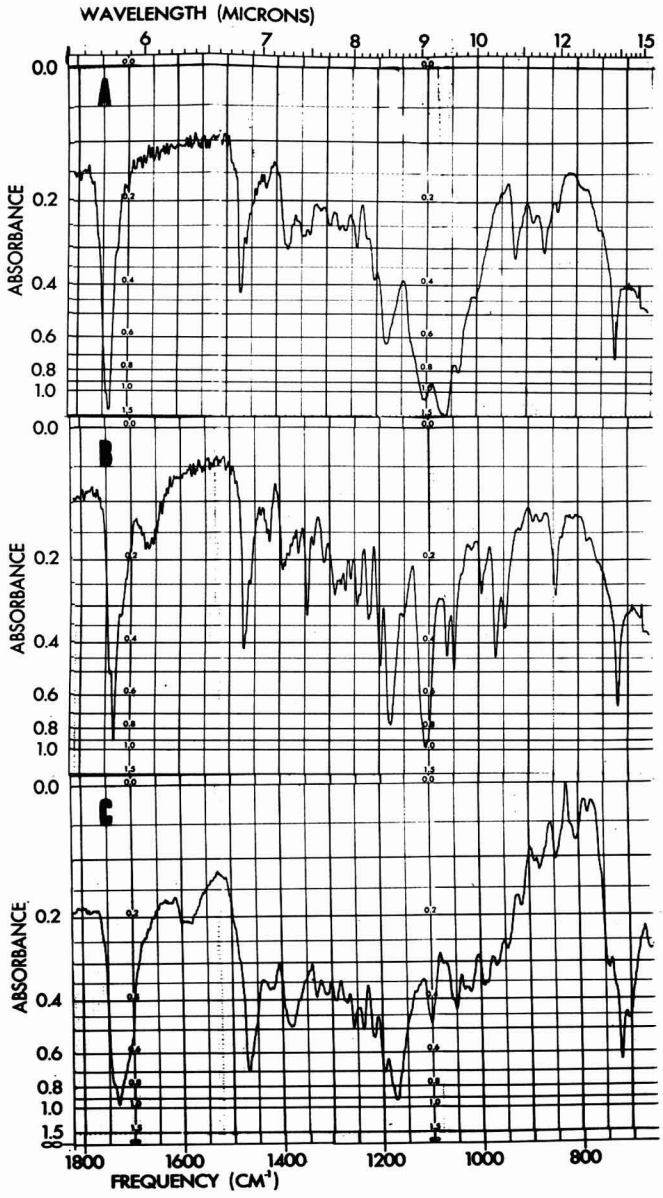


FIG. 5. Spectra of wax coatings of three commercial products.

plate and recording the spectrum. The resulting spectrum is that of the vehicle since that is the major component in such preparations. Minor components are not observable due to the large area of the IRS plate covered by the vehicle relative to the minor components. Figure 7 shows the IRS spectra of a vehicles used in a cream preparation and an ointment.

An extension and possible use of this technique in the analysis of toothpastes has been reported (*1*). Figure 8 is the IRS spectrum of a toothpaste. The spectrum was obtained by smearing the toothpaste on an

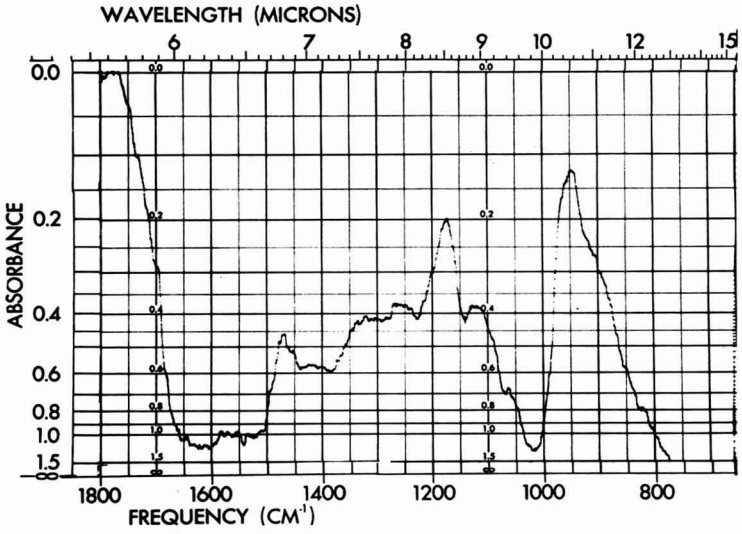


FIG. 6. Spectra of liver tissue.

IRS plate and then recording the spectrum. The strong absorptions at 1410 cm^{-1} and the doublet at 860 and 885 cm^{-1} indicate presence of carbonate.

From these examples it can be seen that IRS would be a useful tool in the analysis of creams, ointments, and other jell-like preparations.

Multidose Vial Lids

Infrared spectroscopy offers a means of identifying and maintaining a control on the quality of multidose vial lids. Because of the opaque nature of these materials, however, it is not possible to obtain an infrared transmission spectrum of the lids in their natural state. The availability of the IRS technique does allow one to obtain a characteristic spectrum of a

multidose vial lid. The spectrum can be recorded conveniently and easily with no sample preparation whatsoever. The lids are placed in a sample holder and pressed against an IRS plate. The spectrum thus obtained gives a true picture of the lid as it will be when in actual use. It is possible to identify and differentiate between various grades and types of rubber and plastic lids by use of these spectra. It is also possible to

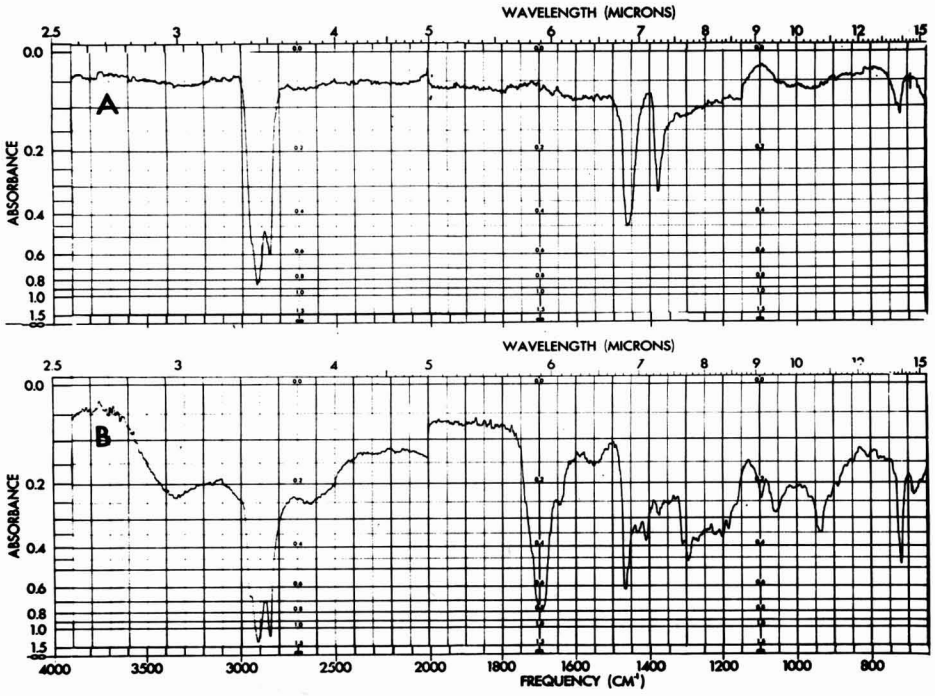


FIG. 7. Spectra of vehicles: A. Ointment; B. Cream.

determine whether or not the lids have been coated, *e.g.* with silicone, as is required in special cases. Figure 9 is the spectrum of a rubber lid from a multidose vial preparation. It is apparent that the spectrum would be sufficient to establish identity of the lid material and serve as a monitor for subsequent batches of lids.

Impurities and Sediments

Impurities and sediments which can be separated from solution by precipitation, filtration, and other separation techniques offer no problem in

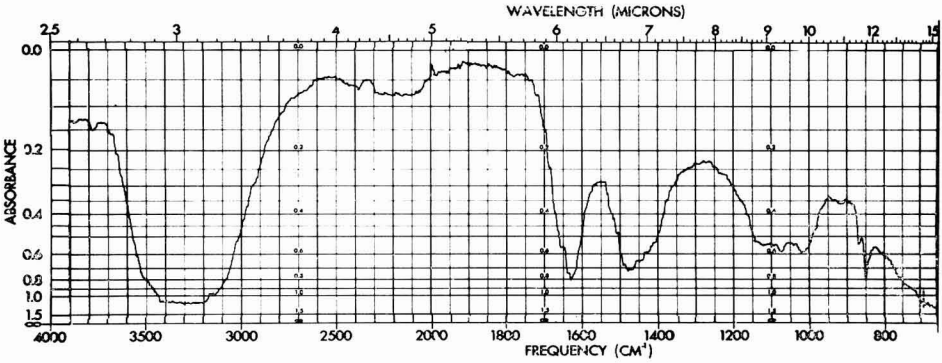


FIG. 8. Spectrum of toothpaste.

obtaining IRS infrared spectra. If the particles are large enough to be separated by a simple extraction procedure, they can be identified by the same procedure outlined for micro-samples. Colloidal suspensions or very fine particulate matter in solution can be separated by millipore filters, and the infrared spectra is obtained by two procedures. In the first, the particles are filtered using the millipore filter and the particles or sediment obtained on the filter are transferred to an IRS plate after suitable washing procedures. The IRS infrared spectrum is then obtained. The second method is used when it is not possible to wash the precipitate from the filter, as when the quantity of material is so limited that further sample handling might result in loss of a sample which is in very short supply. In such cases, the millipore filter containing the sample (or precipitate) is placed on one IRS plate and a blank millipore filter is placed

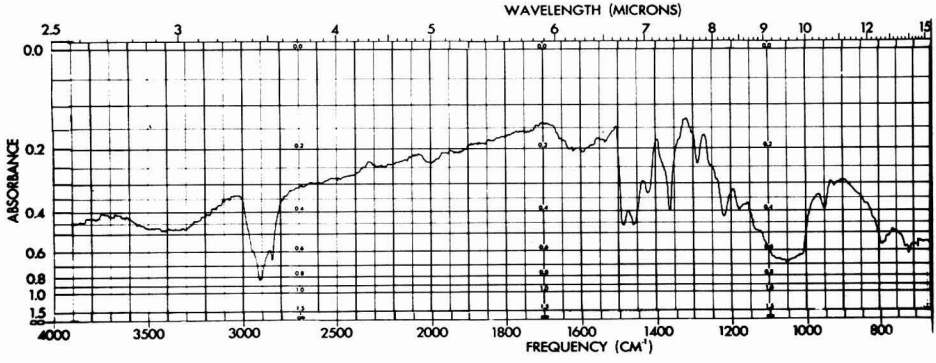


FIG. 9. Spectrum of a rubber lid from a multidose vial.

against the IRS plate in the reference beam of the IR spectrometer. In this way the contribution of the filter is effectively removed and the resulting IRS infrared spectrum is that of the material in question. In practice we have found that it is not always possible to remove completely the contribution of the filter. The filter in the sample beam has undergone treatment in the filtration and washing processes and it is difficult to duplicate the effects of this treatment with a reference filter. The resulting infrared spectrum usually shows evidence of this. This procedure can be used in difficult cases, but washing the sample onto the IRS plate has been the preferred method in our experience. Figure 10 illustrates use

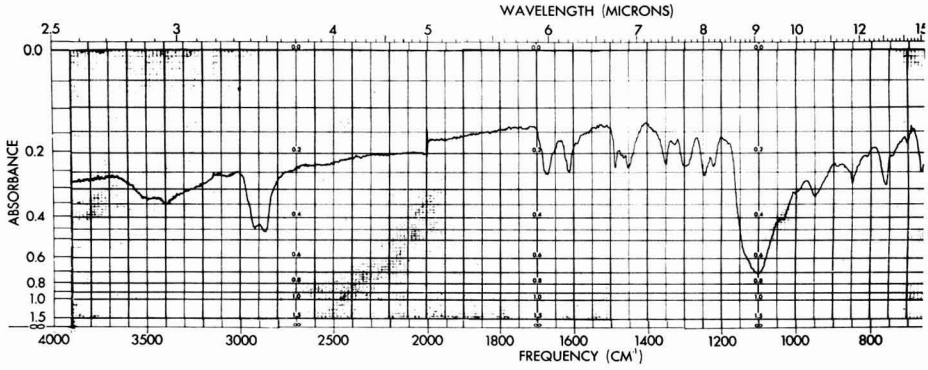


FIG. 10. Spectrum of a haze-like precipitate.

of IRS to identify a haze-like precipitate from solution. The IRS spectrum identified the precipitate as a sulfate.

SUMMARY

Internal reflectance spectroscopy has been shown to be a useful tool in infrared analysis of pharmaceuticals. Sufficient illustrations of its utility have been presented to show its value in current problems as well as its potential in future pharmaceutical analysis.

ACKNOWLEDGMENT

The authors are grateful to Mrs. Eleanor Cherry for technical assistance in obtaining the spectra here.

REFERENCES

1. PUTTMAN, N. A., LEE, L., AND BAXTER, B. H., Application of attenuated total reflectance IR spectroscopy to toilet articles and household products. I. Qualitative analysis. *J. Soc. Cosmetic Chemists* **16**, 607 (1965).

MICROCHEMICAL JOURNAL **12**, 568-583 (1967)

A Thirty Minute Direct Oxygen Analysis

HUGH J. BARTON AND CLYDE W. NASH

Rohm and Haas Company
Bristol, Pennsylvania 19007

Received July 12, 1967

INTRODUCTION

For many years, the oxygen content of an organic compound was calculated as the difference between 100% and the sum of the other elements present. This system led to numerous errors since an element other than oxygen might be present. Inaccuracies also occur in the calculation of oxygen due to the accumulation of possible errors involved in the other elemental determinations. Therefore, a direct method for the analysis of organic oxygen is preferred in most instances.

The gravimetric method as proposed by Steyermark (*1*) was introduced into this laboratory in 1961. Several modifications were made in the early stages of operation.

A carrier gas purification system was added consisting of a caustic pyrogallol gas washing bottle, followed by a concentrated sulfuric acid gas washing bottle. No internal blank was present after this modification.

A second absorption tube was used on the combustion train for the control of any external blank, which might be caused by varying temperature and humidity conditions between laboratory and balance room. This has proved beneficial to the operation of the method.

The time of the reverse sweep prior to combustion was reduced to 5 minutes.

With the carrier gas rate still maintained at 10 ml/minute, it was found that satisfactory results could be obtained in all cases with a displacement of 300 ml of water from the Mariotte bottle. This reduced the sweep time to 30 minutes.

These early modifications made possible a complete oxygen determination in 50 minutes.

Through the use of additional flow control valves and a combustion

and sweep rate of 20 ml/minute, the time for the displacement of 300 ml of water from the Mariotte bottle was further reduced to 15 minutes, thus reducing the time for a complete oxygen analysis to only 25 minutes. This procedure is applicable to all organic compounds with the exception of those containing phosphorous and/or fluorine. Some 700 samples were analyzed.

The standard deviation of this method was found to be $\pm$ 0.17%.

MATERIALS AND METHODS

The method used for the determination of oxygen in organic compounds is based on the combustion of the sample in an inert atmosphere of prepurified nitrogen. The pyrolysis products are swept through a section of specially prepared carbon black at 1100-1170°C. The temperature must be held to $\pm$ 5°C. All oxygen present in the compound is converted to carbon monoxide. After passage through various absorbers for the removal of acid by-products, the CO is converted to CO_2 by sweeping through copper oxide at 670°C. The CO_2 is then dried, absorbed on Mikohbite, and determined gravimetrically.

Reagents

Prepurified nitrogen, used as the inert atmosphere in the entire system.

Caustic pyrogallol, used as a scrubber for the prepurified nitrogen. Dissolve 50 gms of KOH pellets in 100 ml of distilled water. After cooling to room temperature, add 5 g of pyrogallol. Stir until dissolved.

Sulfuric acid, conc., used as a scrubber for the prepurified nitrogen and in the bubble counter.

Mikohbite® (NaOH on exploded mica) 10-30 mesh (G. Frederick Smith Chemical Company), used for the absorption of CO_2 and acidic products formed from the combustion of samples containing sulfur and halogens.

Dehydrite® (magnesium perchlorate) (A. H. Thomas Company) for absorption of water.

Hydrogen (prepurified) lecture bottle (Matheson).

Carbon black (pelletized) (A. H. Thomas Company). That part which passes through a 30 mesh sieve but is retained by an 80 mesh sieve is used in the combustion tube.

Quartz wool, used to hold the carbon black in place in the combustion tube.

Quartz chips, used in the combustion tube as a filler.

Reduced copper gauze (30 x 30 mesh, 0.014 inch diam. wire) (Newark Wire Cloth Co.), used for removing traces of oxygen from the prepurified nitrogen, and for removing sulfur compounds formed during the combustion in the high temperature furnace.

Reagent copper oxide wire (Baker analyzed) 1-3 mm in length, used to convert the purified CO to CO_2 in the conversion furnace at 670°C.

Kronig cement (for absorption tubes) (A. H. Thomas Company).

Hydrofluoric acid, conc.

Apparatus

Mettler Micro Balance M 5

Matheson, 2 stage regulator, Mod. No. 8, CGA connection No. 580

Brinkmann — Hereaus micro combustion apparatus No. 60 00 00 with micro long burner No. 60 00 05 capable of maintaining 1200°C and short burner No. 60 00 20 capable of maintaining 900°C (Brinkmann Instruments, Inc.).

Nupro meter valve (Penn Valve and Fitting Company).

Nupro cross pattern meter valve (Penn Valve and Fitting Company).

Whitey toggle valve No. 1 GM4 (Penn Valve and Fitting Company).

Furnace capable of maintaining 900°C No. 5675-B (A. H. Thomas Company).

Standard glassware and supports for purification and combustion sections and purification furnace. (A. H. Thomas Company under No. 6444-C).

Conversion furnace, approximately 20 cm long, capable of maintaining 670°C.

Vycor combustion tube No. 3883-A2, Dumas Tube (A. H. Thomas Company).

Quartz combustion tube No. 3883-A8, Dumas Tube (A. H. Thomas Company).

Pregl micro absorption tubes, 100 mm length, No. 3854P (A. H. Thomas Company).

Absorption tube rack, No. 3854W (A. H. Thomas Company).

Drying tube (guard tube), No. 4744-A2 (A. H. Thomas Company).

Powerstats (Superior Electric Company).

Plastic tubing (PVC tubing) $\frac{1}{4}$ inch $\times$ $\frac{1}{16}$ inch—$\frac{3}{8}$ inch $\times$ $\frac{1}{16}$ inch—$\frac{3}{16}$ inch $\times$ $\frac{1}{16}$ inch.

Glass adapters (for joining glass tubing of different diameters).

Mariotte bottle with support, No. 2270-K (A. H. Thomas Company).

Silicone rubber tubing, No. 8849M (A. H. Thomas Company) (for connecting absorption tubes).

Support jack, No. 9359-P, large size (A. H. Thomas Company).

Graduated cylinder - 10 ml; graduated cylinder - 500 ml.

Stopwatch.

Quartz or Vycor tubing, 40 cm long $\times$ 0.5 cm o.d.

Gas washing bottles, two.

Platinum holder, a piece of sheet platinum cut to about 13 $\times$ 40 mm and bent in the middle longitudinally; used for holding glass capillaries containing the samples in the combustion tube.

Platinum boats, No. 8308-M small size (A. H. Thomas Company).

National air-gas torch (Mod. 3-A).

Forceps.

Glass Tee.

Chamois, flannel; petri dish and cover.

Pyrometer (chromel alumel couple) Mod. No. 301 (Weston Elec. Inst. Corp.).

Preparation of Apparatus

A. *Packing of purification drying tube* (*T-1*, see Fig. 1). A clean, dry, drying tube is used. A small glass wool plug is placed in the bottom of the tube. A small section of Dehydrite is placed in the tube; on top of this Dehydrite is placed a small plug of glass wool. Mikohbite is then added; and another plug of glass wool is added. A rubber stopper with a hole in it is used to close the tube. Through this stopper is inserted a short 5 mm o.d. glass tube bent at a 90° angle.

B. *Preparation and packing of nitrogen purification tube* (*T-2*). A clean, dry purification tube is needed. A piece of copper gauze is cut 9 cm wide and just long enough to fill the inside diameter of the tube when rolled tightly. This copper gauze is reduced by heating to 500°C in a current of hydrogen. After cooling, it is placed in the tube; the tube is inserted into the cold purification furnace Fig. 1; and the furnace is turned on.

C. *Preparation and filling of the bubble counter and U-tube.* Mikohbite is placed in the base of the U-tube and extends slightly up the side arms of the U. A plug of glass wool is added to each of the arms and

pushed down on top of the Mikohbite. Dehydrite is then added to each of the arms, keeping the top level below the inlet and outlet arms; and a plug of glass wool is placed on top of the Dehydrite.

Two ml of conc. sulfuric acid is added to the bubble counter section of a clean dry bubble counter U-Tube by means of a hypodermic syringe. Once the sulfuric acid has been added to the bubble counter, care must

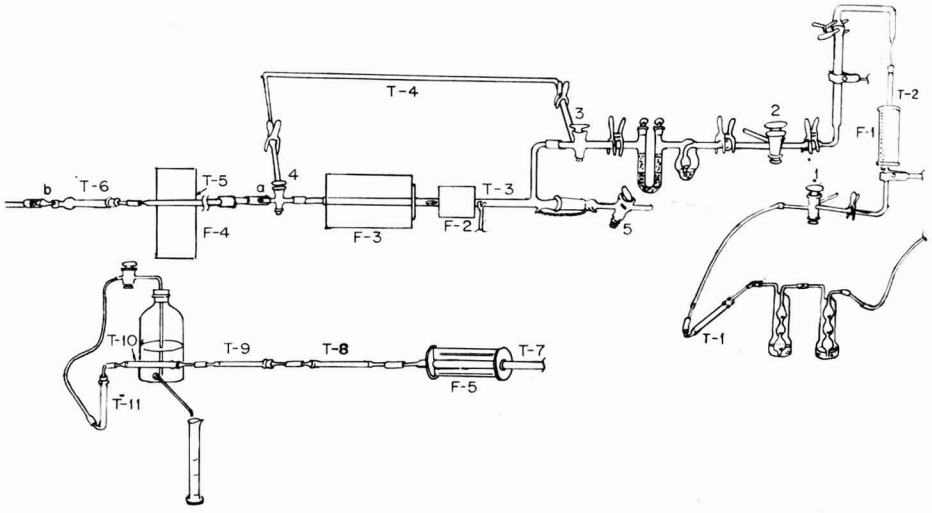


FIG. 1. 1. Stopcock; 2. stopcock; 3. stopcock; 4. stopcock; 5. stopcock; F-1. purification furnace (500°C); F-2. movable sample furnace; F-3. stationary combustion furnace (1120°C); F-4. sulfur scrubber tube furnace (900°C); F-5. $CO \rightarrow CO_2$ conversion furnace (670°C); T-1. purification drying tube; T-2. reduced copper purification tube; T-3. combustion tube; T-4. by-pass tube; T-5. sulfur scrubber tube (reduced copper); T-6. scrubber Tube (Mikohbite); T-7. $CO \rightarrow CO_2$ conversion tube; T-8. drying tube; T-9. CO_2 absorption tube; T-10. blank tube; T-11. guard tube; a. b. glass reducer fittings.

be taken in the manipulation of the unit to prevent the acid from entering the U-Tube section.

The glass caps are warmed over a microburner, rolled on a stick of Kronig cement, and placed in the U-Tube. A good seal is necessary.

D. *Preparation and packing of combustion tube (T-3).* A new quartz direct oxygen combustion tube is washed with hydrofluoric acid, rinsed well with distilled water and methanol, and dried. Quartz chips are also washed with conc. hydrofluoric acid, distilled water, and methanol, and dried. The combustion tube is marked so that the carbon packing will be

positioned 2 cm inside the ends of the combustion furnace. Enough quartz chips are added to accomplish this. Quartz wool, previously heated in a flame, is inserted to form a small plug. Then pelletized carbon is added. The carbon section should be about 12 cm long in the 16 cm furnace. After light tamping, a small plug of quartz wool is added to hold the carbon in place.

E. *Preparation and packing of the reduced copper scrubbing tube* (*T-5*). A clean Dumas quartz combustion tube is used (overall length of 40 cm). A section of quartz wool, approximately 2-3 cm long, is placed in the end of the tube with the reduced tip, to act as a spacer. Then two sections of rolled copper gauze, each 9 cm long, are added. This gauze was reduced in the same manner as the copper gauze (in Section B). The tube is then inserted into the (900°C) furnace, when the furnace is cold.

F. *CO scrubber tube* (*T-6*). A large, clean, dry U-Tube or $CaCl_2$ drying tube is used. If a drying tube is used, a plug of glass wool is placed in the bottom. It is then filled with Mikohbite only, and covered with a plug of glass wool. The drying tube is sealed with a rubber stopper through which is inserted a length of 7 mm o.d. glass tubing. The outside end of this glass tubing is pulled out to a smaller tip about the size of the Dumas tube to which it is attached.

If a U-Tube is used, it is filled with Mikohbite only which is held in place by glass wool plugs. The glass stoppers are warmed, rolled in Kronig cement, and placed in the arms of the U.Tube (as in Section C).

G. *Preparation and packing of* $CO \rightarrow CO_2$ *conversion tube* (*T-7*). A clean, dry Vycor Dumas combustion tube is used (overall length of 35 cm). It is packed with a quartz wool plug first. Then ground copper oxide wire is added. This wire has been screened, and only that retained on a No. 40 mesh sieve is used. A quartz wool plug is placed on the copper oxide wire. The packing may be 2 cm more than the length of the furnace. The tube should be inserted into the furnace when the furnace is cold.

H. *Preparation and packing of absorption tubes.* Absorption tubes should be thoroughly cleaned and dried before packing. Benzene is used to remove Kronig Cement. Water, a stiff micro brush, and methanol, are used to clean the tubes. Occasionally, chomic acid cleaning solution is necessary.

1. *Water tube* (*T-8*). A small amount of glass wool is placed in a clean, dry absorption tube at the capillary end. The tube is filled with medium size particles of Dehydrite, and the outside of the tube is tapped gently

to insure good packing. Enough space is left at the cap end of the tube to insert a wad of glass wool to completely cover the Dehydrite. The cap of the absorption tube is heated gently in a micro flame. While hot, the cap is rolled over a stick of Kronig cement, inserted in the tube, and turned slowly to insure a good seal. More heat may be required to remove all lines from the seal. The cap is held firmly in the tube until the cement sets. Excess cement around the seal is removed with benzene-soaked cotton wrapped around a knurled wire.

2. *CO_2 tube* (*T-9, T-10*). A little glass wool is inserted into a clean, dry absorption tube at the capillary end. A section of the tube about 2 cm long is filled with Dehydrite. Enough glass wool is placed over the Dehydrite to prevent the Mikohbite from being mixed with the Dehydrite. Mikohbite is added, and the sides of the tube are tapped gently to insure good packing. Enough space is left at the cap end of the tube to insert a layer of glass wool. The cap is inserted into the tube as described under *Water Tube*. A second absorption tube (called the blank tube) is filled in the same manner with Dehydrite and Mikohbite.

I. *Guard tube* (*T-11*). A plug of glass wool is inserted into a clean, dry guard tube. A small section of Dehydrite is added; another small plug of glass wool is inserted. A section of Mikohbite is added; and after tapping down, a glass wool plug is inserted. The tube is fitted with a rubber stopper with a hole in it. A short piece of 3 mm o.d. glass tubing bent at a 90° angle is passed through the rubber stopper.

Apparatus Assembly

All glassware should be washed with chromic acid cleaning solution, rinsed well with both distilled water and methanol, and then dried.

A. *Furnace temperature adjustments*. While the glassware is drying, the furnaces can be set up and the temperature checked with a pyrometer. The nitrogen purification furnace (F-1) should operate at approximately 500°C. The temperature is factory set, but should be checked. The high temperature combustion furnace should operate at approximately 1120°C ± 5°C (F-3). (The furnace must operate between 1100°C-1170°C.) The reduced copper furnace should operate at approximately 900°C (F-4). The conversion furnace (F-5) should operate at approximately 670°C. All furnaces are then allowed to cool.

B. *Gas flow regulation* (See Fig. 2). A Matheson 2-stage regulator, Mod. No. 8, is attached to a cylinder of prepurified nitrogen. A Matheson

shutoff valve is next installed. A brass check valve is then attached to the shutoff valve. A Nupro meter valve is then installed, and to this a Nupro cross pattern meter valve (4MX) is attached. A Whitey toggle valve is connected to the side exit port of the cross pattern valve. The cross pattern valve does not contol gas flow through the side ports. Only the bottom port is metered by the valve. Plastic tubing is run from the

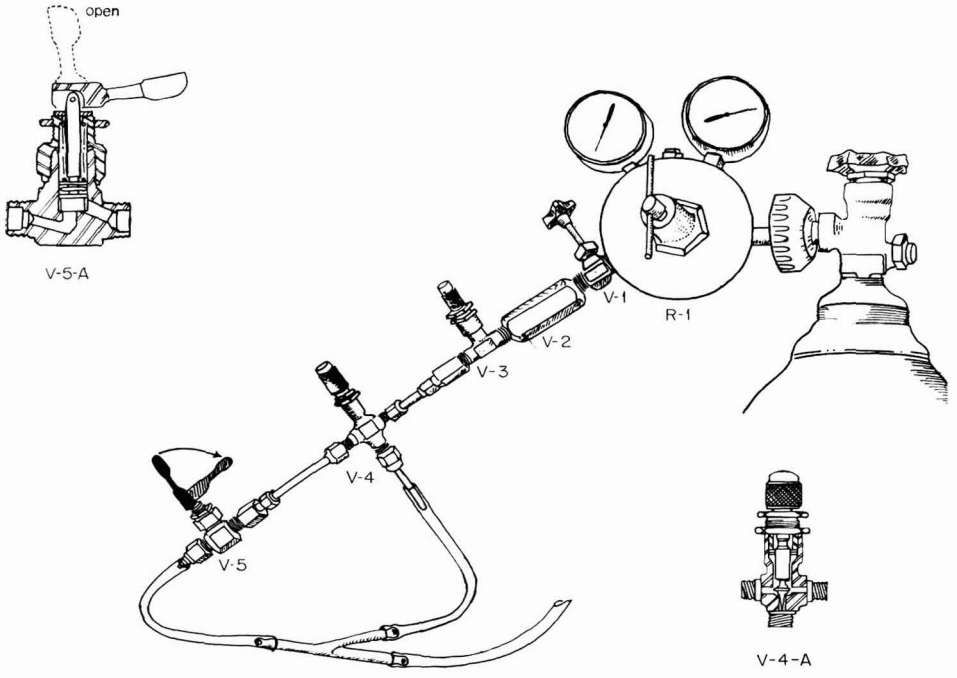


FIG. 2. R-1 Matheson, 2-stage regulator, Mod. No. 8; V-1 Matheson shutoff valve; -2 Matheson check valve, brass; V-3 Nupro meter valve; V-4 Nupro cross pattern meter valve (4 MX); V-5 Whitey toggle valve; V-4-A cross pattern meter valve (4 MX), Cross Section; V-5-A Whitey toggle valve, Cross Section.

bottom port of the cross pattern valve and from the exit side of the Whitey toggle valve. These two pieces of tubing are connected to a glass tee. The third leg of the tee is connected to the gas purification system.

With all the valves in the closed position, the cylinder valve is opened and the threaded stem regulator is adjusted to 10 lb pressure. The small shutoff valve is turned on. The Nupro meter valve is opened and adjusted to a flow rate of 20 ml/minute. The Nupro cross pattern valve is opened

and adjusted to 10 ml/minute at the bottom port. When the Whitey toggle valve is in the closed position, the rate of gas flow is 10 ml/minute. When the toggle valve is opened, the rate increases to 20 ml/minute. This rate is maintained by the Nupro meter valve.

C. *Purification section.* The glassware is assembled. Plastic tubing is used to make connections where necessary. Ball and socket joints are sealed with Lubriseal stopcock grease.

Starting at the glass tee, the gas first passes through two gas scrubbing bottles. The first contains caustic pyrogallol, the second bottle contains conc. sulfuric acid.

Approximately 15 ml of caustic pyrogallol solution is transferred by dropper to the scrubber bottle. This should be done as quickly as possible, and then the scrubber attached to the plastic tubing from the glass tee. Concentrated sulfuric acid is placed in the second scrubber, which is then attached to the first scrubber. The outlet of the second scrubber is attached to the purification drying tube packed with Mikohbite and Dehydrite. The drying tube in turn it attached to the 3 way stopcock No. 1. All connections from nitrogen cylinder to No. 1 stopcock are made with plastic tubing.

The purification furnace (F-1) tube, packed as described is inserted into the cold furnace. The standard glass connections are made through 3-way stopcock No. 2 to a bubble counter U-Tube packed as described.

D. *Furnace section.* The previously packed quartz direct oxygen combustion tube is now inserted into the cold combustion furnace (F-3). It is carefully positioned so that the attachment of the by-pass tube can be easily made. A 3-way stopcock No. 4 is attached to the thick-walled capillary tip of the combustion tube with plastic tubing. Stopcock No. 3 is now fastened to the sidearm of the combustion tube with plastic tubing. This should be a glass-to-glass joint. Stopcock No. 3 is attached to the bubble counter U-Tube with a ball and socket joint using Lubriseal grease.

A glass adapter (a) is attached to stopcock No. 4 with plastic tubing. The other end of the adapter is attached to the previously packed Dumas combustion tube (reduced copper scrubbing tube) with plastic tubing. The tube is inserted in the cold high temperature furnace (F-4). The by-pass tube is connected; the ball and socket joints are greased with Lubriseal.

The CO scrubbing tube is attached to the capillary tip of the reduced copper scrubbing tube with narrow bore silicone rubber tubing.

The $CO \rightarrow CO_2$ conversion tube is placed in the cold furnace and at-

tached to the CO scrubber by means of the glass adapter (b). A Pregl absorption tube, previously packed with Dehydrite, is now attached to the conversion tube by means of silicone tubing. A guard tube previously packed is now attached to the water absorption tube. The flow rate of the nitrogen from the cylinder, which has been turned on, is now checked with a flow meter.

A flow rate of 8 to 10 ml/minute is established with the Whitey toggle valve closed. When the valve is opened, the rate should increase to 16 to 20 ml/minute. An overnight sweeping period is desirable before the analysis of standard samples is attempted.

PROCEDURE

The CO_2 absorption tube and the blank absorption tube are connected to each other with a 1-inch length of silicone rubber tubing. The other end of the CO_2 absorption tube is connected to the tip of the water absorption tube with silicone tubing and the guard tube is attached to the tip of the blank absorption tube and the Mariotte bottle. The Mariotte bottle is filled with water and the sidearm lowered halfway down into a graduate cylinder. The absorption tubes are swept for about 10 minutes before the first analysis of the day. The short movable furnace is turned on at this time—making sure that the furnace is not on the combustion tube and is properly shielded from the combustion tube with asbestos. Then, the two tubes are removed from the train with a chamois, placed on a tube rack, carried to the balance room, and weighed prior to analysis (see Procedure, Section A).

A solid sample is weighed out in a platinum boat. The sample sizes can vary from 5 to 10 mg. A glass capillary is used for liquid samples whenever possible. When the sample has been weighed, it is brought to the apparatus. If the sample is in a capillary, it is first centrifuged; the end is broken off, and both pieces are placed in a platinum holder. The Whitey toggle valve is placed in the closed position (rate of 10 ml/minute).

Stopcock No. 3 is turned to direct the nitrogen flow through the by-pass tube. Stopcock No. 4 is turned to direct the nitrogen flow from the by-pass tube through the combustion tube, in reverse direction of the normal gas flow. Stopcock No. 5 is opened to allow the system to flush itself out to the atmosphere. Only then can the combustion tube cap be removed. The boat or platinum holder is placed in the combustion tube and positioned approximately 7-9 cm from the main furnace by means of a glass rod with

platinum wire. The cap is then replaced and the system allowed to purge itself for at least five minutes with stopcock No. 5 still open to the atmosphere.

The absorption tubes are weighed during this purge time. The first tube weighed is the CO_2 pickup tube. The blank tube is then weighed. Small external changes on the blank tube have an effect on the results which cannot be ignored. Stopcock No. 5 is closed, then Stopcock No. 3 is positioned to close off the by-pass tube line and allow the carrier gas to pass through the combustion tube in the normal direction. Stopcock No. 4 is positioned to allow the carrier gas to leave the combustion tube and continue on through the train.

The absorption tubes are immediately attached. The asbestos shields are then removed from the movable furnace, and it is placed about 4 cm behind the sample. The sidearm of the Mariotte bottle is lowered.

The Whitey toggle valve is opened, and the rate is increased to 20 ml/minute. The furnace travel speed is permanently set at 15 mm/minute.

The furnace should reach the end of the tract in about 7 minutes. An alarm will sound and the furnace drive is shut off. The movable furnace (F-2) is placed as tightly as possible against the main furnace (F-3). The movable furnace is allowed to sit in this position for an additional 5 minutes. It is then returned to its starting position, opened and pushed back off the tube and reshielded from the tube with asbestos. An air-gas torch is used to heat the combustion tube in the area where the two furnaces meet. The tube should be heated to redness for approximately 2 minutes. When 300 ml of water have been displaced from the Mariotte bottle (approximately 15-18 minutes), the tubes are disconnected.

A. *Absorption Tube Weighing Procedure.* After the tubes are disconnected from the train, they are placed on the rack and carried to the balance room. The silicone rubber connections are removed from the tubes and each tube is wiped with a damp chamois. They are placed back on the rack for approximately 5 minutes. During this time, the sample is weighed and placed in the combustion tube. The first tube weighed is the CO_2 pickup tube. The second tube weighed is the blank tube. Any variations on the blank tube are due to external conditions. Because of the small tube weight differences obtained, these small variations are important. If the blank tube picks up weight, this weight gain is subtracted from the CO_2 pickup tube. If the blank tube loses weight, this weight loss is added to the CO_2 pickup tube. These weight differences are usually less than 30 μg, but have been noted as high as 100 μg.

After the tubes are weighed, the silicone rubber connections are replaced and the tubes are returned to the combustion train.

Calculations:

$$\frac{(\text{Wt. of } CO_2 \pm \text{Wt. of blank tube}) \times 0.3636 \times 100}{\text{Wt. of sample}} = \% \text{ Oxygen}$$

Maintenance Procedures

A. *Reduction of copper in nitrogen purification tube.* After a period of time it is necessary to reduce the copper gauze in the purification section. Red safety flags are positioned in the area. No open flames are allowed in the immediate area. A small beaker of water is placed on the bench under Stopcock No. 2. A piece of rubber tubing is attached to the atmospheric outlet of Stopcock No. 2; the end of the hose is placed in the water; and Stopcock No. 2 is opened to the atmospheric outlet. Stopcock No. 1 is positioned to open the inlet to the atmosphere. The valve on the lecture bottle of hydrogen is cracked open and the rate is checked by running a hose into the beaker of water.The hydrogen bottle is attached by a small piece of rubber hose to Stopcock No. 1. The copper gauze will be reduced almost instantly, with the formation of a large amount of water. The valve is closed and the bottle is disconnected. Stopcock No. 1 is turned so that the cylinder of nitrogen is back in the circuit. The system is flushed out to the atmosphere for about 10 minutes through Stopcock No. 2. Then Stopcock No. 2 is returned to its original position.

B. *Removal of carbon deposit from quartz direct oxygen combustion tube.* The deposit of carbon, which gradually accumulates on the inside wall of the combustion tube just before the main furnace, must be periodically removed. Stopcock valves 3, 4, and 5 are placed in the position for the reverse flow of nitrogen through the combustion tube. A piece of quartz or Vycor tubing (40 cm long and 0.5 cm o.d.) is connected to an oxygen source, and the oxygen flow is adjusted by a flow meter to 8 ml/minute or less. The springs are disconnected and the ground glass cap containing Stopcock No. 5 is removed from the end of the combustion tube. Insert the quartz or Vycor tube into the combustion tube to where the carbon deposit begins. Apply a gas-air torch flame to the outside of the combustion tube and gradually burn off the carbon deposit, all the way to the main furnace. After this has been done, replace the cap and continue to back flush with nitrogen for 15 minutes. Then, run an unweighed sample to reestablish operating conditions in the system.

C. *Replacing of copper in the reduced copper scrubbing tube in the 900° Furnace (F-4).* This reduced copper section occasionally needs replacing. New Copper gauze is cut to proper size and rolled. (It is reduced as under Preparation of apparatus, Section B). The furnace is turned off, and after cooling, the tube is removed and the copper sections replaced. The tube is reinserted and after the air has been replaced with nitrogen, the furnace is turned back on. The furnace should be allowed to reach operating temperature before any analysis is attempted.

D. *Repacking of CO scrubber tube.* It may become necessary to repack the CO scrubber tube; especially when results are erroneously high. This is done by disconnecting the tube, repacking with fresh Mikohbite, and replacing it in the train. The tube should be swept for at least $\frac{1}{2}$ hour before operation is resumed.

E. *Repacking of $CO \rightarrow CO_2$ conversion tube with CuO.* The $CO \rightarrow CO_2$ conversion unit occasionally needs repacking. When the copper oxide wire has been almost completely reduced (determined visually), a fresh packing is needed. Extremely low results indicate exhaustion of the packing. The tube should be changed if a large amount of discoloration is present.

New copper oxide wire is ground; and all wire retained on a No. 40 mesh sieve is used. The tube is packed and inserted in a cold furnace, flushed with nitrogen for $\frac{1}{2}$ hour, and then the furnace is turned on. Operations can be resumed when the furnace reaches operating temperature.

RESULTS

Many analyses have been run on a National Bureau of Standards sample of benzoic acid. This is the usual standard used for the direct oxygen method. The standard deviation established for the rate of 20 ml/minute compares very favorably with this standard deviation found for the rate of 10 ml/minute (see Table 1).

Standard samples containing sulfur, bromine, and iodine were also analyzed (Table 2). Research samples containing chlorine were analyzed (Table 3). These elements sometimes cause high results by the exhaustion of the scrubber in the combustion train. It can be seen by the results in Table 2 that the scrubber involved was able to remove these interfering elements, even at this high rate of gas flow. Standard deviations were not determined for these materials. These standards are only used when running samples containing sulfur or halogens to determine the condition of the scrubbers.

TABLE 1
STANDARD DEVIATION

Run	Theory O_2	O_2 found 10 ml/min	O_2 found 20 ml/min	Class of compound
1	26.20	26.26	26.12	NBS Benzoic acid[a]
2		26.52	26.14	
3		26.18	25.98	
4		25.81	26.33	
5		26.07	26.04	
6		26.63	26.01	
		± 0.27	± 0.17	Standard deviation

[a] National Bureau of Standards.

TABLE 2
STANDARD SAMPLES CONTAINING HALOGENS AND SULFUR

Run	Theory O_2	O_2 Found 20 ml/min	Class of compound	Comments
1	15.92	15.84	B.D.H. p-Bromo Benzoic Acid[a]	Sample contains 40% bromine
2	12.90	12.79	N.B.S. Iodobenzoic Acid[b]	Sample contains 51% iodine
3	28.03	28.23	B.D.H. Sulphonal	Sample contains 28% sulfur
4	49.43	49.38	B.D.H. Sulphamic Acid	Sample contains 33% sulfur

[a] British Drug Houses.
[b] National Bureau of Standards.

TABLE 3
SAMPLES CONTAINING HALOGENS

Run	Theory O_2	O_2 Found 10 ml/min	O_2 Found 20 ml/min	Total of all elements	Class of compounds
1	—	11.61	11.36	100.3	Co-polymers
2	26.3		26.03	99.80	
3	17.81		12.41	100.13	
4	7.64		7.56	100.28	
5	11.34		12.82	99.60	
6	9.11		9.35	100.11	
7	5.05		5.20	100.03	
8	5.68		7.33	100.1	
9	6.28		6.11	99.86	
10	10.23		10.25	100.03	

Numerous research samples were run and the results can be found in Tables 3, 4, and 5. All samples were analyzed with a flow rate of 10 ml/minute as well as 20 ml/minute, except when the oxygen analysis was theory and the total of all elements was within the acceptable limits of ± 0.50 of 100%.

This modified system has been in constant use for eight months. There

TABLE 4
SAMPLES CONTAINING POLYMERS

Run	Theory O_2	O_2 Found 10 ml/min	O_2 Found 20 ml/min		Total of all elements	Class of compounds
1	29.60		30.13		100.2	Co-polymers
2	29.60	29.90	30.61[a]	30.17	99.95	
3	20.0	21.94	21.85			
4	19.4	21.16	20.72			
5	29.60	31.37	30.43[a]	31.46	100.4	
6	4.69		4.90		100.13	
7	5.69		5.86		100.06	
8	29.62		29.43		99.72	
9	30.0		31.54		99.61	
10	30.0		31.26		99.54	
11	21.0		20.67		99.70	
12	20.0		21.15		100.04	
13	29.60		29.65		100.07	
14	29.60		29.75		99.98	
15	29.60		29.74		99.60	
16	29.60		31.90		100.05	
17	23.09		24.19		100.0	

[a] These analyses were included in this report for purposes of complete information, but are considered suspect.

TABLE 5
MISCELLANEOUS SAMPLES

Run	Theory O_2	O_2 Found 10 ml/min	O_2 Found 20 ml/min	Total of all elements	Class of compounds
1	23.85		23.44	100.4	Misc.
2	24.7	25.28	25.58	—	
3	25.2		16.80	99.6	
4	12.58		14.32	100.0	
5	12.58		14.14	99.5	
6	12.58		14.74	100.3	
7	12.58		14.78	100.3	

have been no problems encountered either in the maintenance of the equipment or in the reliability of the results.

The total cost of the equipment to accomplish this change was less than $20.

SUMMARY

The method for direct oxygen analysis as described by Steyermark, has been modified through the use of special flow control valves and a combustion and sweep rate of 20 ml/minute. A micro analyst can complete an oxygen analysis in 30 minutes with no sacrifice of precision and accuracy.

ACKNOWLEDGMENTS

The authors are thankful to Mr. Ronald Mason for his drawings of the valves and apparatus.

REFERENCES

1. STEYERMARK, A., "Quantitative Organic Microanalysis," 2nd ed., Academic Press, New York, 1961.

MICROCHEMICAL JOURNAL **12**, 584-592 (1967)

Microdetermination of Carbon, Hydrogen, and Nitrogen by Thermal Conductivity Measurement

GERALD KAINZ AND EUGEN WACHBERGER

Analytical Institute of the University of Vienna, Vienna, Austria

Received March 21, 1967

INTRODUCTION

We have recently reported (*3*) on a new method for the determination of carbon, hydrogen and nitrogen in organic samples. This method has now been improved with respect to procedure and apparatus. At the same time, factors causing error have been studied.

The micro or ultramicro sample is burned in a measured volume of oxygen. Excess oxygen and nitrogen oxides are retained on copper. Water and carbon dioxide are retained separately on specific storage units while nitrogen is determined directly. Carbon dioxide and water are then desorbed in sequence and detected in that order.

The special advantage of this new method is that gas chromatography is avoided, eliminating the need for the simultaneous injection of all combustion products necessary for good separation. The kinetics of oxidation do not influence the complete separation of combustion products, so that long and short combustion times are tolerated.

MATERIALS AND METHOD

1. Carrier gas (Fig. 1). The pressure of helium carrier gas is set at 0.4 atmosphere. Interfering impurities are eliminated by means of an absorption tube containing Anhydrone and a molecular sieve. The rate of flow of the helium is adjusted to 50 ml/minute, using a needle valve and rotameter. A Brooks (Model ELF 8943) flow-controller keeps the flow constant. A three-way stopcock (or a three-way magnetic valve) follows, allowing the helium to be turned off during the combustion. During this period, the helium is vented to the atmosphere. In order to keep the flow constant after venting, the resistance of the exit line has been made equal to that of the measuring line by means of a precision pinch clamp.

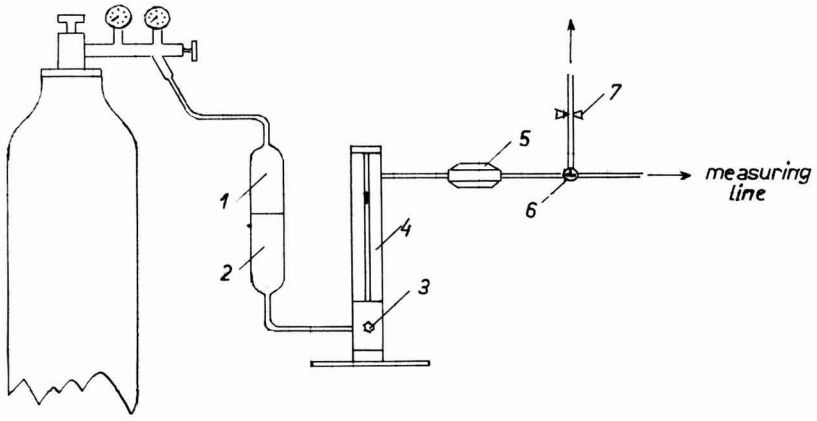


FIG. 1. Flow Diagram: 1. Molecular sieve; 2. Anhydrone; 3. Needle valve; 4. Rotameter; 5. Flow controller; 6. Three-way stopcock; 7. Precision pinch clamp.

2. Combustion train (Fig. 2). The combustion train consists of an injection device for a measured amount of oxygen, sample chamber, and combustion chamber. An oxide layer, a silver wool layer, and a layer of copper follow.

Injection of oxygen. The flow of oxygen from a tank is adjusted to about 10 ml/minute. The oxygen is fed into the injection pipette through a three-way stopcock. The pipette carries an exit tube at the 20-ml mark. When the plunger is retracted beyond this mark, the pipette is flushed with oxygen. To inject oxygen, the three-way stopcock is turned to the position leading to the combustion tube and the plunger is pushed in all the way. After injection, the three-way stopcock is turned back to the

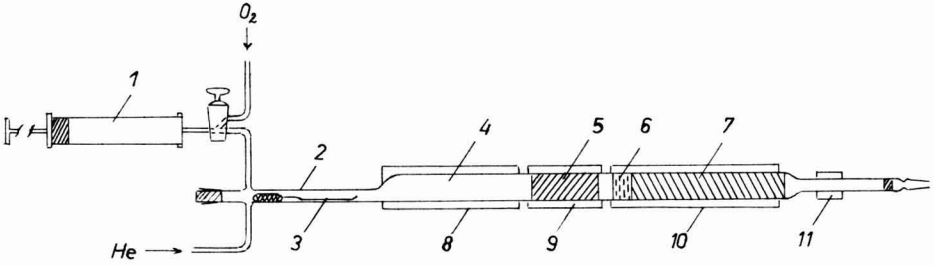


FIG. 2. Combustion Train: 1. Gas pipette for injecting oxygen; 2. Sample chamber; 3. Sample holder; 4. Combustion chamber; 5. Oxide layer; 6. Silver wool; 7. Copper layer; 8. 900-1000°C oven; 9. 700°C oven; 10. 500°C oven; 11. 350°C oven.

flush position and the plunger is retracted in preparation for the next analysis.

Sample introduction device. The sample is introduced into the heated chamber (900-1000°C) by a magnet. The combustion boat is positioned on a platinum "shovel." A glass coated iron core is cemented into the handle of the "shovel."

Combustion chamber. This consists of a quartz cylinder, 20 mm in diameter and 100 mm long. Since oxygen may reach the sample from all directions, combustion is complete.

3. Separation unit (Fig. 3). Separation of water, carbon dioxide, and nitrogen takes place in that order. Water is absorbed in a finely ground mixture of calcium chloride and quartz sand in a quartz tube fused

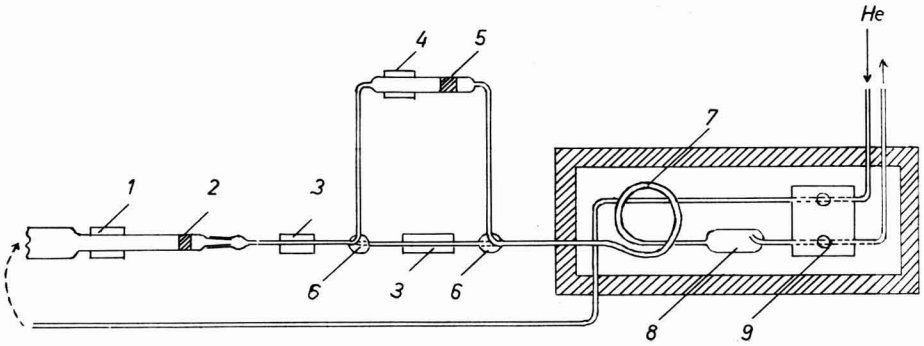


FIG. 3. Separation Unit: 1. Water desorption oven; 2. Water adsorbent; 3. Oven for heating connecting section; 4. Oven for desorption of carbon dioxide; 5. Carbon dioxide adsorber; 6. Magnetic valve; 7. Delay spiral; 8. Mixing tube; 9. Detector.

directly onto the combustion tube. Carbon dioxide is adsorbed in a second tube containing molecular sieve 5A. To desorb CO_2, a special desorption oven heated to 300°C may be moved over the molecular sieve. After the detector has registered the carbon dioxide signal, another special desorption oven at 300-380°C is moved over the water absorber, and the resultant water detected.

The desorbed water must avoid the molecular sieve; this is achieved by means of a bypass through two three-way magnetic stopcocks (Asco C3153). These are hooked up so that either the carbon dioxide adsorber or the detector is available to the flow of gas. A Beckman two-column switching valve (cat. No. 906610) may be used instead of magnetic stopcocks; the molecular sieve would be hooked to column 1, and the bypass to column 2.

4. Detector (Fig. 3). A metallic mixing tube precedes the detector (volume 10 ml). It is shaped like a spray trap (Kjeldahl connecting tube) hooked up in reverse. The function of this tube is to dilute the component being measured sufficiently to insure linear response. The delay coil slows up the presentation of the component to the detector until the bulk of the component is desorbed.

The detector consists of 2 cells, each containing a thermistor. These are connected to a bridge circuit. One thermistor senses the gas from the separation system and acts as a measuring transducer. The reference thermistor is connected between the flow-controller and the combustion tube.

5. Integrator. The signal from the detector is integrated by means of an electronic digital integrator. An automatic slope indicator signals the beginning and end of the peak integration.

6. Evaluation of the integrated signal. Calibration factors for carbon, hydrogen, and nitrogen are determined on the basis of standard substances. These are determined by dividing the integrated signal by the amount of carbon, hydrogen, and nitrogen contained in the particular sample. Figure 4 shows the calibration factors obtained.

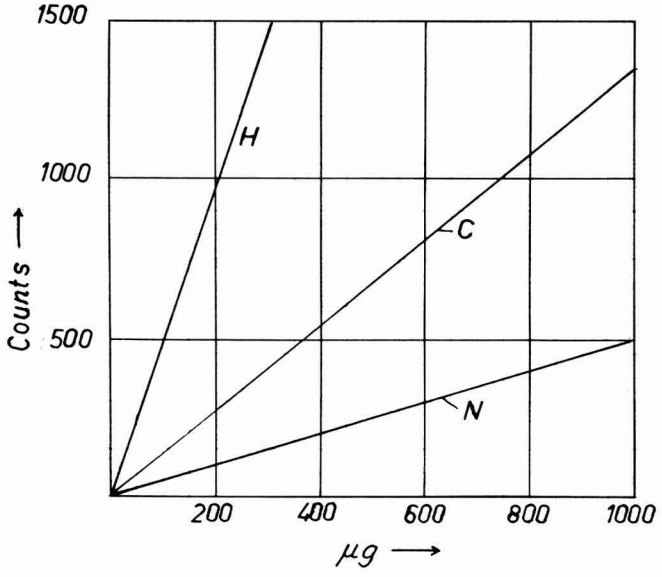


FIG. 4. Calibration Factors for carbon, hydrogen, and nitrogen.

DISCUSSION

1. Complete oxidation of the sample. The combustion train is a combination of the "empty tube" (with oxygen) system with the oxide packed tube concept. Experiments have shown that resistant carbon residues are avoided by insuring that sufficient oxygen reaches the sample. For this reason, a tube diameter of 20 mm was chosen. Smaller diameters lead to carbon residues on micro samples (Kainz and Scheidl (*2*)), that may or may not burn off.

2. Separation of combustion products. For quantitative absorption of water and carbon dioxide, layers of each absorber 10 to 15 mm long are sufficient since they are regenerated after every analysis. The absorbents must be renewed after a few hundred analyses since they slowly lose activity. They may not be heated above 400°C.

3. Evaluation of the signals. The signal measured at the bridge may be either recorded on a potentiometric recorder or integrated. Peak height is used in the fist instance, peak area in the second. We have tested both methods. Peak height measurements are simpler instrumentally, but a good deal less exact. We recommend electronic integration.

a. Evaluation of peak height. A nearly linear relationship between peak height and amount was found for substances whose combustion products are first adsorbed from the gas stream and then serially desorbed. The above mentioned absorbents were used for absorption and desorption of water and carbon dioxide, but nitrogen was absorbed at —80°C on molecular sieve 5A (10 cm long) and desorbed at 100°C. The carrier gas was turned off during desorption. The upper limit of the linear region was found at 7 ml of carbon dioxide, 1 ml of nitrogen and 3 mg of water. The values were reproducible at only 1%.

Nonlinearity resulted when the combustion products were allowed to reach the detector as they were formed by combustion, instead of being collected on the absorbent. Compounds that burned slowly showed lower peak heights than those burning quickly.

We offer the following explanation: The recorder reads out continously the concentrations of the particular component during the analysis. Maximum peak height is a measure of maximum concentration, which depends upon the rate of introduction of the component into the carrier gas. The higher the rate of introduction from a constant weight of sample, the higher the peak concentration, and thus peak height. If the rate of

combustion limits the rate of introduction, and this is different from sample to sample, then peak height is not a measure of sample components.

Peak height readings become more accurate when the rates of introduction are equalized by desorbing stored components of the combustion mixture. The degree of accuracy attained, however, does not suffice for the demands of elemental analysis.

b. Evaluation through integration. This approach gave the best results. Three conditions are essential for accurate integration of concentration peaks:

1. Linear relationship between concentration and signal.
2. Constant base line.
3. Constant carrier gas velocity.

The first condition was investigated by running the individual combustion products (carbon dioxide, nitrogen, and water), mixed with helium, to the detector heated to 80°C. The response to carbon dioxide was linear to 20% in helium, corresponding to a signal of 10 mv. Nitrogen was linear through the same concentration range, giving a signal of only 7.5 mv (see Fig. 5). Water showed a linear response up to 18 mg water in 100 ml helium, at a maximum allowable signal of 5.5 mv (see Fig. 6). The linear range found is appreciably greater than that expected from the formula of Walisch (*4*).

A mixing tube was introduced before the detector to reduce the maximum concentration sufficiently to permit remaining in the linear range. The concentration of a component may also be limited by controlling the temperature of desorption; the higher the temperature, the higher the maximum concentration.

The second condition is attained by exposing both the measuring and the reference thermistor to the same gas stream. Velocity changes thus are balanced out. The separation method described affords both a constant and zero baseline between peaks.

The third condition can be satisfied by keeping constant the moles per unit time which pass the detector; in our tests, 50 ml of helium plus component per minute were used. It is well known that the rate of flow through a column is proportional to the difference between inlet and outlet pressure. It was shown experimentally that the rate of flow was not

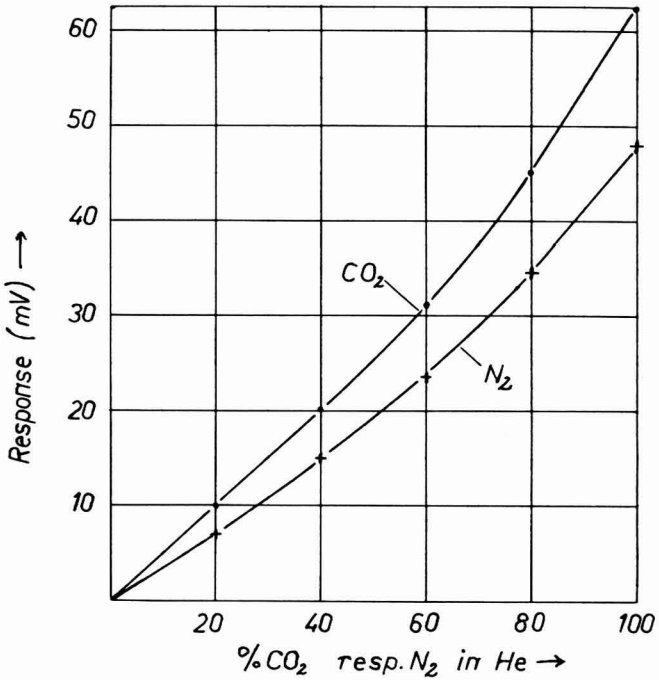


FIG. 5. Relationship between signal, CO_2, and N_2 concentration, respectively (in helium).

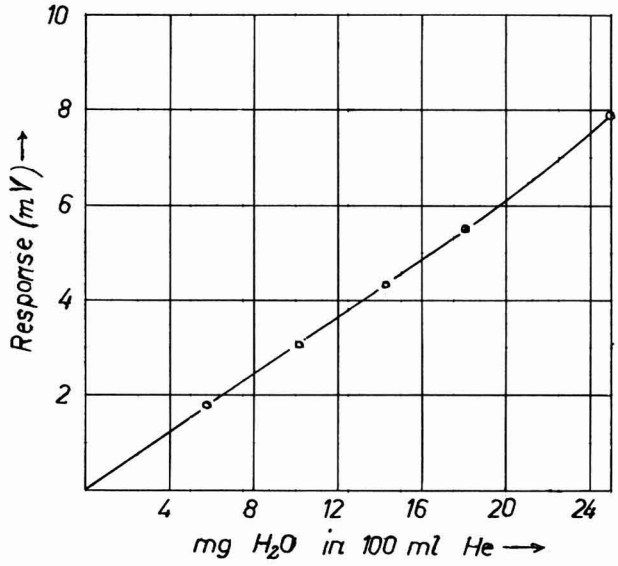


FIG. 6. Relationship between signal and concentration of water in helium.

appreciably changed during heating of the short adsorption layers. However, desorption causes a transient increase in the rate of flow. The time delay spiral largely neutralized this transient change. The dimensions of this spiral are such that the front of the component does not reach the detector before equalization of the transient.

Separation method and constancy of calibration

Since in our method only one component at a time (mixed with helium) reaches the detector, constancy of calibration for each component is assured; but constancy is not attained by all separation methods. For example, if carbon dioxide were determined by difference through measurement of the sum of carbon dioxide, nitrogen, and helium in the first catharometer, then, after removal of carbon dioxide on alkali-asbestos, the sum of nitrogen and helium were determined on a second catharometer, the carbon dioxide signal would be less in the presence of higher amounts of nitrogen. The same applies to the determination of water in a mixture or helium, carbon dioxide, and nitrogen, when after removal of the water on magnesium perchlorate, the remaining helium, carbon dioxide, and nitrogen mixture is used as a reference gas. The water signal becomes smaller as the carbon dioxide and nitrogen increase. According to Dal Nogare and Juvet (*1*), the signal E is proportional to the moles of carbon dioxide but is also a function of the reference mixture composition.

$$E = \text{prop. } x_{CO_2} \, (1/K_{CO_2} - 1/K_{\text{Reference gas}})$$

SUMMARY

A previously described method for the determination of carbon, hydrogen, and nitrogen has been improved with respect to procedure and apparatus. The sample is burned in a measured amount of pure oxygen. Excess oxygen is absorbed on copper. Nitrogen oxides are reduced. Water is absorbed on a calcium chloride-quartz mixture, and carbon dioxide on a layer of molecular sieve 5A. Nitrogen is detected first, and the signal integrated on an integrator with digital read out. Carbon dioxide is then desorbed through heating and is similarly quantitated. Finally, water is desorbed and determined. Alternate peak height methods were evaluated, but were not accurate enough for micro elemental analysis. The necessary conditions for accurate integration are discussed.

REFERENCES

1. Dal Nogare, St., and Juvet, R. S., Jr., "Gas Liquid Chromatography, Theory and Practice," 450 pp. Wiley (Interscience), New York, 1962.
2. Kainz, G. and Scheidl, F., Ueber die quantitative Oxydation organischer Verbindungen im "leeren Rohr." II. Mitt. Einfluss der Versuchsvariablen. *Mikrochim. Acta* **1963**, 902-910.

3. KAINZ, G. AND WACHBERGER, E., Ueber eine neue Mikro-und. Ultramikrobestimmung von Kohlenstoff, Wasserstoff und Stickstoff organischer Verbindungen mit Hilfe der Waermeleitfaehigkeit. *Z. Anal. Chem.* **222**, 271-278 (1966).
4. WALISCH, W., Eine Ultramikroschnellmethode zur gleichzeitigen Bestimmung des C-, H- and N-Gehaltes organischer Verbindungen. *Chem. Ber.* **94**, 2314-2327 (1961).

MICROCHEMICAL JOURNAL **12**, 593-605 (1967)

The Determination of Phosphorus in Organic Compounds on the Centimilligram Scale[1]

A. J. CHRISTOPHER AND T. R. F. W. FENNELL

Royal Aircraft Establishment, Farnborough, Hants, England.

Received April 3, 1967

INTRODUCTION

Kirsten and Carlsson (*5*) compared various methods for the determination of phosphorus in organic compounds. Mineralization procedures included open tube wet digestion (with a mixture of perchloric, sulphuric and nitric acids), sealed tube digestion (with diluted sulphuric acid) and oxygen flask combustion. Colorimetric finishes, either as yellow phosphomolybdic acid measured at 400-460 mμ or as molybdenum blue measured at 710-720 mμ, were used after extraction of the colored species into amyl acetate. Although open tube digestion was recommended for general use, sealed tube digestion was preferred for the centimilligram scale. Belcher *et al.* (*1*), however, reported low recoveries following sealed tube digestion, using fuming nitric acid, which they attributed to adsorption of phosphate on the tube walls. These authors recommended oxygen flask combustion followed either by titrimetric determination of precipitated quinoline phosphomolybdate or by a colorimetric finish as molybdenum blue.

Salvage and Dixon (*8*) decomposed samples, in the 30-500 μg range, with a mixture of perchloric and sulphuric acids in a 1-ml flask subsequently used for development *in situ* of the phosphovanadomolybdate complex; the color was measured at 430 mμ in micro cuvettes.

We have applied the three mineralization techniques outlined in an earlier paper (*3*) and open tube digestion to the determination of phosphorus in centimilligram samples, some of which also contained fluorine. The resulting phosphate was determined spectrophotometrically, either as molybdenum blue at 735 mμ or as phosphovanadomolybdate at 315 mμ.

[1] British Crown Copyright, reproduced with the permission of the Controller, Her Britannic Majesty's Stationery Office.

SPECTROPHOTOMETRIC DETERMINATION OF PHOSPHORUS

The most sensitive simple method available for the determination of microgram amounts of phosphorus appeared to be that where the absorption of the phosphovanadomolybdate complex is measured at 315 mμ (*7*). Only one reagent is added; the complex forms rapidly and is stable for a considerable time; because the sensitivity is 10-15 times that at 430 mμ, neither extraction nor the use of special small-scale apparatus is required. On the other hand, measurement in the ultraviolet renders the method liable to interference from "colorless" molecules or ions and requires the use of expensive, though quite common, equipment.

For absorption in the visible region of the spectrum, only molybdenum blue methods are available. The simplest, sensitive, nonextractive method appeared to be that of Levine *et al.* (*6*). It is less sensitive than the phosphovanadomolybdate method, involves the addition of more reagents and requires 30 minutes for color development. However, measurement at 735 mμ requires less expensive photometric equipment and should not be so liable to interference as measurement at 315 mμ.

MINERALIZATION PROCEDURES

Open Tube, Acid Digestion

Salvage and Dixon (*8*) have reported good results after using a simpler acid mixture than that used by Kirsten and Carlsson (*5*). We confirmed that the perchloric-sulphuric acid mixture was very satisfactory although, in order to keep the final acidity down to the level recommended by Michelsen (*7*) for the phosphovanadomolybdate finish, we added only half the quantity of sulphuric acid used by Salvage and Dixon. We established, using triphenylphosphine as a test material, that heating for 15 minutes at 275°C was sufficient for complete digestion.

The results obtained using this procedure for mineralization of a number of solid and liquid compounds, with either the ultraviolet or visible spectrophotometric finish, are given in Table 1. These results are satisfactory for unfluorinated compounds; it is seen, however, that recoveries tended to be slightly low for some fluorocompounds and very low for others (cf. Fennell *et al.* (*2*)).

Open Tube, Hydrogen Peroxide Digestion

The use of 50% hydrogen peroxide has been advocated by Whalley (*10*) and by Taubinger and Wilson (*9*) for the decomposition of organic

TABLE 1

ANALYSIS OF ORGANIC MATERIALS: OPEN TUBE $HClO_4$-H_2SO_4 DIGESTION

Compound	Ultraviolet finish			Visible finish		
	Wt. taken (μg)	Phosphorus (%)		Wt. taken (μg)	Phosphorus (%)	
		Found	Error		Found	Error
Triphenylphosphine	35.12	11.70	−0.11	42.85	11.62	−0.19
11.81% P	43.91	11.77	−0.04	43.79	11.81	0.00
	62.59	11.66	−0.15	47.51	11.79	−0.02
	78.21	11.87	+0.06	49.14	11.88	+0.07
	83.57	11.80	−0.01	50.79	11.73	−0.08
	92.94	11.75	−0.06	55.07	11.46	−0.35
	95.64	11.73	−0.08	61.84	11.85	+0.04
				62.21	12.04	+0.23
$C_{20}H_{20}NO_3P$	42.47	8.78	+0.01	69.62	8.99	+0.22
8.77% P	51.41	8.83	+0.06	81.08	8.84	+0.07
$C_{19}H_{19}N_2O_2P$	44.71	8.97	−0.18	69.54	9.49	+0.34
9.15% P	68.30	9.14	−0.01	86.57	9.32	+0.17
$C_8H_{10}NO_5P$	38.76	13.44	+0.04			
13.40% P	44.61	13.45	+0.05			
$C_{28}H_{42}Br_2MgO_8P_2$	46.31	8.25	+0.02			
8.23% P	67.17	8.31	+0.08			
Tri-*m*-cresyl	45.07	8.48	+0.07			
phosphate[a]	62.02	8.43	+0.02			
8.41% P	64.71	8.34	−0.07			
	69.50	8.42	+0.01			
	101.66	8.41	0.00			
	116.44	8.28	−0.13			
$C_{38}H_{52}O_9P_2Si_2$[a]	28.07	7.87	−0.17			
8.04% P	34.03	8.02	−0.02			
	74.02	8.02	−0.02			
$C_{18}H_{18}F_7N_2O_2P$	19.02	6.20	−0.56			
6.76% P	27.25	6.75	−0.01			
	54.45	6.61	−0.15			
	61.72	6.64	−0.12			
	63.59	6.42	−0.34			
	68.11	6.75	−0.01			
	113.42	6.74	−0.02			
	118.96	6.60	−0.16			
$C_{14}H_{14}F_3N_2O_2P$	45.54	8.59	−0.79			
9.38% P	46.57	9.15	−0.23			
	53.18	9.33	−0.05			
	62.09	9.34	−0.04			
	73.87	9.27	−0.11			

TABLE 1 (*Continued*)

Compound	Ultraviolet finish			Visible finish		
	Wt. taken (μg)	Phosphorus (%)		Wt. taken (μg)	Phosphorus (%)	
		Found	Error		Found	Error
$C_{14}H_{10}F_{14}NO_3P$	74.04	4.08	−1.69			
5.77% P	92.51	3.45	−2.32			
$C_{15}H_{12}F_{14}NO_3P$	70.66	3.18	−2.44			
5.62% P	71.16	3.02	−2.60			

[a] Liquid.

materials. Although it was recognized that phosphate might be used as a stabilizing agent, we did not think that the small amount of reagent required would contain sufficient phosphate to interfere seriously.

The results obtained using this method of decomposition are given in Table 2. Although these are generally satisfactory, those for triphenylphosphine tend to be low; it is confirmed that fluorocompounds were, again, not completely mineralized.

Sealed Tube, Acid Digestion

We used our usual technique (*3*) for sealed tube digestion, with a nitric and sulphuric acid mixture (95 + 5). Table 3 shows the results with both ultraviolet and visible finishes. In both cases, correction had to be made for a reagent blank. Results for triphenylphosphine tend to be low but it is notable that two of the fluorocompounds which had not been amenable to mineralization by the open tube methods gave reasonable recoveries after sealed tube digestion.

TABLE 2

ANALYSIS OF ORGANIC MATERIALS: OPEN TUBE H_2O_2-H_2SO_4 DIGESTION

Compound	Wt. taken (μg)	Phosphorus (%)	
		Found	Error
Triphenylphosphine	51.50	11.57	−0.24
11.81% P	53.37	11.60	−0.21
	58.51	11.45	−0.36
$C_{19}H_{19}N_2O_2P$	54.46	9.14	−0.01
9.15% P	54.59	9.25	+0.10
	58.16	9.20	+0.05
	61.63	9.02	−0.13
	63.77	9.20	+0.05
	74.53	9.11	−0.04

TABLE 3
ANALYSIS OF ORGANIC MATERIALS: SEALED TUBE HNO_3-H_2SO_4 DIGESTION

Compound	Ultraviolet finish			Visible finish		
	Wt. taken (μg)	Phosphorus (%)		Wt. taken (μg)	Phosphorus (%)	
		Found	Error		Found	Error
Triphenylphosphine	42.49	11.27	−0.54	47.97	11.57	−0.24
11.81% P	43.60	11.40	−0.41	55.68	11.66	−0.15
	48.70	11.46	−0.35			
	48.99	11.57	−0.24			
	49.04	11.13	−0.68			
$C_{20}H_{20}NO_3P$	62.83	8.58	−0.19	59.43	8.78	+0.01
8.77% P	67.49	8.59	−0.18	62.30	8.65	−0.12
	68.13	8.56	−0.21	72.94	8.82	+0.05
				79.44	8.70	−0.07
				82.46	8.89	+0.12
$C_{19}H_{19}N_2O_2P$	38.63	9.01	−0.14	52.87	9.17	+0.02
9.15% P	40.33	9.00	−0.15	86.29	9.21	+0.06
	44.12	9.04	−0.11			
	66.79	9.07	−0.08			
	72.82	8.91	−0.24			
$C_{14}H_{10}F_{14}NO_3P$	72.94	5.40	−0.37	106.98	5.72	−0.05
5.77% P	82.61	5.48	−0.29	129.55	5.73	−0.04
$C_{15}H_{12}F_{14}NO_3P$	86.23	5.31	−0.31	110.13	5.48	−0.14
5.62% P	94.96	5.29	−0.33	113.38	5.40	−0.22
	97.00	5.34	−0.28			

Sodium Peroxide Fusion

It was known that, on the semimicro scale, fusion with sodium peroxide resulted in complete decomposition of the "difficult" fluorocompounds (*2*). Attempts were made, therefore, to apply the technique to the centimilligram scale. Unfortunately, severe interference was found with both spectrophotometric finishes. It is suspected that this interference was caused by impurities in the sodium peroxide and by random adsorption and desorption of phosphorus in the nickel capsules.

Hot Flask Combustion

Kirsten's hot flask technique (*4*) was tried, using sodium hypochlorite as absorbent. Liquid and solid compounds, including the "difficult" fluoro-

compounds, were successfully decomposed by this technique (see Table 4). No blank was found but it was essential to heat the absorbent, after acidification, presumably to convert *meta-* into *ortho*-phosphate.

TABLE 4
ANALYSIS OF ORGANIC MATERIALS: HOT FLASK COMBUSTION

Compound	Wt. taken (μg)	Phosphorus (%)	
		Found	Error
Triphenylphosphine	32.27	10.97	−0.84
11.81% P	45.39	11.65	−0.16
	55.34	12.20	+0.39
	61.87	11.56	−0.25
	76.42	11.63	−0.18
$C_{20}H_{20}NO_3P$	57.36	8.32	−0.45
8.77% P	57.88	8.66	−0.11
	70.99	8.59	−0.18
	71.19	8.68	−0.09
	72.83	8.91	+0.14
	76.23	8.78	+0.01
Tri-*m*-cresyl	29.35	8.35	−0.06
phosphate[a]	50.80	8.52	+0.11
8.41% P	56.93	8.61	+0.20
	71.08	8.26	−0.15
	79.54	8.65	+0.24
	83.45	8.40	−0.01
	120.08	8.04	−0.37
$C_{38}H_{52}O_9P_2Si_2$[a]	28.10	8.54	+0.50
8.04% P	48.27	8.06	+0.02
	66.06	7.69	−0.35
	72.60	7.41	−0.63
$C_{14}H_{10}F_{14}NO_3P$	59.12	6.11	+0.34
5.77% P	80.86	5.43	−0.34
	81.27	5.73	−0.04
	89.67	5.69	−0.08
	110.39	5.66	−0.11
$C_{15}H_{12}F_{14}NO_3P$	50.24	5.95	+0.33
5.62% P	54.45	5.85	+0.23
	66.94	5.38	−0.24
	83.60	5.74	+0.12
	91.01	5.47	−0.15

[a] Liquid.

ANALYTICAL PROCEDURES

Weighing of Sample

Solids. Transfer sufficient of the compound to give 3-7 μg of phosphorus into a tared platinum boat and weigh on a quartz-fiber balance. Tip the sample into the decomposition vessel and reweigh the boat.

Liquids. Transfer 400-500 μg of liquid into the bottom of a small (4 mm × 4 mm) tared silica cup and weigh. Dip one end of a piece of cotton thread, about 5 mm long, into the liquid so that some is absorbed up the thread. Drop the thread into the decomposition vessel and reweigh the cup.

Mineralization

1. *Open tube, acid digestion.* A test tube, 7.5 mm diameter and 7.5 cm long, is used as digestion vessel.

Add, from graduated capillaries, 15 μl of concentrated sulphuric acid and 5 μl of 70% perchloric acid to the sample in the test tube. Centrifuge to drive all acid to the bottom of the tube and then place it, in a hole drilled in a 1.5 inch deep aluminum block, on a hotplate at 275°C. Digest for 15 minutes, remove the tube from the block and allow it to cool. Dilute the acid to about 1 ml with water and transfer it quantitatively into a 25-ml volumetric flask.

2. *Open tube, hydrogen peroxide digestion.* A test tube, 7.5 mm diameter and 7.5 cm long, is used as digestion vessel.

Add 1 drop of concentrated sulphuric acid and 1 drop of 50% hydrogen peroxide to the sample in the test tube. Clamp the tube obliquely and heat the liquid gently with a small microburner flame. After the initial, very vigorous reaction, gradually drive off the hydrogen peroxide until oily drops of sulphuric acid are refluxing in the tube. Allow the tube to cool, dilute the acid with water and transfer it quantitatively into a 25-ml volumetric flask.

3. *Sealed tube, acid digestion.* A hard glass test tube, 9 mm id, 12.5 mm od, 8 cm long, is used as digestion vessel.

Add 0.1 ml of digestion mixture (nitric and sulphuric acids, 95 + 5) to the sample in the tube and seal the tube in a blowpipe flame. Place the sealed tube vertically in an aluminium rack and transfer to a furnace at 300°C. After 1 hour, remove the rack and tube and allow to cool. Release pressure in the digestion tube by heating the tip in a blowpipe flame until a small hole appears. Remove volatile material by heating on a

steam bath and under reduced pressure (water pump) for 10 minutes. Allow to cool, and cut off the top of the tube. Rinse the contents of the tube into a 25-ml volumetric flask with water.

4. *Hot flask combustion.* The horizontal silica flask and sample holder described by Kirsten (*4*) are used. The organic sample is placed directly into the silica cup of the sample holder.

Clamp the combustion flask horizontally so that the closed end is held in a tube furnace at 850°C and allow it to heat for 10 minutes.

Pipette 0.5 ml of water and 3 drops of sodium hypochlorite (laboratory reagent grade, 10-14% w/v available chlorine) into the absorption bulb and sweep out the flask with oxygen for 30 seconds. Moisten the ground joint of the sample holder with water, quickly insert it into the combustion flask and fix it with the springs. After 1 minute, remove the whole apparatus from the furnace and allow it to cool (10 minutes) in a horizontal position. Shake the flask to wet the walls and allow it to stand vertically for a few minutes. Remove the sample holder and wash it thoroughly, allowing the washings to drain into the flask. Add 1 ml of 0.5*N* sulphuric acid and heat the flask in boiling water for 15 minutes. Cool the flask and transfer its contents into a 25-ml volumetric flask.

Spectrophotometry

A. Phosphovanadomolybdate Method

The instrument used was a Hilger Uvispek fitted with a quartz prism.

Vanadomolybdate reagent. Dissolve 1.6 g of ammonium vanadate and 50 g of ammonium molybdate in about 700 ml of water at about 60°C, filter if not completely clear and allow to cool. Add cautiously, with stirring, 150 ml of concentrated sulphuric acid, allow to cool and dilute to 1 liter with water. Dilute 20 ml of this solution to 1 liter.

Procedure. To the liquid in the 25-ml flask add 3 ml of vanadomolybdate reagent and dilute to the mark with water. Read the absorbance (4 cm silica cuvettes), after 2 minutes, at 315 mμ against a reference solution prepared by diluting 3 ml of reagent, 15 μl of concentrated sulphuric acid and 5 μl of 70% perchloric acid to 25 ml. Compute the phosphorus content from a calibration curve prepared by treating portions of a standard (1 μg P/ml) potassium dihydrogen phosphate solution as above.

B. Molybdenum Blue Method

The instrument used was a Hilger Uvispek fitted with a glass prism.

Acid molybdate reagent. Dissolve 10 g of ammonium molybdate in about 200 ml of water. Add 170 ml of concentrated hydrochloric acid, allow to cool and dilute to 500 ml.

Stannous chloride reagent. Dissolve 5 g of stannous chloride in 10 ml of diluted hydrochloric acid (1 + 1) and dilute to 100 ml. Dilute 10 ml of this stock solution to 100 ml. This reagent solution should be freshly prepared each day.

Procedure. To the liquid in the 25-ml flask add 3 ml of acid molybdate reagent and 0.2 ml of stannous chloride. Dilute to the mark and allow to stand for 30 minutes. Read the absorbance (4-cm glass cuvettes at 735 mμ against a reagent reference solution. Compute the phosphorus content by reference to a calibration curve.

DISCUSSION

In order to avoid lengthy repetition, the various procedures used will be identified by the following code: *Decomposition:* 1. Open tube, acid digestion; 2. Open tube, hydrogen peroxide digestion; 3. Sealed tube, acid digestion; 4. Hot flask combustion. *Finish:* A. Phosphovanadomolybdate, at 315 mμ; B. Molybdenum blue, at 735 mμ.

Owing to the anomalous behaviour of some fluorocompounds, these will be discussed separately.

Unfluorinated Compounds

The results obtained by the various methods for the analysis of triphenylphosphine are summarized in Table 5. From these data, it may be

TABLE 5
Comparison of Methods for Analysis of Triphenylphosphine, 11.81%P

Method	No. of results	Phosphorus (%)			
		Mean	SD	Bias	SD of bias
1A	7	11.75	0.07	—0.06	0.03
1B	8	11.77	0.18	—0.04	0.06
2A	3	11.54	0.08	—0.27	0.05
3A	5	11.37	0.17	—0.44	0.08
3B	2	11.62	—	—0.19	—
4A	5	11.60	0.44	—0.21	0.20

inferred that (a) finish A is significantly more precise than B, (b) decomposition methods 2 and 3 have a significant negative bias, and (c) decomposition method 1 leads to significantly higher precision than method 4. In respect to both accuracy and precision, therefore, method 1A is preferred.

The results from analyses of a number of compounds are summarized in Table 6. Here, except for method 3A, the mean recoveries are satis-

TABLE 6
COMPARISON OF METHODS FOR ANALYSIS OF UNFLUORINATED COMPOUNDS

Method	Time, min.		No. of compounds	Total no. of results	Phosphorus recovery (%)	
	Decomposition[a]	Color[b]			Mean	SD
1A	25	2	7	24	99.73	0.80
1B	25	30	3	12	100.53	1.84
2A	20	2	2	9	99.26	1.40
3A	90	2	3	13	97.43	1.38
3B	90	30	3	9	99.72	1.06
4A	40	2	4	22	99.30	3.62

[a] Estimate of time taken, for a single determination, between completion of weighing to transference of solution to volumetric flask.

[b] Standing time between making up to volume and measurement of absorbance.

factory for all the methods; but the differences in precision are, except for method 3B, statistically significant compared with method 1A. Also shown are the estimated times required for the decomposition processes and color development. The most rapid, for a single determination, are clearly 1A and 2A. However, decomposition process 1 may be done in multiple whereas process 2 must be done individually.

In both Tables 5 and 6, it will be seen that lower results are found by method 3A than by 3B. For both methods a blank was determined by treating a sample of benzoic acid exactly as in the procedure for phosphorus compounds, adding 5 μg of phosphorus (as potassium dihydrogen phosphate solution) and recording the absorbance after normal color development. It is clear that the blank so found is too high for method 3A, possibly caused by absorption of organic residues which absorb in the ultraviolet but not in the visible region.

Although reported results vary widely in scope and manner of presentation, our preferred method 1A may be compared with other published procedures by reference to Table 7, where the statistics of the various procedures are listed. It is clear that our proposed method has advantages

TABLE 7
COMPARISON OF PUBLISHED METHODS: UNFLUORINATED COMPOUNDS

Method	Total no. of results	No. of compounds	Precision (%P)	Accuracy (%P)	
			Pooled SD	Bias	SD of bias
Salvage and Dixon	32	14	0.30	−0.11	0.06
Kirsten and Carlsson	6	2	0.24	−0.14	0.10
Belcher, et al.	44[a]	7	0.23[c]	+0.07	0.07
	14[b]	3	0.38[c]	−0.13	0.05
Method 1A	24	7	0.07	−0.02	0.02

[a] Titrimetric finish.
[b] Colorimetric finish.
[c] Estimate of SD computed from the ranges quoted in the original paper.

as regards both accuracy and precision over the other published procedures. We are also of the opinion that it is manipulatively simpler and, apart from the method of Salvage and Dixon, more rapid.

It is interesting to note that although Belcher *et al.*, reported low results after sealed tube digestion with fuming nitric acid, we have not had this experience with the nitric-sulphuric acid mixture; Kirsten and Carlsson stated a preference for sealed tube digestion, with 75% sulphuric acid, for very small amounts of phosphorus. It would appear that the presence of diluted sulphuric acid prevents the adsorption suspected by Belcher *et al.*

Fluorocompounds

Neither of the open tube digestion procedures was completely successful for certain fluorocompounds and difficulty was experienced in trying to apply sodium peroxide fusion. Sealed tube digestion and hot flask combustion, however, both gave reasonably satisfactory results for the compounds available. The results obtained for the two compounds which gave the worst results by open tube digestion are summarized in Table 8.

TABLE 8
COMPARISON OF METHODS FOR ANALYSIS OF TWO FLUOROCOMPOUNDS

Method	No. of results	Phosphorus (%)	
		Bias	SD of bias
1A	4	−2.24	0.20
3A	5	−0.32	0.02
3B	4	−0.11	0.04
4A	10	−0.01	0.08

Method 3A again gave low recoveries although with excellent precision. Of the remaining two successful procedures, we would recommend method 4A, in spite of its poorer precision, on grounds of time, ease of manipulation, and absence of blank. It is possible that oxygen flask combustion might also be successfully applied to these materials but it has the disadvantage that an absorption time of 90 minutes is required after combustion. The results quoted by Belcher *et al.* (*1*) indicate that this combustion method might have no better precision than the hot flask procedure.

SUMMARY

Open tube digestion with a perchloric-sulphuric acid mixture followed by spectrophotometric measurement, at 315 mμ, of the phosphovanadomolybdate complex provides an accurate, precise, and rapid method for the determination of phosphorus in organic compounds on the centimilligram scale. This method appears to be superior to other published procedures. An alternative finish by production of molybdenum blue and measurement at 735 mμ may be used with some loss of precision and speed.

Certain fluorocompounds are not amenable to open tube digestion. These materials may be mineralized by sealed tube digestion with a nitric-sulphuric acid mixture or by hot flask combustion.

ACKNOWLEDGMENT

We are indebted to Mr. J. H. Cadwell for the statistics shown in Table 7.

REFERENCES

1. Belcher, R., Macdonald, A. M. G., Phang, S. E., and West, T. S., Submicromethods for the analysis of organic compounds. XX. The determination of phosphorus and arsenic. *J. Chem. Soc.* **1965**, 2044-2048.
2. Fennell, T. R. F. W., Roberts, M. W., and Webb, J. R., The semi-micro determination of phosphorus in fluorinated organic compounds. *Analyst.* **82**, 639-643 (1957).
3. Fennell, T. R. F. W., and Webb, J. R., Analysis of organic materials on the centimilligram scale. *Microchem. J.* **10**, 456-460 (1966).
4. Kirsten, W. J. Hot-flask combustion methods for decimilligram determination of halogen and other elements in organic compounds. *Microchem. J.* **7**, 34-40 (1963).
5. Kirsten, W. J., and Carlsson, M. E., Investigation of the determination of phosphorus in organic compounds. *Microchem. J.* **4**, 3-31 (1960).
6. Levine, H., Rowe, J. J., and Grimaldi, F. S. Molybdenum blue reaction and determination of phosphorus in waters containing arsenic, silicon and germanium. *Anal. Chem.* **27**, 258-262 (1955).
7. Michelsen, O. B., Photometric determination of phosphorus as molybdovanadophosphoric acid. *Anal. Chem.* **29**, 60-62 (1957).
8. Salvage, T., and Dixon, J. P., The colorimetric determination of phosphorus in organic compounds on the microgram scale. *Analyst.* **90**, 24-28 (1965).

9. TAUBINGER, R. P., AND WILSON, J. R., The use of 50 per cent. hydrogen peroxide for the wet oxidation of organic materials. *Analyst.* **90**, 429-431 (1965).

10. WHALLEY, C., Hydrogen peroxide as an analytical reagent. *In* "Analytical Chemistry 1962" (P. W. West, A. M. G. Macdonald and T. S. West, ed.), pp. 397-404. Elsevier, Amsterdam, 1963.

MICROCHEMICAL JOURNAL **12**, 606-608 (1967)

The Microdetermination of Sulfur in Organic Compounds Containing Phosphorus

R. B. BALODIS,[1] A. COMERFORD, AND C. E. CHILDS

Parke, Davis and Co., Research Laboratories, Ann Arbor, Michigan

Received April 7, 1967

INTRODUCTION

The determination of sulfur in phosphorus-containing compounds has always been a problem in organic analysis due to the phosphorus interference, and several procedures have been developed to overcome this (*2*, *3*). The methods employing the oxygen flask combustion and subsequent titration with a barium salt usually lead to high results due to the slightly soluble barium phosphate formed. It is necessary, therefore, to eliminate the phosphate produced in the course of the combustion before an accurate measurement of sulfur can be made.

In the present method the phosphate ions are masked with ferric ions, since the latter chelate more readily with phosphate than sulfate ions in an acidic solution, and the excess ferric ions are back-titrated with EDTA (*1*, *5*). The sulfate is titrated in the usual way (*4*).

EXPERIMENTAL

Reagents

1. Aqueous solution of 0.02N $BaCl_2$.
2. 0.025M Disodium ethylene dinitrilotetraacetate·$2H_2O$ (EDTA).
3. 5% sulfosalicyclic acid solution.
4. 0.1% Thorin.
5. 0.0125% Methylene blue.
6. 30% Hydrogen peroxide.
7. Ferric chloride, reagent grade.

[1] Present address: Chemical Abstract Service, Ohio State University, Columbus, Ohio.

Procedure

A 5-7 mg sample is burned in a 300 ml oxygen flask over 10 ml of triple-distilled water containing 7 drops of 30% hydrogen peroxide. The solution is transferred to a 100 ml beaker using absolute alcohol (ethanol) to rinse out the flask, and boiled down to 5 ml. The solution is then acidified with 1 drop of 0.1N HCl and buffered with 1 drop of 5%

TABLE 1
TYPICAL ANALYTICAL RESULTS

Compound	Sulfur found	Sulfur calculated
$C_4H_{11}O_2PS_2$-NH_3 Salt	31.57%	31.49%
	31.49	
$C_{14}H_{19}N_2O_4PS$	9.46	9.34
	9.56	
$C_{13}H_{19}Cl_3NO_2PS$	8.20	8.19
	8.16	
$C_{13}H_{19}Cl_3NO_2PS$ (Isomer)	8.25	8.19
	8.31	
$C_{12}H_{17}Cl_3NO_2PS$	8.27	8.50
	8.52	
S-benzylthiuronium chloride	15.69	15.82
	15.97	
	16.03	
	16.03	

sulfosalicylic acid. Enough $FeCl_3$ crystals (several mgs) are added to make the solution dark red and the excess ferric ions are back-titrated with 0.025M EDTA (.5-.8 ml) until the solution becomes pale yellow. The solution is then transferred to a 30 ml titration cell using absolute alcohol as the rinse with a final volume of 30 ml. Titration is carried out on an illuminated stand equipped with a masking plate (A. H. Thomas Co.) using .02N $BaCl_2$ as the titrant with 2 drops of thorin and 4 drops of methylene blue as the combined indicator. The end point is a color change from green to grey. Overtitration produces a grey pink.

RESULTS

The results of a group of known compounds are shown in Table 1.

DISCUSSION

The main advantage of this procedure is that no special separation of phosphorus is required. As the results indicate, the method is quite accurate

and simple, but there may be some difficulty in seeing the final end-point. With a little practice, however, it is readily distinguishable. An illuminated stand and masking plate are necessary to concentrate the light on the solution, and a colorless titration cell is mandatory. The titration was not tried on a regular titration stand but might be possible if a good light source were used.

SUMMARY

An oxygen-flask procedure is described for the microdetermination of sulfur in phosphorus-containing compounds. The phosphate formed is masked with ferric ions and the sulfate is titrated directly with barium chloride.

REFERENCES

1. CHENG, K. L., Increasing selectivity of analytical reactions by masking. *Anal. Chem.* **33**, 783-790 (1961).
2. FRITZ, J. S., AND YAMAMURA, S. S., Rapid microtitration of sulfate. *Anal. Chem.* **27**, 1461-1464 (1955).
3. JASELSKIS, B., AND VAS, S. F., Titrimetric determination of semimicro amounts of sulfate in the presence of phosphate. *Anal. Chem.* **36**, 1965-1967 (1964).
4. THOMAS, ARTHUR H. COMPANY, Determination of Sulfate, Technological Service Bulletin 6470E-G, **5** (1958).
5. VAN NIEUWENBURG, C. J., AND VAN LIGTEN, J. W. L., "Qualitative Chemical Microanalysis," pp. 120-121, Elsevier, Amsterdam, 1963.

MICROCHEMICAL JOURNAL **12**, 609-611 (1967)

New Titrimetric Microdetermination of Osmium

O. C. SAXENA

Chemical Laboratories, University of Allahabad, Allahabad, India

Received May 1, 1967

INTRODUCTION

Literature concerning the determination of osmium, titrimetrically, does not seem to be very rich. Syrokomskii (*3*) determined osmium indirectly with sodium or ammonium vanadate. Crowell and Baumbach (*1*) determined it by titrating against a standard solution of chromium(II).

The present method deals with the determination of osmium in micro quantities with potassium ferrocyanide. A known excess of potassium ferrocyanide is added to an acid solution of osmium tetroxide when a green-colored complex, $[Os^{VIII} \cdot O_5 \cdot Os^{VI} \cdot \{Fe(CN)_6\}_2]^{2-}$, is formed. The remaining excess of potassium ferrocyanide is titrated against a standard solution of ceric sulfate using *N*-phenyl anthranilic acid as indicator. It has been observed that the results agree with those obtained by standard method (*2*) and give concordant and precise values.

The green-colored complex is formed in the ratio of 1:1, confirmed by potentiometric titrations. The above mentioned complex in the mixed valency state has been confirmed by magnetic measurements, which show that the complex is paramagnetic in nature ($\chi = 2.967 \times 10^{-6}$/g).

It has been observed that Na^+, K^+, Li^+, Ca^{++}, Sr^{++}, Ba^{++}, Cd^{++}, Mg^{++}, Th^{+4}, Te^{+4}, and Se^{+6} do not interfere, but Ag^+, Tl^+, Co^{++}, Ni^{++}, Pb^{++}, Pd^{++}, Zn^{++}, Bi^{+3}, Rh^{+3}, Ru^{+3}, Au^{+3}, In^{+3}, La^{+3}, Pt^{+4}, and Zr^{+4} do.

EXPERIMENTAL

Chemicals Employed

1. OsO_4 (J. M. grade sample).
2. Potassium ferrocyanide (ANALAR B.D.H. grade sample).
3. Ferrous ammonium sulfate (ANALAR B.D.H. grade sample).
4. Sulfuric acid (ANALAR B.D.H. grade sample).

5. Hydrochloric acid (ANALAR B.D.H. grade sample).
6. Sodium carbonate (ANALAR B.D.H. grade sample).
7. Ceric sulfate (Technical B.D.H. grade sample).
8. *N*-Phenyl anthranilic acid (B.D.H. grade sample).

PROCEDURE AND RESULTS

A known excess of a standard solution of potassium ferrocyanide (standardized against a standard solution of ceric sulfate using *N*-phenyl anthranilic acid as indicator) is added to an aqueous solution of osmium tetroxide to which 10-20 ml of 0.1N HCl is added. The mixture, thus formed, is shaken well and the remaining potassium ferrocyanide is titrated against a standard solution of ceric sulfate (in 8N H_2SO_4) (standardized against a standard solution of ferrous ammonium sulfate in 1N H_2SO_4) using the above mentioned indicator. At the endpoint, a red-brown color is observed. A deep red color appears at the end-point when strong solutions of osmium tetroxide are used, but a fine red-brown color is obtained when the solution contains osmium in microquantities. It has been observed that very good results are obtained when osmium is present in microamounts. Ranges in which osmium has been estimated vary from 14.18 mg to 0.68 mg. See Table 1 for complete discussion of results in tabular form.

DISCUSSION

It is observed that in the presence of acid potassium ferrocyanide, a green-colored complex, $[Os^{VIII} \cdot O_5 \cdot Os^{VI} \cdot \{Fe(CN)_6\}_2^{2-}]$ is in solution in the ratio of 1:1. Probably the following reaction takes place:

$$2OsO_4 + 2K_4Fe(CN)_6 \rightarrow K_2Os^{VIII} \cdot O_5 \cdot Os^{VI} \cdot \{Fe(CN)_6\}_2 + 3K_2O$$

It is observed that the complex, so formed, remains unaffected by ceric sulfate as long as potassium ferrocyanide remains in excess.

The estimation of osmium(VIII) by this method is carried out at room temperature and at higher acid concentrations. Speciality of this method is that it is simple, less time consuming, accurate, and under these experimental conditions no particular precautions are necessary.

SUMMARY

New titrimetric method for microdetermination of osmium has been described with potassium ferrocyanide. An excess of potassium ferrocyanide solution is added to an acid or aqueous solution of osmium tetroxide and the remaining potassium ferrocyanide is titrated against a standard solution of ceric sulfate using *N*-phenyl an-

TABLE 1

RANGE OF REAGENTS AND AMOUNTS OF CHEMICALS USED

OsO_4	$K_4Fe(CN)_6$	$Ce(SO_4)_2$	$Ce(SO_4)_2$ used with:	
0.015N taken ml	0.0176N taken ml	0.01N added ml	0.01N $K_4Fe(CN)_6$ that reduced OsO_4 ml	Amount of osmium found (mg)
—	10	17.60	—	—
0.5	10	16.84	0.76	1.44
1.0	10	16.12	1.48	2.81
1.5	10	15.44	2.16	4.10
2.0	10	14.56	3.04	5.78
3.0	10	13.22	4.38	8.33
5.0	10	10.14	7.46	14.18
0.0015N				
—	5	8.80	—	—
2.5	5	8.44	0.36	0.68
5.0	5	8.04	0.76	1.44
7.5	5	7.70	1.10	2.09
10.0	5	7.32	1.48	2.81
15.0	5	6.56	2.24	4.26

thranilic acid as indicator. A green-colored complex, $[Os^{VIII} \cdot O_5 \cdot Os^{VI}\{Fe(CN)_6\}_2^{-2}]$ is formed in the ratio of 1:1, confirmed by potentiometric titration. The mixed valency state of the complex is confirmed by magnetic measurements, which show that the isolated complex being paramagnetic in nature. It is observed that Na^+, K^+, Li^+, Ca^{++}, Sr^{++} Ba^{++}, Cd^{++}, Mg^{++}, Th^{+4}, Te^{+4}, and Se^{+6} do not interfere, but Ag^+, Tl^+, Co^{++}, Ni^{++}, Pb^{++}, Pd^{++}, Zn^{++}, Bi^{+3}, Rh^{+3}, Ru^{+3}, Au^{+3}, In^{+3}, La^{+3}, Pt^{+4}, and Zr^{+4} do. Ranges in which osmium has been estimated vary from 14.18 mg to 0.68 mg.

ACKNOWLEDGMENT

The author is grateful to Dr. Bal Krishna, Dr. M. P. Singh and Dr. M. N. Srivastava for their kind interest; and also to the C.S.I.R. (Govt. of India) for providing financial assistance.

REFERENCES

1. CROWELL, W. R., AND BAUMBACH, H. L., Determination of osmium with Chromium(II) *J. Amer. Chem. Soc.* **57**, 2607 (1935).
2. SCOTT, WILFRED W., "Standard Methods of Chemical Analysis," 5th ed., p. 750. Van Nostrand, Princeton, New Jersey (1939).
3. SYROKOMSKII, V. S., Determination of osmium with sodium or ammonium vanadate. *Doklady Akad. Nauk.* **46**, 307 (1945).

MICROCHEMICAL JOURNAL **12**, 612-613 (1967)

Book Reviews

(546)

Spectroscopy. By D. H. WHIFFEN. Wiley, New York, 1966. vii + 205 pp. $4.25.

Whiffen's "Spectroscopy" covers the basic theory of nuclear magnetic resonance and quadrupole resonance, electron spin resonance, Mössbauer (γ-ray) spectroscopy, microwave spectroscopy, infrared and Raman spectroscopy, and visible and ultraviolet spectroscopy of atoms and molecules. The book will be found especially useful by students who have to acquire a knowledge of general theory in the above subjects for examinations. Basic quantum mechanical concepts are covered, but detailed derivations are omitted. Almost all of the basic ramifications of each subject are covered at least by a mention, and sketchy examples are given. One could suggest the inclusion of some things which are not there, such as the NMR of heavy atoms like vanadium and the associated quadrupole effects. Some practical details and applications are also briefly discussed. However, for example, no attempt is made to expand on applied aspects like the wide utility of infrared, ultraviolet-visible, and fluorescence spectrophotometry in organic and analytical chemistry. Mass spectroscopy is absent.

A most useful set of working problems and answers are given at the end of the book together with an excellent bibliography of advanced texts in spectroscopy and those aspects of chemical physics required for a thorough physical background in spectroscopy. The book is accurate and instructive, and could be made more useful in future editions if, perhaps by means of a summary chapter, more emphasis were placed on the important molecular parameters that can be obtained through spectroscopic measurements.

J. G. PRITCHARD, *IIT Research Institute, Chicago, Illinois*

The Determination of Crystal Structures. Volume III. By H. LIPSON AND W. COCHRAN. Cornell University Press, Ithaca, 1960. viii + 414 pp. $14.00.

Crystallographers and structural chemists will welcome this new and enlarged edition of Volume III of the series, "The Crystalline State," edited by Sir Lawrence Bragg. The purpose of this volume, first published in 1953, is to describe the procedures by means of which a crystal structure can be determined from a set of measured intensities. The book, therefore, does not cover introductory crystallography or the theory of X-ray diffraction, which were adequately treated in Volumes I and II of the series, nor does it deal with experimental techniques.

Most of the content of the nine chapters of the original edition has been retained in

this revision, although there has been some rearrangement of the material. The chapter on direct methods has been rewritten by Professor M. M. Woolfson, and a new chapter on intensity statistics has been contributed by Dr. A. Hargreaves. The various discussions of Fourier transforms and optical methods have now been collected in a single chapter. Other new chapters are concerned with thermal vibration, neutron and electron scattering, and anomalous scattering. The appendices of the first edition have been omitted.

The main defect of this new edition is that it seems to give only grudging recognition to the impact of the computer on X-ray crystallography since 1953. We thus find it stated that full matrix least-squares programs have "sometimes" been used. While the pedagogical merit of some hand calculation must be conceded, it does seem to this reviewer that most of the material on calculation of structure factors and summation of Fourier series is unnecessary. Beevers-Lipson strips are perhaps of historical importance, but programming a computer to do the calculations has both practical and instructional value. The space devoted to pre-computer topics could have been better used for more complete and detailed discussions of the minimum function and direct methods.

The book is well written, and it is to be highly recommended as an excellent summary of many practical aspects of structure determination. A particularly valuable feature is a reference and name index containing about 400 selected literature references, of which somewhat more than 20% are of 1960 or later vintage.

DONALD E. SANDS, *Department of Chemistry, University of Kentucky, Lexington, Kentucky*

Notice to Subscribers

The subscription rate for Microchemical Journal, Volume 13, 1968, is $22.00. This new rate is necessitated by the increased number of pages planned for the coming year.

Information about reduced prices for personal subscriptions placed by members is available from the American Microchemical Society.

THE PUBLISHERS

Author Index, Volume 12

Microchemical Journal

devoted to the application of microtechniques in all branches of science

VOLUME 12

1967

Published under the auspices of the American Microchemical Society by

ACADEMIC PRESS

New York and London

MADE IN THE UNITED STATES OF AMERICA

CONTENTS OF VOLUME 12

NUMBER 1, MARCH 1967

NUMBER 2, JUNE 1967

CONTENTS OF VOLUME 12

CONTENTS OF VOLUME 12

NUMBER 3, SEPTEMBER 1967

NUMBER 4, DECEMBER 1967

CONTENTS OF VOLUME 12

Statement of ownership, management and circulation required by the Act of October 23, 1962, Section 4369, Title 39, United States Code; of

MICROCHEMICAL JOURNAL

Published quarterly at 35 New Street, Worcester, Massachusetts 01605 for Academic Press Inc., 111 Fifth Avenue, New York, N.Y. 10003. Editor-in-Chief: Al Steyermark, Hoffmann-La Roche, Inc., Nutley, New Jersey 07110.

Owned by Walter J. Johnson, Thekla Johnson, and Kurt Jacoby, 111 Fifth Avenue, New York, N.Y. 10003. Known bondholders, mortgagees, and other security holders owning or holding 1 percent or more of total amount of bonds, mortgages or other securities: None.

Paragraphs 2 and 3 include, in cases where the stockholder or security holder appears upon the books of the company as trustee or in any other fiduciary relation, the name of the person or corporation for whom such trustee is acting, also the statements in the two paragraphs show the affiant's full knowledge and belief as to the circumstances and conditions under which stockholders and security holders who do not appear upon the books of the company as trustees, hold stock and securities in a capacity other than that of a bona fide owner. Names and addresses of individuals who are stockholders of a corporation which itself is a stockholder or holder of bonds, mortgages or other securities of the publishing corporation have been included in paragraphs 2 and 3 when the interests of such individuals are equivalent to 1 percent or more of the total amount of the stock or securities of the publishing corporation.

Total no. copies printed. average no. copies each issue during preceding 12 months: 1500; single issue nearest to filing date: 1500. Paid circulation (a) to term subscribers by mail, carrier delivery or by other means, average no. copies each issue during preceding 12 months: 1145; single issue nearest to filing date: 1191. (b) Sales through agents, news dealers, or otherwise, average no. copies each issue during preceding 12 months: 0; single issue nearest to filing date: 0. Free distribution by mail, carrier delivery, or by other means, average no. copies each issue during preceding 12 months: 14; single issue nearest to filing date: 14. Total no. of copies distributed, average no. copies each issue during preceding 12 months: 1159; single issue nearest to filing date: 1205.

(Signed) J. B. Chertok, Vice President

A MULTI-VOLUME TREATISE

The Chemistry of Non-Aqueous Solvents

Edited by **J. J. Lagowski**
Department of Chemistry
University of Texas, Austin, Texas

Volume 2
ACIDIC AND BASIC SOLVENTS

CONTENTS:

1967, 398 pp., $18.50

Volume 1
PRINCIPLES AND TECHNIQUES

CONTENTS:

1966, 403 pp., $16.50

AP 1912

Academic Press
NEW YORK AND LONDON
111 FIFTH AVENUE, NEW YORK, N.Y. 10003
BERKELEY SQUARE HOUSE, LONDON W.1

COMPARATIVE PHYTOCHEMISTRY

Edited by **T. Swain**

Low Temperature Research Station
University of Cambridge, England

Comparative phytochemistry deals with the distribution and biogenesis of chemical substances in plants. The subject is now well established and has proved of interest not only to plant taxonomists, but also, by indicating interesting taxa and biosynthetic sequences, to natural product chemists.

This volume, based on papers presented at a 1965 NATO sponsored meeting of the Phytochemical Group in Cambridge, can be regarded as an important up-to-date account of the present state of the art and the progress made in this field in the last few years.

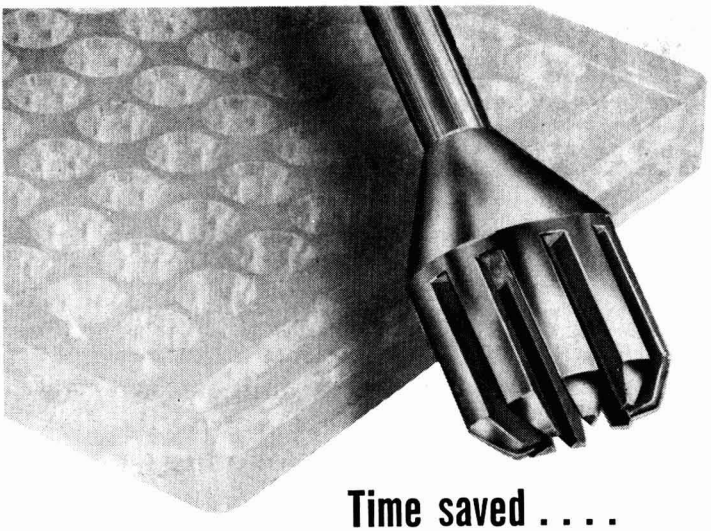
CONTRIBUTORS:

V. H. Heywood, C. Mentzer, R. E. Alston, A. G. Douglas, G. Eglinton, J. D. Bu'Lock, G. Weissmann, T. W. Goodwin, Elizabeth Percival, E. C. Bate-Smith, T. Swain, H. W. L. Ruijgrok, Anders Kjaer, E. A. Bell, R. Hegnauer, Tom J. Mabry, C. Mathis, J. B. Harborne, A. H. Williams, H. Wagner.

1966, 360 pp., $14.75

AP 1852

Academic Press
NEW YORK AND LONDON
111 FIFTH AVENUE, NEW YORK, N.Y. 10003
BERKELEY SQUARE HOUSE, LONDON W.1



Time saved . . . .
is money earned

Microtiter®

The Microtiter system for making serial dilutions has proved to be an essential tool in many of the leading hospitals, research centers, and universities. Because of savings in time, space, and reagents — this self-contained system is equally essential in private laboratories of microbiologists, immunologists, or virologists. It can perform eight serial dilutions simultaneously in minutes. The saving in reagents and sera alone is eight-fold.

When special tests are not feasible with the macro technique, or for routine tests, the Microtiter system provides absolute reliability and reproducibility.

For more information
and a complete bibliography of test procedures write:

COOKE ENGINEERING COMPANY
MEDICAL RESEARCH DIVISION
735 North Saint Asaph Street
Alexandria, Virginia

INFORMATION FOR AUTHORS

The MICROCHEMICAL JOURNAL will invite research papers on all phases of chemistry, including clinical and biochemical, which involve small-scale manipulation. This will include both organic and inorganic work on preparation, purification, separation, detection, determination, trace analyses, and all types of instrumentation.

Address. Manuscripts should be submitted to Al Steyermark, Hoffmann-La Roche Inc., Nutley, New Jersey 07110.

Manuscripts should be concise and consistent in style, spelling, and use of abbreviations. At least two copies should be submitted, each copy to include all figures and tables. The original copy must be typewritten, double-spaced, on one side of white bond paper, about 8½ x 11 inches in size, with one-inch margins on all sides. Each manuscript should have a separate title page noting title of article, authors' names (without degrees), a running title (not exceeding 35 letters and spaces), and address to which proof should be mailed. Please number all pages of manuscript.

Authors are encouraged to use descriptive subheadings in this order: Introduction, Materials and Methods, Results, Discussion, Summary, Acknowledgments, and References.

Units of weights, measures, etc. when used in conjunction with numerals should be abbreviated and unpunctuated (e.g., 10%, 50 ml, 3 g, 8 cm, 5 μg, 2 μl).

Figures. Figures should be numbered consecutively with Arabic numerals in order of mention in the text; each figure should have a descriptive legend. Legends should be typed together on a separate sheet, double-spaced. All illustrations should be in finished form ready for the engraver. A convenient size for drawings is 8½ x 11 inches. Drawings should be made with India ink on tracing linen, smooth surface white paper, or bristol board. Graph paper, if used, should be ruled in blue. Grid lines that are to show in the final engravings should be inked in black.

Photographs must be kept to a minimum and should be glossy prints with strong contrasts; the magnification should be indicated by a scale where possible.

Tables should be typed on separate pages, numbered consecutively with Arabic numerals in order of mention in the text. All tables should have descriptive headings, typed (double-spaced) above the table.

Footnotes should be designated in text by superscript numbers and listed on a separate sheet; in tables, by superscript letters and placed at bottom of page containing table.

References should be arranged alphabetically and numbered in the list in sequence. The text citations may then consist of either just the italic number of the reference enclosed in parentheses and set on the text line or of the name (or names) of the author(s) followed by the italic number enclosed in parentheses. In the text citation, if there are more than two authors of a given reference use the surname of the first author followed by "*et al.*," "and co-workers," or "and associates." All authors' names should be included in the reference list itself.

For abbreviations of journal titles in the reference list please follow the forms used in *Chemical Abstracts* (Vol. 55, 1961).

Pattern the styling, punctuation, and capitalization after these examples, noting the particular style for journal articles, books, and edited (multi-author) books.

1. AMES, S. R., SWANSON, W. J., AND HARRIS, P. L., Biochemical studies on vitamin A. XIV. Biopotencies of geometric isomers of vitamin A acetate in the rat. *J. Am. Chem. Soc.* **77**, 4134-4136 (1955).

2. PEASE, D. C., "Histological Techniques for Electron Microscopy," 274 pp. Academic Press, New York, 1960.

3. STERN, K. G., Electrophoresis and ionophoresis. *In* "Physical Techniques in Biological Research" (G. Oster, ed.), Vol. 2, pp. 61-121. Academic Press, New York, 1956.

"Personal communication" and "unpublished observations" should be cited as such in the text.

Galley proofs will be sent to the authors with reprint order forms. Fifty reprints of each article will be provided free of charge.

the updated second edition . . .

Kinetics and Thermodynamics in Biochemistry

By **H. Geoffrey Bray**
Department of Physiological Chemistry
University of Birmingham
Birmingham, England

Kenneth White
Computer Services
University of Birmingham
Birmingham, England

This updated second edition presents the most important recent developments in aspects of kinetics and thermodynamics in biochemistry. Two new chapters cover the thermodynamics of irreversible processes, and include applications of isotopic tracers to biological problems. Advanced students and teachers in biochemistry, physiology and related biological sciences, and physical chemists interested in biological problems will welcome this new edition.

CONTENTS:

1967, 413 pp., $15.00
Available from Academic Press in the United States and South America with the exception of Brazil.

AP 1844

Academic Press
NEW YORK AND **LONDON**
111 FIFTH AVENUE, NEW YORK, N.Y. 10003
BERKELEY SQUARE HOUSE, LONDON W.1