Volume 15, Number 3, September 1970

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

Microchemical Journal

devoted to the application of microtechniques in all branches of science

Volume 15, Number 3, September 1970

Published under the auspices of the American Microchemical Society by

ACADEMIC PRESS

New York and London

CONTENTS

Published quarterly at Exchange Place, Hanover, Pa. 17331
by Academic Press, Inc.,
111 Fifth Avenue, New York, New York 10003
In 1970, Volume 15 (4 issues) will be published. Price: $28.00
In 1971, Volume 16 (Quarterly) will be published. Price: $30.00
(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 at 111 Fifth Ave., New York, N.Y. 10003
Send notices of change of address to the office of the Publishers at least 4 weeks in advance. Please include both old and new address.
Second class postage paid at New York, N.Y. and other mailing offices

Microchemical Journal, Vol. 15, No. 3

Briefs

Simultaneous Determination of Carbon, Hydrogen, and Nitrogen by Means of Gas Chromatography. VLASTIMIL REZL, *Institute of Instrumental Analytical Chemistry, Czechoslovak Academy of Sciences, Brno, Leninova 82, Czechoslovakia.*

The sample is pyrolized in a tube containing a Dumas filling. The combustion products are diluted with helium in a cylindrical chamber with a pneumatically controlled piston. Chromatographic separation of the nitrogen, carbon dioxide, and water follow and are detected by means of a katharometer. Two records are kept, one for the adsorbed gases and one when they are eluted.

Microchem. J. **15,** 381 (1970).

Direct Titrimetric Microdetermination of L-Arginine. I. Direct Estimations of L-Arginine and DL-Valine, and L-Arginine and DL-Alanine; and L-Arginine, DL-Valine, and DL-Alanine Together in One Solution Without Separating. O. C. SAXENA, *Chemical Laboratories, University of Allahabad, Allahabad, India.*

Direct titration with ferrous ammonium sulfate using chromazural red S as indicator is described. The reaction results in the formation of a 1:2 complex between the ferrous ion and L-arginine.

Microchem. J. **15,** 391 (1970).

Indirect Determination of Nitrate, Nitrite, and Nitro Groups by Atomic Absorption Spectrophotometry. MARILYN E. HOUSER AND MAE I. FAUTH, *Research Directorate, Naval Ordnance Station, Indian Head, Maryland 20640.*

Complexation is done with copper(I) neocuproine and the complexed copper measured by atomic absorption spectroscopy. Nitro and nitrite are oxidized to nitrate by cerium(IV) sulfate or potassium permanganate and the nitrate is determined. Reduction of nitro and nitrite to elemental nitrogen by sulfamic acid permits the determination of nitrate in the presence of the other two types.

Microchem. J. **15,** 399 (1970).

The Coulometric Microdetermination of Iodide After Oxygen Flask Combustion of Organic Iodine Compounds. The Use of Iodate, Biiodate, and Iodide as Primary Standards. G. E. SECOR AND L. M. WHITE, *Western Regional Research Laboratory, Agriculture Research Service, U. S. Department of Agriculture, Albany, California 94710.*

Comparative results are given to show that potassium iodate, biiodate, and iodide can replace National Bureau of Standards 2-iodobenzoic acid, which is no longer available, as a standard source of iodide for determining the coulometric titration constant for iodide.

Microchem. J. **15,** 409 (1970).

Applications Involving the Iodide Ion. VI. Determination of Thallium(I) and Analysis of Its Mixtures with Some Metal Ions, H. KHALIFA AND Y. M. ISSA, *Faculty of Science, Cairo University, Giza, U. A. R.*

The potentiometric method is based on the precipitation of the slightly soluble iodide and back titration of the excess iodide, after removal of the precipitate, with mercury(II) using silver amalgam as the indicator electrode.

Microchem. J. **15,** 415 (1970).

Submicrogram Determination of Manganese with Other Elements by Polarography. V. FANO, *Istituto di Fisica dell'Università, Parma, Italy.*

Manganese was detected in concentrations of the order of $10^{-6}\%$. Copper, lead, cadmium, zinc, and nickel do not influence the height of the peaks. However, if nickel is present with copper or lead, or copper and lead, the curve for nickel overlaps that for copper and lead.

Microchem. J. **15,** 422 (1970).

Detection of Various Alicyclic Compounds Via the Komarowsky Reaction. HARVEY W. YUROW AND SAMUEL SASS, *Chemical Research Laboratory, Edgewood Arsenal, Maryland 21010.*

A considerable number of cycloalkanols, cycloalkyl ketones, and cycloalkenes have been found to give colors on condensation with aromatic aldehydes in strong sulfuric acid.

Microchem. J. **15,** 428 (1970).

Tripositive Copper as a Titrant: Determination of Some Sugars. P. K. JAISWAL, *Department of Chemistry, M.M.M.V. Bhat Par Rani, Deoria, India.*

Six sugars were determined by titration with Cu(III), carbon dioxide and water being the end products of the oxidations.

Microchem. J. **15,** 434 (1970).

Determination of the Minimal Amount of Antigen Detected by the Electroprecipitin Test on Cellulose Acetate. FREDERICK A. ZYDECK, *Department of Comparative Medicine, Wayne State University, School of Medicine, Detroit, Michigan 48207.*

This study reports the quantitative comparison of the electroprecipitin test to the precipitin technique of double diffusion in one dimension in agar gel, utilizing a crystallized egg albumin–antiegg albumin system. The electroprecipitin test detected antigen at a concentration of 0.00001 mg per ml.

Microchem. J. **15,** 438 (1970).

Spectrophotometric Determination of Silicon in the Presence of Germanium. F. A. SORRENTINO, NANCY J. WITIAK, AND J. PAUL, *Chemistry Department, University of Bridgeport, Bridgeport, Connecticut 06602.*

The method involves the precipitation of germanium with tannic acid and the solvent extraction of the excess of the latter by isoamyl alcohol. The silicon is then converted to silicomolybdic acid, which is reduced with 1-amino-2-naphthol-4-sulfonic acid in the presence of perchloric acid. The maximum color develops in 1 hour and readings are made at 690 mμ.

Microchem. J. **15,** 441 (1970).

Simultaneous Determination of Arsenic, Germanium, Phosphorus, and Silicon. F. A. SORRENTINO AND J. PAUL, *Chemistry Department, University of Bridgeport, Bridgeport, Connecticut 06602.*

Germanium is determined as the phenylfluorone complex and selectively extracted with isoamyl alcohol. Arsenic and phosphorus are determined in the aqueous phase by selective formation of the arseno- and phosphomolybdic acids after polymerization of the soluble silica with perchloric acid followed by the selective extraction of the phosphomolybdic acid in the presence of arsenomolybdic acid by isobutyl acetate. Silicon is determined by a differential procedure after selective destruction of arseno- and phosphomolybdic acids in the presence of silicomolybdic acid.

Microchem. J. **15,** 446 (1970).

Semimicrodetermination of Oxalate with a Lead-Specific Electrode. WALTER SELIG, *Lawrence Radiation Laboratory, University of California, Livermore, California 94550.*

Oxalate is titrated potentiometrically with standard lead perchlorate in 40% *p*-dioxane solution at pH 3.5 to 10.5. A lead-ion specific electrode in conjunction with an expanded-scale pH meter is used to monitor the emf. Anions forming insoluble lead salts interfere. An excess of formate, acetate, propionate, and phthalate does not interfere.

Microchem. J. **15,** 452 (1970).

Microdetermination of Glycolic Acid with Guanidine Carbonate as a Titrant. A. K. SAXENA, *Chemistry Department, University of Allahabad, Allahabad, India.*

Glycolic acid was determined by titration with guanidine carbonate using bromcresol purple as the indicator. Determinations were carried out in the range of 0.380–0.038 mg.

Michrochem. J. **15,** 459 (1970).

Coulometric Microdetermination of Oxygen in Organic Compounds. KAN-ICHI NAKAMURA, *Central Research Laboratories of Sankyo Co., Ltd., Tokyo, Japan;* MASA-AKI NISHIMURA AND TETSUO MITSUI, *Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Japan.*

Coulometry has been applied to the microdetermination of oxygen in organic compounds, making use of a newly designed Pt–P_2O_5 electrolytic cell. Platinized carbon and reduced copper are used and the resulting carbon monoxide is converted to carbon dioxide by means of copper oxide. The carbon dioxide is converted to water by means of lithium hydroxide. The water is then absorbed onto the electrolytic cell and electrolyzed.

Michrochem. J. **15,** 461 (1970).

Microdetermination of the Hydrazine Function: A New Gasometric Method Based on Oxidation with Benzoquinone. S. S. M. HASSAN AND M. T. M. ZAKI, *Research Microanalytical Laboratories, Department of Chemistry, Faculty of Science, A'in Shams University, Cairo, U.A.R.*

The gasometric method is based on the oxidation of the hydrazine group to elemental nitrogen with *p*-benzoquinone in 5% disodium hydrogen phosphate solution.

Microchem. J. **15,** 470 (1970).

A Microchemical Study of the Photodegradation of 4,4′-Bis(diethylamino) benzophenone Oxime on Silica Gel. E. J. POZIOMEK, *Physical Research Laboratory, Edgewood Arsenal, Maryland 21010,* AND A. F. FENTIMAN AND R. H. POIRIER, *Battelle Memorial Institute, Columbus, Ohio 43201.*

This paper describes a microchemical study of the photoinduced decomposition of the oxime mentioned in the title. The degradation products include 4,4′-bis (diethylamino)benzophenone, 4,4′-bis(diethylamino)benzanilide, *N*-(*p*-ethylaminophenyl)-*p*-diethylaminobenzamide, and 4,4′-bis(diethylamino)benzophenone imine.

Microchem. J. **15,** 475 (1970).

An Automatic Microdetermination of Carbon, Hydrogen, and Nitrogen in Organic Compounds Using Data Printing System. KEIICHIRO HOZUMI, *Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan;* OSAMU TSUJI AND HIDEYA KUSHIMA, *Research and Development Division, Yanagimoto Mfg. Co. Ltd., Kyoto, Japan.*

An automatic instrumentation for the microdetermination of carbon, hydrogen, and nitrogen in organic compounds involving an automatic sample charger, a double action pump, and a data printing system is described. Twelve samples are placed in the apparatus and sequentially charged into the combustion tube. Helium is used as the carrier gas and pushes the combustion products to a series of three differential thermal conductometers. An analog-digital converter is used to print out the successive signals from the thermal conductometers.

Microchem. J. **15,** 481 (1970).

MICROCHEMICAL JOURNAL 15, 381–390 (1970)

Simultaneous Determination of Carbon, Hydrogen, and Nitrogen by Means of Gas Chromatography

VLASTIMIL REZL

Institute of Instrumental Analytical Chemistry of Czechoslovak Academy of Sciences, Brno, Leninova 82, Czechoslovakia

Received September 30, 1969

INTRODUCTION

The utilization of gas chromatographic separation in the elemental analysis of carbon, hydrogen, and, if need be, nitrogen has been studied by a number of authors (*1, 7, 8, 13, 14, 15, 17*). Some automatic instruments, now commercially available (*3, 9*), are also based on the above principle. The initial difficulties associated with rapid single-step separation of nitrogen, carbon dioxide, and water have been eliminated by the introduction of new sorbents, based on vinylethylbenzene–divinylbenzene (*10, 11*), which enhanced considerably the attractivity of gas chromatographic separation for the purpose of elemental organic analysis. However, to meet the standard of precision of $\pm 0.3\%$, accepted in elemental analysis, it is necessary to strictly observe the conditions of constant carrier gas flow rate through the column as well as the temperature of the latter. The use of a thermal conductivity detector has associated with it further limitations, such as the linearity range of response (necessity of working with submiligram sample sizes) and also the detector time constant. After all, the method of processing and recording of the detector signal presents also a contribution to the resultant error of the determination, irrespective of the expensiveness of the final instrumentation. In order to avoid some of the above limitations, a method has been suggested and tested which is simple and gives satisfactory results.

MATERIALS AND METHOD

A scheme of the instrumental arrangement is illustrated in Fig. 1. The sample weighed out is put on a spoon at the end of a quartz rod, *A*, introduced into the combustion tube, and closed tightly in the tube by means of a cap nut with silicone rubber sealing. After purging the combustion space by helium, led through the needle valve J_2 and

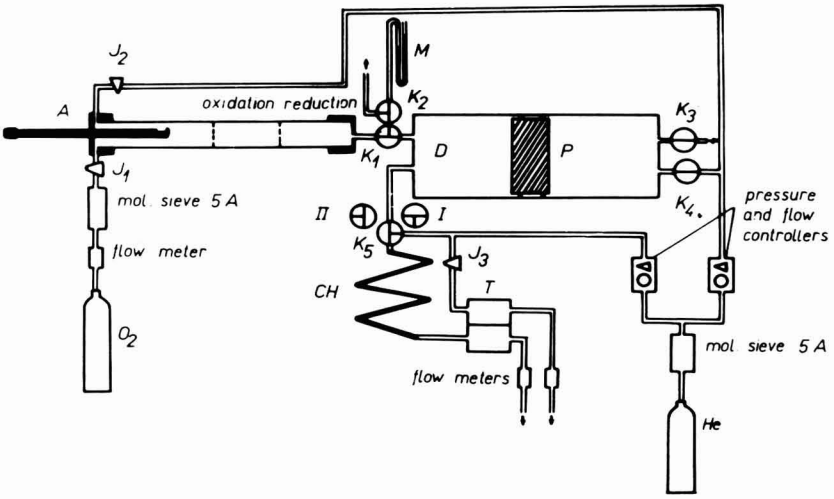


FIG. 1. Scheme of the instrumental arrangement.

stopcocks K_1 and K_2, the combustion is carried out, at either static or dynamic conditions, by inserting the sample into the pyrolyzing zone, while adding or after having added oxygen, if need be, via the needle valve J_1. The combustion products are carried by the helium through the stopcock K_1, while having connected the manometer, *M*, into a chamber, *D*, with a piston, *P*, which is in the left extreme position at the beginning of analysis. Upon the originated excess pressure of the gas, the piston *P* is shifted towards the right extreme position, the stopcocks K_3, K_4, and K_5 being in off, on and *I* positions, respectively. As soon as the piston arrives at the right extreme position, the pressure in the chamber *D* starts rising. After reaching a chosen value of the excess pressure (the same in all cases, for example 200 mm Hg) the chamber is closed off by means of the stopcocks, K_1 and K_3, and let stand for some time (2–3 minutes) to allow diffusional equilibration. Meanwhile, helium is streaming at a constant flow rate (about 20 ml/minute) through the stopcock K_5, chromatographic column, *CH*, and measuring part of the thermal conductivity detector, *T*. After attaining diffusional equilibrium and setting the pressure in the chamber equal to that at the column inlet, by turning off the stopcock K_4, the stopcock K_5 is turned to the position *II*, and the content of the chamber is pushed by the piston into the column *CH* and the measuring part *T*. The chromatographic column is packed with Porapak P or Q (Waters Associates, Inc., Framingham, Mass.). The separation of nitrogen, carbon dioxide, and water proceeds by frontal chromatography (*4*), and the heights of the individual concentration steps recorded are proportional to the

concentrations of the respective components in the chamber *D*. After that, pure helium starts again being introduced through the stopcock K_5 upon its turning off, into the column, from which the combustion products previously sorbed are gradually eluted in a form of desorption concentration steps. The shape of the sorption–desorption record is evident from Fig. 2. During the desorption, it is possible to combust another sample in the same way as described above, as the combusting and diluting parts operate independently of the chromatographic part. The pressure and gas flow controls are carried out in the conventional way, using differential regulators, the purifying of the gases being accomplished by passing them through a layer of activated molecular sieve 5 *A*, or in another convenient way.

Combustion Part

A 50-cm long quartz combustion tube of 10-mm inner diameter was packed by the decomposition product of silver permanganate (*12*) over a length of 5 cm, and by reduced copper, of about 20 mesh in particle size, over a length of 20 cm, to capture free oxygen and reduce nitrogen oxides. Samples were weighed out in a small boat of aluminum sheet by a Cahn Gram-Electrobalance balance (Cahn Instr. Co. Paramount, California). The sample weighed out was placed on a 20-mm long quartz spoon of 5 mm in width, provided at the end of a 25-cm long quartz sampling rod of 6-mm outer diameter. The sample was covered by powdered Co_3O_4 to achieve perfect combustion. However, the combusting part may be modified successfully in various ways (cf., e.g. *2,6,16*).

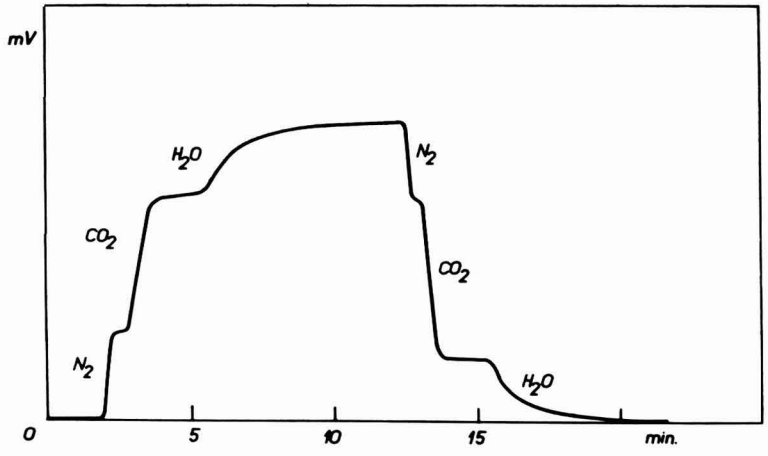


FIG. 2. Sorption–desorption record.

Diluting Chamber

A 22-cm long cylindrical chamber of 4.2-cm inner diameter was made of stainless steel and finely polished inside. Removable stainless faces, provided with inlets of 1 mm in the open diameters, rendered tight closures through use of screws and silicone rubber gaskets. The Teflon piston (4.1-cm diameter and 2.5-cm height) was provided with two grooves for silicone rubber sealing rings ensuring the tightness of the piston. The perfection of tightness of the piston as well as the frictional resistance of the latter depend on the perfection of manufacturing the above grooves. A higher attention has to be paid also to the dead volume in the space between the piston in its left extreme position and the cylinder face, which causes, along with the inlets, holding up the residues of combustion products from the previous analysis. In our case, the corresponding space was interconnected with the combustion part by means of the stopcock K_1 during purging of the former, so that most of the combustion products were removed by diffusion from the dead space. The inner surface of the chamber was smeared by silicone oil 550, which decreased considerably the friction of the piston, enhanced the tightness of the latter, and prevented water from its sorption on the wall. The piston was easy to move by as low overpressure as 20–40 mm Hg. The tightness of the piston was tested by an overpressure of 400 mm Hg.

Chromatographic Column

The column was made from a stainless tube of 2-mm inner diameter, was 200-cm long and wound into a helix. As a packing, Poropaks P or Q of 100–120 mesh in particle size were found convenient.

Detection System

A common type of four filament katharometer with flow-through cells served as a dector, namely, a C8 type of detector, source of stabilized 12-V tension, and Wheatstone bridge, (all products of the ÚACHP, Prague-Satalice). The detector response was recorded by an EZ-3 recorder (Laboratory Instruments, Prague) providing for switching the measuring range over 5 scales to the right or left. This rendered it possible to expand the signal over several scales (mainly in cases of the determination of carbon) and thus a more precise determination. The records were processed by measuring, both at sorption and desorption part of the chromatogram, the heights of the individual steps, corresponding to the nitrogen, carbon dioxide, and water.

Thermostat and Other Accessories

The diluting chamber, chromatographic column, and detector were placed in an ordinary liquid thermostat with a temperature stability of ±0.05°C. For work with Porapak P, an optimum temperature of 75°C was found; this temperature was determined mainly by the degree of separation of nitrogen and carbon dioxide. When determining only carbon and hydrogen, it is possible to raise the column temperature up to 120°C, which obviously shortens considerably the time of analysis. All interconnections in the instrument were made from stainless and copper capillaries of 1-mm open diameter. The function of valves was supplied by glass two- and three-way stopcocks.

CALCULATION

The height of a concentration step in the chromatographic record is proportional to the concentration of a component chromatographed only if there occurs no competitive sorption on the sorbent used, and provided the response of the thermal conductivity detector is linear. The above assumptions can be met only in a region of low concentrations, as in our case. The responses to the individual components, determined by measuring the heights (mm) of the respective steps in the chromatographic record, have to be corrected for the normal pressure. Then

$$h_i = h_i'(P + \Delta P)/760$$

where h_i is the corrected response to a component $_i$(mm), h_i' is the measured, noncorrected, response to the component (mm), P is the atmospheric pressure (mm Hg), and ΔP is the excess pressure in the diffusion chamber (mm Hg).

It is necessary to subtract from h_i' the height of the background signal, measured in a blank experiment. An advantageous procedure is that with the use of a calibration curve [the dependence of μg(i) on h_i], in which case

$$\%(i) = 100\mu g(i)/w,$$

where w is the amount of sample weighed out (μg). In case that the state conditions in the diffusion chamber as well as the conditions in the thermal conductivity detector (voltage applied, filament resistance, gas flow rate—particularly with through-flow cells) are reproducible, the sensitivity $S(\mu g/mm)$ defined by

$$S_i = \mu g(i)/h_i,$$

can be assumed to be the proportionality constant between the percentage content, % (i), and the corrected response h_i. Then

$$\%(i) = 100S_i h_i'(P + \Delta P)/760w.$$

It is appropriate to plot calibration curves, or, if need be, to determine the sensitivities separately for the sorption and desorption parts of the record, as the flow conditions are rarely identical in both phases of analysis. In very high precision measurements, it is necessary to carry out the corrections of the responses h_i for the changes in mole fractions of the components, coming about during the sorption-desorption process, as described in the selective absorption process (*5*).

RESULTS

It is apparent from Figs. 3,4, and 5 that the responses to nitrogen, carbon, and hydrogen are proportional to their amounts. Table 1 shows, at the same time, the results of a series of analyses of different samples. With amounts weighed out of within 0.5–2.5 mg, the standard deviation of a single determination of C and H, estimated from 18 measurements; and of N, from 13 measurements; amounted to 0.15, 0.15, and 0.2, respectively.

DISCUSSION

The above-described method of elemental analysis of carbon, hydrogen, and nitrogen has several interesting assets:

1. Two records are obtained from a single sample.

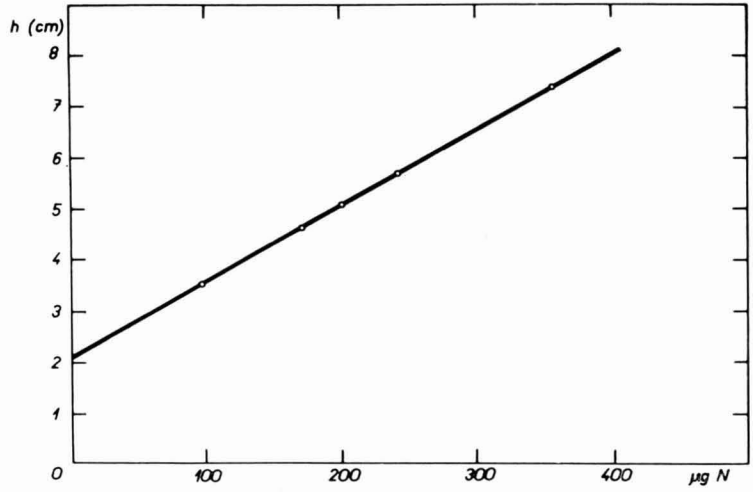


FIG. 3. Checking the linearity of response to N.

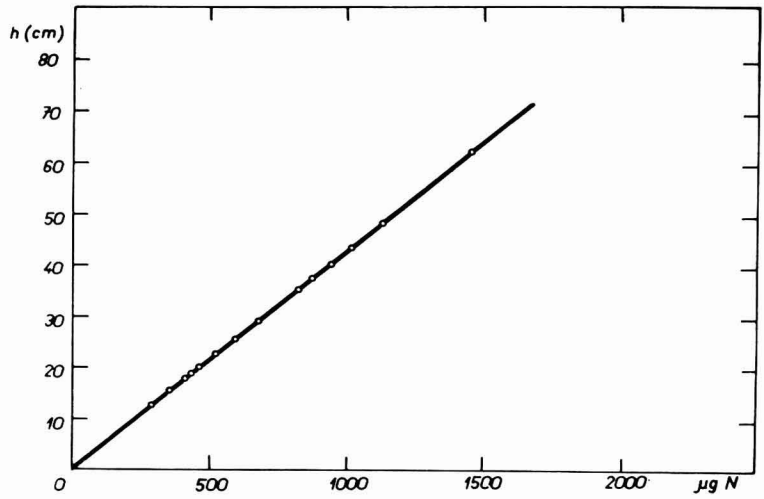


FIG. 4. Checking the linearity of response to C.

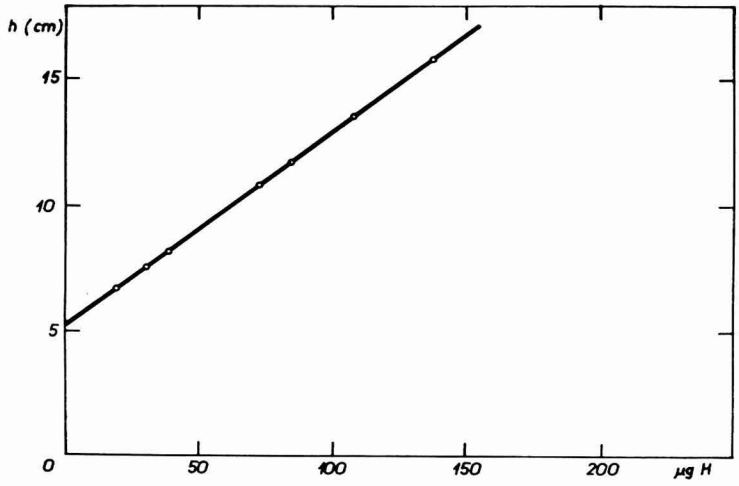


FIG. 5. Checking the linearity of response to H.

2. No integrator is necessary, the measurement of the detector response is simple.

3. The set-up is simple and apt for automation.

4. The arrangement renders a possibility of a more universal utilization, e.g., the determination of oxygen.

The following factors affect the resultant error: weighing out; setting the pressure, temperature, and volume in the diluting chamber; recording of the signal and its measuring by a rule; and fluctuation of the voltage applied to the detector and of the carrier gas flow rate,

TABLE 1

SIMULTANEOUS DETERMINATION OF CARBON, HYDROGEN AND NITROGEN

Sample	Sample wt (mg)	C (%)		H (%)		N (%)	
		Found	Deviation	Found	Deviation	Found	Deviation
Sucrose	0.8155	42.06	−0.04	6.62	+0.14		
	2.0495	42.05	−0.05	6.44	−0.04		
	1.3075	42.14	+0.04	6.61	+0.13		
	0.7235	42.15	+0.05	6.70	+0.22		
	0.8355	42.25	+0.15	6.22	−0.26		
TAMH II [a]	1.0490	50.90	−0.15	3.71	−0.15	17.73	−0.13
	0.4350	51.03	−0.02	3.68	−0.18	17.93	+0.07
	1.0000	51.10	+0.05	4.00	+0.14	17.70	−0.16
	1.3740	50.95	−0.10	3.88	+0.02	17.87	+0.04
	2.0575	51.05	±0.00	3.86	±0.00	17.88	+0.05
	1.0910	51.23	+0.18	3.85	−0.01	17.41	−0.42
Phenacetin	1.2895	67.15	+0.13	7.33	+0.02	7.83	+0.01
	0.8685	66.78	−0.24	7.36	+0.05	7.61	−0.22
	0.7275	66.94	−0.08	7.56	+0.25	7.50	−0.32
	2.2380	67.11	+0.09	7.21	−0.10	7.80	−0.02
	1.1170	67.32	+0.30	7.34	+0.03	7.90	+0.08
Azobenzene	1.5385	79.03	−0.06	5.36	−0.17	15.40	+0.03
	0.7245	79.36	+0.27	5.38	−0.15	15.61	+0.24

[a] 2-(2-thiazolylazo)-4-methoxyphenol.

irrespective of the effect of temperature on the sorption equilibrium in the column. When employing a detector with semidiffusion or diffusion cells, the effects of carrier gas flow fluctuation can be neglected. With precise temperature control ($< 0.05°C$), the effect of temperature fluctuations within the thermostat on the sorption equilibrium may also be neglected. The question of a statistical analysis of the errors associated with the individual factors has been dealt with, for a similar type of elemental analyzer, by Clerc *et al.* (*5*). Analogously, if there holds in our case for the percentage of a component being determined

$$X(\%) = khPV/wTE^3,$$

where k is a proportionality factor; h is the measured height of the signal recorded (mm), corresponding to X; w is the sample amount weighed out (μg); P is the pressure in the diluting chamber (mm Hg); V is the volume of the diluting chamber; T is the temperature in the diluting chamber (°K); E is the voltage applied to the katharometer (V). Hence there holds for the variance S^2 of the determination of component X, provided both sorption and desorption records have been evaluated,

$$S_X^2 = X^2\left[\frac{S_h^2}{2h^2} + \frac{S_l^2}{2l^2} + \frac{9S_E^2}{2E^2} + \frac{S_P^2}{P^2} + \frac{S_V^2}{V^2} + \frac{S_T^2}{T^2} + \frac{S_w^2}{w^2}\right],$$

where l is the height of the signal records, as measured with a rule (mm). In case of evaluating only one record, there holds

$$S_X^2 = X^2\left[\frac{S_h^2}{h^2} + \frac{S_l^2}{l^2} + \frac{9S_E^2}{E^2} + \frac{S_P^2}{P^2} + \frac{S_V^2}{V^2} + \frac{S_T^2}{T^2} + \frac{S_w^2}{w^2}\right].$$

The above relations make it possible to estimate the errors of the final determination of the individual components if the respective conditions are known and, on the other hand, to set the conditions for a designated error.

SUMMARY

The method described of elemental analysis of carbon, hydrogen, and nitrogen is based on the oxidation pyrolysis of sample in a tube containing Dumas' filling; dilution of the combustion products with helium in a cylindrical chamber with pneumatically controlled piston; setting of the diffusional equilibrium at constant state conditions in the chamber; chromatographic separation of the nitrogen, carbon dioxide, and water by the frontal technique; and detection of the above substances by a katharometer. Two records, those of sorption and desorption, are obtained from a single sample. The use of both records makes it possible to decrease considerably the error of determination, brought about by the chromatographic, detection, and recording parts of the instrument. The expanding of the detector signal over several recorder scales is used to an advantage. The

method provides for the combustion under both static and dynamic conditions, as well as work on submicro-, micro-, and, if need be, semimicroscales.

REFERENCES

1. Belcher, R., Dryhurst, G., MacDonald, A. M. G., Majer, J. R., and Roberts, G. J., The submicrodetermination of carbon, hydrogen and nitrogen by a gas-chromatographic method. *Anal. Chim. Acta* **43,** 441–450 (1968).
2. Belcher, R., and Ingram, G., A rapid micro-combustion method for the determination of carbon and hydrogen. *Anal. Chim. Acta* **4,** 118–129 (1950).
3. Carlo Erba Scientific Instruments Div., "Elemental Analyzer," Model 1100.
4. Claesson, S., Theory of frontal analysis and displacement development, *Discuss. Faraday Soc.* **7** (1949); repr. by Butterworths, London, 34–38 (1963).
5. Clerc, J. T., Dohner, R., Sauter, W., and Simon, W., Vollautomatische Anlage zur gleichzeitigen Bestimmung von Kohlenstoff, Wasserstoff und Stickstoff in organischen Verbindungen an Milligram- und Submilligram-Mengen unter Verwendung einer selbstintegrierenden Wärmeleitfähigkeitmessmethodik. *Helv. Chim. Acta* **46,** 2369–2388 (1963).
6. Culmo, R., Automatic microdetermination of carbon, hydrogen, and nitrogen: improved combustion train and handling techniques. *Mikrochim. Acta* **1969,** 175–180.
7. Duswalt, A. A., and Brandt, W. W., Carbon-hydrogen determination by gas chromatography. *Anal. Chem.* **32,** 272–274 (1960).
8. Haber, H. S., and Gardiner, K. W., A rapid instrumental method for the microdetermination of carbon and hydrogen in organic compounds. *Microchem. J.* **6,** 83–90 (1962).
9. Hewlett-Packard-F & M Scientific Div., Bulletin 1850–2.
10. Hollis, O. L., Separation of gaseous mixtures using porous polyaromatic polymer beads. *Anal. Chem.* **38,** 309–316 (1966).
11. Hollis, O. L., and Hayes, W. V., Gas separations on microporous polymers. *In* "Gas Chromatography 1966" (A. B. Littlewood, ed.) Inst. of Petrol., pp. 57–74. London, 1966.
12. Körbl, J., Verwendung des thermischen Zersetzungsproduktes von Silberpermanganat in der organischen Elementaranalyse. *Mikrochim. Acta* **1956,** 1705–1721.
13. Maresch, C., Sundberg, O. E., Hofstader, R. A., and Gerhard, G. E., Microdetermination of carbon, hydrogen and nitrogen by gas chromatography. *Microchem. J. Symp. Ser.* **2,** 387–396 (1962).
14. Nightingale, C. F., and Walker, J. M., Simultaneous carbon—hydrogen–nitrogen determination by gas chromatography. *Anal. Chem.* **34,** 1435–1437 (1962).
15. Sundberg, O. E., and Maresch, C., Application of gas chromatography to microdetermination of carbon and hydrogen. *Anal. Chem.* **32,** 274–277 (1960).
16. Večeřa, M., The isothermic and adiabatic combustion of organic substances. *Fresenius' Z. Anal. Chem.* **208,** 15–26 (1965).
17. Vogel, A. M., and Quattrone, J. J., Rapid gas chromatographic method for determination of carbon and hydrogen. *Anal. Chem.* **32,** 1754–1757 (1960).

MICROCHEMICAL JOURNAL 15, 391–398 (1970)

Direct Titrimetric Microdetermination of L-Arginine

I. Direct Estimations of L-Arginine and DL-Valine, and L-Arginine and DL-Alanine; and L-Arginine, DL-Valine, and DL-Alanine Together in One Solution Without Separating

O. C. SAXENA

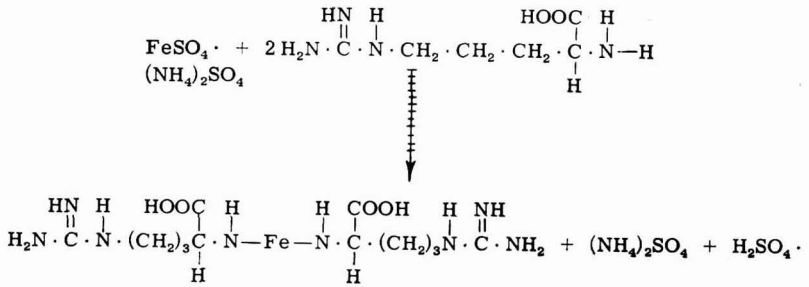
Chemical Laboratories, University of Allahabad, Allahabad, India

Received September 30, 1969

INTRODUCTION

Available literature, concerning the determination of L-arginine, fails to show direct titrimetric methods separately. Until now, only Libicky and Wunsch (*5*) have been successful in producing a direct titrimetric method for the determination of certain amino acids with cupric sulfate solution. However, L-arginine has also been determined by a mixture of $CuCl_2$ and Na_2HPO_4 (*1*); potentiometrically (*2*); by the substitution of oxime for 1-naphthol by Macpherson's procedure (*3*); with ceric sulfate (*4, 15*); by ion-exchange chromatography (*6, 7*); by oxidizing it to components with alkaline gold chloride and then back-titrating the remaining gold (*8*); and spectrophotometrically (*13, 14*).

Present type of work has been proposed to solve the problem of quantitative determination of amino acids separately and in presence of each other. In the first part of this work, L-arginine has been determined directly in micro amounts by titrating against ferrous ammonium sulfate using chromazural red S as indicator. Potentiometric titrations and data of analysis show that a complex between ferrous ammonium sulfate and L-arginine is formed in the ratio of 1:2. Probably the following reaction takes place:



Second part of the work deals with the determination of L-arginine and DL-valine; L-arginine and DL-alanine; and L-arginine, DL-alanine, and DL-valine together in the form of mixtures without separating by titrating directly. DL-alanine and DL-valine have already been directly titrated against gold chloride (*9*) and potassium tellurite (*10*), respectively. Gold chloride (chlorauric acid $HAuCl_4$) and potassium tellurite form complexes with DL-alanine and DL-valine in the ratios of 1:3 and 1:4, respectively.

EXPERIMENTAL METHODS

Reagents Used: L-arginine, DL-valine, DL-alanine, and potassium tellurite (E. Merck grade); ferrous ammonium sulfate (ANALAR B. D. H. grade); chlorauric acid (Palmston grade); chromazural red S, xylenol orange, and Congo red (B.D.H. grade).

Apparatus used: Micropipette and microburettes used had least count = 0.01 ml.

Standard DL-valine, DL-alanine, L-arginine, and ferrous ammonium sulfate solutions were prepared by dissolving the exactly weighed amount in distilled water. Chlorauric acid and potassium tellurite solutions were prepared by dissolving an exact quantity in distilled water—which were further standardized by methods in Refs. (*14*) and (*15*), respectively. Xylenol orange, Congo red, and chromazural red S solutions were prepared by dissolving 0.1 g in 100 ml of distilled water.

Procedure

I. Direct determination of L-*arginine separately.* A known volume of standard L-arginine solution was taken in a beaker, through a micropipette, and diluted to 30 ml by adding distilled water. A few drops (2 to 3) of chromazural red S solution were added and the solution became a light yellow color. This solution was titrated against a standard ferrous ammonium sulfate (in 0.1 *N* H_2SO_4) solution till the color finally changed to purple.

II. Direct determination of L-*arginine and* DL-*valine together in one solution without separating.* Known volumes of a standard L-arginine and DL-valine solutions were taken in a beaker, through a micropipette, and diluted to 30 ml by adding distilled water. Thus, this formed a mixture of two amino acids, to which a few drops (2 to 3) of chromazural red S solution were added and the whole solution mixture became a light yellow color. In such a solution mixture, L-arginine is first titrated by addition (from a microburette) of a standard ferrous ammonium sulfate (in 0.1 *N* H_2SO_4) solution until the color sharply changes to purple violet at the end point. At this stage, a few drops (2 to 3)

of Congo red solution were added and the entire solution became violet. Now, DL-valine was titrated by adding a standard potassium tellurite solution until the color sharply changed to purple at the end point.

Xylenol orange may also be used as an indicator. It is used after titrating L-arginine by adding ferrous ammonium sulfate and using chromazural red S. On adding a few drops (2 to 3) of xylenol orange solution to the violet colored solution mixture of xylenol orange solution to the violet colored solution mixture of amino acids, DL-valine was then titrated by adding potassium tellurite solution till the appearance of a pink color at the end point.

III. Direct determination of L-*arginine and* DL-*alanine together in one solution without separating.* Known volumes of L-arginine and DL-alanine were taken in a beaker and diluted to 30 ml by adding distilled water, which forms a solution mixture of two amino acids. In this mixture, if L-arginine is to be titrated first, then a few drops (2 to 3) of chromazural red S solution were added and the solution mixture became a light yellow color. Then standard ferrous ammonium sulfate (0.1 *N* H_2SO_4) solution was run, through a microburette, into the beaker, containing the above mentioned solution, until the color changed pink and finally violet. At this stage, DL-alanine was titrated in the same solution mixture, without adding any other indicator, by running into the beaker, containing violet colored solution, a standard chlorauric acid ($HAuCl_4$) solution until the appearance of a blue color sharply.

If xylenol orange solution is added to the solution mixture then a pink color appears and DL-alanine is first titrated against standard gold chloride solution till the appearance of very light yellow color at the end point. Then, in the same solution L-arginine is titrated against a standard ferrous ammonium sulfate solution until the appearance of a pink color at the end point.

Congo red solution may also be used as indicator if DL-alanine solution in the mixture is to be titrated first. In this case, the solution mixture assumes a red color on adding a few drops (2 to 3) of Congo red solution, which is titrated for DL-alanine against standard chlorauric acid solution until the appearance of an orange color. Then, in the same solution L-arginine is titrated against ferrous ammonium sulfate solution until the orange color changed completely to a violet color.

IV. Direct determination of L-*arginine,* DL-*valine and* DL-*alanine in the form of a mixture without separating.* Known volumes of standard L-arginine, DL-valine, and DL-alanine solutions were placed in a beaker, through a micro-pipette, and diluted to 30 ml by adding distilled water. Thus, this formed a solution mixture of three amino acids. A few drops

(2 to 3) of chromazural red S solution were added and the whole solution mixture became a light yellow color; and L-arginine was first titrated against standard ferrous ammonium sulfate solution (in 0.1 N H_2SO_4) until a purple color appeared sharply at the end point. In the second phase of the titration DL-valine was titrated, in the same solution mixture containing purple color, against standard potassium tellurite solution till the appearance of pink color. Then in the third and last phase of the titration, DL-alanine was titrated, in the same solution containing pink color, against standard chlorauric acid solution till a blue color appeared sharply at the end point. Thus, all three amino acids were titrated in a mixture without separating, of course, in a particular order.

RESULTS AND DISCUSSION

Results have been given in Tables 1, 2, 3, and 4. Ranges in which L-arginine, DL-valine, and DL-alanine have been estimated vary from 1.7072×10^{-4} to 35.5090×10^{-4} mg/liter; from 11.6956×10^{-4} to 29.2390×10^{-4} mg/liter; and from 4.6602×10^{-4} to 23.3010×10^{-4} mg/liter, respectively.

Table 1 shows the titration results, where L-arginine has been separately determined. Maximum error is 1.9%. Table 2 shows that DL-valine and L-arginine have been determined together in one solution without separating. It is observed that L-arginine is first titrated in such a solution mixture. For the titration of DL-valine, in the second last stage, Congo red and xylenol orange indicators have been used; and in both the cases the change at the end point is sharp and perceptible. When Congo red solution was added, after titrating L-arginine, then the entire solution mixture became violet colored, which changed to purple at the end point on adding potassium tellurite. But on adding

TABLE 1

MICRODETERMINATION OF L-ARGININE

L-Arginine 0.02 M (ml)	$FeSO_4 \cdot (NH_4)_2SO_4$ 0.0098 M (ml)	Amount of L-arginine ($\times 10^4$ mg/liter)		Error (%)
		Taken	Found	
0.05	0.05	1.742	1.7072	1.9
0.10	0.10	3.484	3.4144	1.9
0.30	0.31	10.452	10.5844	1.2
0.40	0.41	13.936	13.9987	0.4
0.70	0.72	24.388	24.5831	0.7
1.00	1.04	34.840	35.5090	1.90

TABLE 2

MICRODETERMINATION OF L-ARGININE AND DL-VALINE TOGETHER IN ONE SOLUTION WITHOUT SEPARATING

L-Arginine 0.02 *M* (ml)	$FeSO_4 \cdot (NH_4)_2SO_4$ 0.0098 *M* (ml)	Amount of L-arginine ($\times 10^4$ mg/liter)		DL-valine 0.01 *M*	K_2TeO_3 0.0104 *M*	Amount of DL-valine ($\times 10^4$ mg/liter)	
		Taken	Found			Taken	Found
0.2	0.2	6.968	6.8288	2.5	0.6	29.2875	29.239
0.3	0.3	10.452	10.5844	2.0	0.48	23.4300	23.3912
0.4	0.4	13.936	13.9987	1.5	0.36	17.5725	17.5434
0.5	0.5	17.420	17.0720	1.0	0.24	11.7150	11.6956

TABLE 3

MICRODETERMINATION OF L-ARGININE AND DL-ALANINE TOGETHER IN ONE SOLUTION WITHOUT SEPARATING

L-Arginine 0.02 *M* (ml)	$FeSO_4 \cdot (NH_4)_2SO_4$ 0.0098 *M* (ml)	Amount of L-arginine ($\times 10^4$ mg/liter)		DL-alanine 0.04 *M* (ml)	$HAuCl_4$ 0.0078 *M* (ml)	Amount of DL-alanine ($\times 10^4$ mg/liter)	
		Taken	Found			Taken	Found
0.5	0.5	17.420	17.0720	0.1	0.17	4.686	4.6602
0.4	0.41	13.936	13.9987	0.2	0.34	9.372	9.3205
0.3	0.31	10.452	13.5844	0.3	0.52	14.058	14.2550
0.2	0.20	6.968	6.8288	0.4	0.70	18.744	18.6209
0.1	0.10	3.484	3.4144	0.5	0.85	23.430	23.3010

xylenol orange solution to the solution mixture, after titrating L-arginine, a purple violet color appeared, which changed to pink color at the end point. For DL-valine the maximum error is 0.1%.

Table 3 shows the titration results of L-arginine and DL-alanine together in one solution without separating. It is observed that with chromazural red S solution as indicator, L-arginine is first titrated until the color changes from light yellow to violet, against ferrous ammoninum sulfate. In the same solution, DL-alanine is titrated against chlorauric acid until blue color appeared at the end point. When xylenol orange or Congo red solution is used, then DL-alanine is first titrated. In the case of xylenol orange, the solution mixture first becomes a pink color, which changes to light yellow color on addition of chlorauric acid. In the same solution, ferrous ammonium sulfate solution is added for titrating L-arginine without adding further indicator, and the light yellow color changed to pink rose color. When a few drops (2 to 3) of Congo red solution were added in the solution mixture, then the whole solution became red and DL-alanine was titrated against chlorauric acid until orange color appeared. In the same solution mixture, containing orange color and without adding further indicator, L-arginine was titrated against ferrous ammonium sulfate solution until the appearance of violet at the end point. In the case of DL-alanine, the maximum error is 1.4%.

Table 4 shows that L-arginine, DL-valine, and DL-alanine have been directly titrated together in one solution without separating. The most peculiar feature with these titrations, where these three are present at a time, is that only one indicator, chromazural red S, has been used. The order of titrations start from L-arginine, DL-valine, and then DL-alanine against ferrous ammonium sulfate, potassium tellurite, and chlorauric acid with color changes from light yellow to purple, from purple to pink, and then from pink to blue, respectively.

Since the complexes formed between ferrous ammonium sulfate and L-arginine, chlorauric acid and DL-alanine, and potassium tellurite and

TABLE 4

L-Arginine 0.02 *M* (ml)	$FeSO_4 \cdot (NH_4)_2SO_4$ 0.0098 *M* (ml)	DL-Valine 0.01 *M* (ml)	K_2TeO_3 0.0104 *M* (ml)	DL-Alanine 0.04 *M* (ml)	$HAuCl_4$ 0.0078 *M* (ml)
0.6	0.61	1.0	0.24	0.4	0.70
0.4	0.41	1.5	0.36	0.3	0.52
0.3	0.31	2.0	0.48	0.2	0.34
0.2	0.20	2.5	0.60	0.1	0.17

DL-valine are in the ratios of 1:2, 1:3, and 1:4, respectively, hence the observed values have been multiplied by 2, 3, and 4, respectively.

Excellent reproductibility has been observed in these titrations, which give good results. These methods are better than other methods, described earlier, in the fact that they are simple, reproductible, and very much less time consuming.

ACKNOWLEDGMENT

The author is thankful to the Council of Scientific & Industrial Research (Government of India), New Delhi, for providing financial assistance.

SUMMARY

L-Arginine has been determined quantitatively in micro amounts by direct titration with ferrous ammonium sulfate using chromazural red S as indicator. The reaction results in the formation of 1:2 complex between ferrous ions and L-arginine. Maximum error is 1.9%. L-Arginine has been determined in combinations with DL-valine and DL-alanine, and all together with these three in one solution without separating. Reproducible results are observed.

REFERENCES

1. Hayashi, I. Y., Microdetermination of amino acids. *Saikyo. Daigaku Gakujutsu Hokoku Rigaku Oyobi Kaseigaku.* **2,** 41 (1956).
2. De O'Hauna, R. W., Potentiometric titrations of amino acids in non aqueous medium. *Rev. Fac. Farm., Univ. Cent. Venez.* **4,** (9), 81 (1963).
3. James, J. W., Determination of arginine. *Nature (London)* **177,** 529 (1956).
4. Kimoto, K., Industrial organic analysis with ceric sulfate. *Rep. Inst. Sci. Technol., Tokyo Univ.* **3,** 20 (1952).
5. Libicky, A., and Wunsch, L., Direct titrimetric determination of amino acids with Cu(II) sulfate solution. *Collect. Czech. Chem. Commun.* **26,** 2663 (1961).
6. Lukov, B. J., and Buckley, E. N., Analysis of tyramine by ion exchange chromatography. *J. Cromatogr.* **15** (2), 258 (1964).
7. Roberts, H. R., and Kolor, M. G., Rapid quantitative determination or arginine, histidine and lysine by ion-exchange paper chromatography. *Anal. Chem.* **31,** 565 (1959).
8. Saxena, O. C., Titrimetric microdetermination of L-arginine, L-asparagine, and keto hexoses: gold chloride as oxidizing agent. *Microchem. J.* **13,** 571 (1968).
9. Saxena, O. C., Microdetermination of DL-alanine. II. Microdetermination of L-glutamic acid, DL-valine, *and* DL-alanine in a mixture in one solution without separating. *Microchem. J.* **14,** 343 (1969).
10. Saxena, O. C., Direct titrimetric microdetermination of L-asparagine and DL-valine. I. Determination of L-asparagine and DL-valine in presence of each other without separating. *Microchem. J.* **14,** 385 (1969).
11. Saxena, O. C., A new volumetric for the microdetermination of tellurium and mercury. *Mikrochim. Act* **6,** 1123 (1967).
12. Saxena, O. C., A new volumetric method for the estimation of gold. *Indian J. Appl. Chem.* **30** (1–2), 33 (1967).

13. Satake, K., and Luck, J. M., The spectrophotometric determination of arginine by Sakaguchi reaction. *Bull. Soc. Chim. Biol.* **40,** 1743 (1958).
14. Strocchi, P. M., and Drogo, P., Determination of arginine by photometry. Ann. Chim. (*Rome*) **44,** 836 (1954).
15. Takahashi, T., Kimoto, K., and Minami, S., Volumetric analysis of amino acids. *J. Chem. Soc. Jap., Ind. Chem. Sect.* **56,** 417 (1953).

MICROCHEMICAL JOURNAL 15, 399–408 (1970)

Indirect Determination of Nitrate, Nitrite, and Nitro Groups by Atomic Absorption Spectrophotometry

MARILYN E. HOUSER AND MAE I. FAUTH

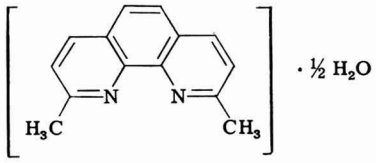
Research Directorate, Naval Ordnance Station, Indian Head, Maryland 20640

Received October 10, 1969

INTRODUCTION

An indirect method for the determination of nitro, nitrite, and nitrate groups has been developed. It is based on complexation of these with copper neocuproine, extraction into methyl isobutyl ketone (MIBK), and measurement of complexed copper by atomic absorption spectroscopy (AAS). This is a modification of method of Kumamaru *et al.* (*7*).

Neocuproine, $(CH_3)_2C_{12}H_6N_2 \cdot 1/2H_2O$, is 2,9-dimethyl-1,10-phenanthroline, FW 217.27:



Wilson and Wilson (*14*) state that the exclusive selectivity of the reagent for copper is due to steric hindrance of the methyl groups. Kumamaru *et al.* (*7*) determined the chemical formula of the extracted nitrate species to be:

$$Cu[(CH_3)_2C_{12}H_6N_2 \cdot \tfrac{1}{2}H_2O]_2 \cdot NO_3.$$

This species is preferentially soluble in the organic phase. Collinson and Boltz (*4*) used a modification of the method to determine perchlorate ions.

The following work has shown that both organic and inorganic nitrate, nitrite, and nitro compounds can be determined by using a modification of the above method. It was found that aromatic compounds gave negative results for C–NO bonding, and varying results up to 10% recovery for a $C–NO_2$ bond. Aliphatic compounds such as nitropropane, as well as inorganic compounds, gave very good results. The positive reaction of the aliphatic compounds is probably due to the ease of cleavage of the carbon–oxygen or carbon–nitrogen bond in the presence of sulfuric acid, whereas these bonds in an aromatic compound are

harder to cleave due to both resonance stabilization and shorter bond length (*3, 5, 9, 10, 12*).

In this work, a Perkin-Elmer Model 303 atomic absorption spectrophotometer with a digital readout system was used. The concentration was set while aspirating the standard nitrate solution; the unknowns were then aspirated and the concentration (ppm) was read directly. MIBK was used as the blank rather than the neocuproine–MIBK solution to eliminate error due to the evaporation of the volatile MIBK and subsequent concentration of the solution. A correction was made for the absorption of the reagents in the solution. A copper hollow-cathode lamp (3247A) was used as the light source. The concentration of the copper ion in the extracted chelate was measured by atomic absorption spectrophotometry (AAS).

MATERIALS AND METHODS

A. Reagents

All materials used were of "reagent grade" or better. Water double-distilled with quartz apparatus was used in making all the reagents.

1. Copper(II) sulfate ($CuSO_4 \cdot 5H_2O$), 0.01 *M*.
2. Hydroxylammonium sulfate ($(NH_3OH)_2SO_4$), 5% by weight.
3. Ammonium dihydrogen phosphate ($NH_4H_2PO_4$), 0.5 *M*.
4. Neocuproine in MIBK, 0.002 *M*.
5. Cerium(IV) sulfate ($Ce(SO_4)_2$), 0.1 *N* in 10% H_2SO_4.
6. Sulfamic acid (NH_2SO_3H), 2% by weight in 10% H_2SO_4.
7. Potassium permanganate ($KMnO_4$), 0.1 *N* in 10% H_2SO_4.
8. Nitrate standard stock solution: purified silver nitrate.
   Solution A, 0.01575 g of $AgNO_3$ in 1 liter of H_2O = 5748 ppm NO_3^-.
   Solution B, 2 ml of A diluted to 1 liter = 11.50 ppm NO_3^-.
   Solution C, 20 ml of B diluted to 1 liter = 2.30 ppm NO_3^-.

An alternate method for the preparation of a standard stock solution for nitrate would be the use of standard silver nitrate AAS solution.

   Alternate: 1000 ppm silver standard solution for AAS.
   Solution A-1, 1000 ppm Ag standard = 574.8 ppm NO_3^-.
   Solution B-1, 10 ml of A-1 diluted to 1 liter = 5.748 ppm NO_3^-.
   Solution C-1, 40 ml of B-1 diluted to 100 ml = 2.30 ppm NO_3^-.

9. Sample: weigh, dissolve, and dilute so 1 ml of aqueous solution = 0.05 to 2 ppm nitro, nitrite, or nitrate.

B. Procedure

1. SPECIFIC

a. Nitrates (ONO_2^-). nitrate solutions can be determined directly using the general procedure.

b. Nitro or nitrite ($-NO_2$, ONO^-). nitro or nitrite and nitrate mixtures can be determined by selectively oxidizing or reducing the nitro or nitrite to nitrates or to nitrogen; sulfamic acid reduces NO_2^- to free nitrogen, while cerium(IV) sulfate or potassium permanaganate oxidizes it to nitrate (*6, 8, 13*). Therefore, by making two sets of solutions, the nitro or nitrite can be distinguished from the nitrate.

Series 1: $xNO_3^- + yNO^-/NO_2^- + HOSO_2NH_2 = xNO_3^- + \frac{1}{2}N_2$,

Series 2: $xNO_3^- + yNO^-/NO_2^- + Ce(SO_4)_2/KMnO_4 = (x + y)NO_3^-$.

Example: $xNO_3^- + yNO_2^-$ = 0.221 ppm NO_3^- (reacted with ceric sulfate)

xNO_3^- = 0.115 ppm NO_3^- (reacted with sulfamic acid)

Difference yNO_2^- = 0.106 ppm

2. GENERAL

Into five 25-ml volumetric flasks, measure with pipets or syringes:

1 ml of cupric sulfate solution,
1 ml of hydroxylammonium sulfate solution,
5 ml of ammonium dihydrogen phosphate solution,
1 ml of sample solution or standard solution.

Swirl flask to mix contents. Add 10 ml of neocuproine in MIBK; shake flasks for 2 minutes, and allow contents to separate for 5 minutes. Measure the concentration of the upper organic phase against standard solutions made in the same way in a 100-ml volumetric flask, multiplying all additions by 5, and substituting 5 ml of standard solution C or C-1 for the sample. Larger standards can be made by diluting 4, 6, or 8 ml of solution B; or 20, 30, or 40 ml of solution B-1 to give 4.60, 6.90, or 9.20 ppm nitrate standard. An alternative method to adding 1 ml of nitrate solution would be to add any desired measured amount of the sample or standard up to 5 ml, and dilute with water to the 25-ml mark before adding the organic phase and extracting. This alternative method works best for those instruments where percentage absorption or absorbance is read and a calibration curve is constructed.

DISCUSSION

Eight "classical" methods for determination of nitrite or nitrate are listed in Table 1. The four main types are titrimetric, precipitation, colorimetric, and gasometric. This method is a member of a fifth type, atomic absorption spectrometric. Table 2 lists some of the more common interferences, the detection range in milligrams or parts per million, and whether or not the method detects and distinguishes all three types of nitrogen compounds; it also lists the references where these classical methods were found.

Table 3 shows the results of elemental nitrogen analysis and/or metallic content analysis by atomic absorption.

Table 4 gives the results of the determination of the nitrate, nitrite, or nitro groups by the modified Kumamaru method. Twelve compounds were tested. Aromatic compounds containing NO^- or NO_2^- groups, such as nitrosoaniline and nitrobenzene were unsuccessfully tested. The nitroso compounds gave negative results (zero recovery) while the nitro compounds gave varying results up to 10% recovery. The unexpected positive results of the determination of these nitrogen groups in aliphatic compounds was attributed to greater reactivity due to less resonance stabilization and longer bond lengths than in the aromatic compounds (*3, 5, 9, 10, 12*).

TABLE 1

METHODS OF DETERMINATION OF NITRO, NITRITE, OR NITRATE GROUPS

A. Titrimetric
   1. $5NO_2^- + 2KMnO_4 + 3H_2SO_4 = 5NO_3^- + K_2SO_4 + 2MnSO_4 + 3H_2O$
   2. $2NO_3^- + 4FeSO_4 + 2H_2SO_4 = N_2O_3(g) + 2Fe(SO_4)_3 + 3H_2O$

B. Precipitation
   1. $C_{20}H_{16}N_4$[a] $+ HNO_3 = C_{20}H_{16}N_4 \cdot HNO_3$
   2. $C_{12}H_{22}Tl(III)SO_4$[b] $+ 2NO_3^- = C_{12}H_{22}Tl(III)(NO_3)_2 + SO^{2-}$

C. Colorimetric
   1. DPA·H_2SO_4[c] $+ NO_3^- =$ DPA·HNO_3
   2. SNEDD[d] $+ NO_3^- =$ SNEDD·NO_3

D. Gasometric
   1. Nitrometer
   2. Kjeldahl distillation to ammonia

E. Spectrophotometric: atomic absorption
   1. Neocuproine–copper complex

[a] Nitron (diphenylene dianilohydrotriazole).
[b] Dicyclohexyl thallium(III) sulfate.
[c] Diphenylamine in concentrated sulfuric acid.
[d] Sulfanilamide-*N*-(1-naphthyl)-ethylene diamine dihydrochloride.

TABLE 2

COMPARISON OF METHODS IN TABLE 1

Method [a]	Ref.	Range	Interferences	NO_3^-	NO_2^-	NO^-
A-1	(*8*)	1–100 mg	Organic matter	No	Yes	No
A-2	(*4*, *8*)	10–1000 mg	ClO_3^-, IO_3^-, BrO_3^-, NaCl	Yes	Yes	No
B-1	(*7*, *8*)	0.01–1000 mg	Br^-, I^-, Cl^-, ClO_3^-, ClO_4^- CrO_4^{2-}, SCN^-, picrate	Yes	Yes	Yes
B-2	(*1*, *2*)	5–1000 mg	Halides, MnO_4^-, $Fe(CN)_6^{3-}$, $Fe(CN)_6^{4-}$	Yes	Yes	No
C-1	(*8*)	0.1–100 ppm	Any color producers; color fades in organic solvents	Yes	No	No
C-2	(*8*)	1–1000 ppm	Any color producers	No	Yes	Yes
D-1	(*1*, *2*, 7)	20–20,000 ppm	Cannot distinguish between the three groups	Yes	Yes	Yes
D-2	(*1*, *2*, 7)	200–20,000 ppm	Cannot distinguish between the three groups	Yes	Yes	Yes
E-1	(*7*)	0.005–10,000 ppm	None below 5× excess	Yes	Yes	Yes

[a] Number refers to method as listed in Table 1.

The following ions were tested for interference in the method, and found to contribute less than 1% error when present in concentrations of less than 5 times that of the nitrate, nitrite, or nitro compound: acetate, amine, ammonium, cadmium, cerium(IV), cobalt(III), copper(II), cyanide, iron(II, III), lead, lithium, magnesium, perchlorate, permanganate, potassium, silver, sulfate.

The lower limit of detection was found to be 0.001 ppm with the particular instrumental setup used. Reproducibility was 0.007 ppm, and the standard deviation was 0.004 ppm, while the range of deviation was 0.001 to 0.012 ppm in the determination range up to 0.5 ppm. Percentage recovery ranged from 95.56 to 104.26, and the average percentage recovery was 99.97.

Larger concentrations—up to 0.10 g of nitrate—can be determined by taking appropriate aliquots of the MIBK extraction of the sample and the standard, and diluting to a known volume with MIBK. It is

TABLE 3

RESULTS OF ANALYSIS OF COMPOUNDS USED IN THE TESTS

Sample	Mol wt	N (%)		Metal (%)	
		Theoret.	Expt. [a]	Theoret.	Expt. [b]
Cadmium nitrate hexahydrate	406.40			27.66	27.91
Copper (II) nitrate hepta-hydrate	313.54			20.27	20.82
Sodium hexanitrito cobalt (III)	403.94			17.08 14.59	16.98 Na 14.30 Co
Potassium nitrite	85.11			45.94	45.84
Sodium nitrite	69.00			33.32	32.56
Sodium nitrosyl pentacyano ferrate (II)	297.95	28.21	28.07	15.43 18.75	15.06 Na 19.06 Fe
Guanidinium nitrate	122.08	45.89	46.06		
1, 2-Dinitroxypropane, NOS No. 1344	166.00	16.80	16.43		
Urea nitrate	123.07	34.14	33.98		
5-Nitrobarbituric acid·$3\frac{1}{2}$ H_2O	236.12	17.80	17.73		
Nitroglycerine	227.09	18.50	— [c]		
1-Nitropropane	89.10	15.72	15.60		
Neocuproine	217.27	12.89	12.50		

[a] Nitrogen content determined by Mrs. P. P. Wheeler, using a Coleman nitrogen analyzer; accuracy of the method = $\pm 0.3\%$

[b] Atomic absorption analysis by author.

[c] Sample failed stability test before analysis could be completed and was destroyed.

necessary to keep the sample concentration less than that of the highest standard concentration. The optimum working range will, of course, depend on the instrument used, but should be between 0.005 and 5 ppm for the best accuracy.

TABLE 4

RESULTS OF NITRATE, NITRITE, OR NITRO DETERMINATION BY AAS

Sample	(ppm) Added	Found	Recovery (%)
Cadmium nitrate hexahydrate $Cd(NO_3)_2 \cdot 6H_2O$	0.103	0.102	
		0.106	
		0.102	
		0.100	
		0.107	
		0.103	100.00
Copper (II) nitrate heptahydrate $Cu(NO_3)_2 \cdot 7H_2O$	0.134	0.123	
		0.123	
		0.134	
		0.129	
		0.136	
		0.129	95.56
Sodium hexanitrito cobalt (III) $Na_3Co(NO_2)_6$	0.105	0.109	
		0.110	
		0.108	
		0.110	
		0.107	
		0.109	103.81
Potassium nitrite KNO_2	0.103	0.103	
		0.099	
		0.104	
		0.108	
		0.100	
		0.103	100.00
Sodium nitrite $NaNO_2$	0.094	0.096	
		0.092	
		0.095	
		0.097	
		0.104	
		0.098	104.26
Sodium nitrosyl pentacyano ferrate (II) $Na_2FeNO(CN)_5 \cdot 2H_2O$	0.101	0.104	
		0.101	
		0.094	
		0.093	
		0.110	
		0.100	99.01

TABLE 4—(Continued)

RESULTS OF NITRATE, NITRITE, OR NITRO DETERMINATION BY AAS

Sample	(ppm) Added	Found	Recovery (%)
Guanidinium Nitrate	0.100	0.096	
$CH_5N_3HNO_3$		0.096	
(laboratory synthesis)		0.102	
		0.098	
		0.100	
		0.098	98.00
1, 2-Dinitroxypropane	0.110	0.110	
$CH_3CH(ONO_2)CH_2(ONO_2)$		0.112	
(pilot plant synthesis)		0.106	
		0.109	
		0.110	
		0.109	99.09
Urea nitrate	0.123	0.130	
$CO(NH_2)_2 \cdot HNO_3$		0.129	
		0.129	
		0.120	
		0.119	
		0.125	101.95
Nitroglycerine	0.121	0.122	
$CH_2(ONO_2)CH(ONO_2)CH_2(ONO_2)$		0.120	
(pilot plant synthesis		0.120	
undried, unpurified;		0.125	
did not pass Abel heat test		0.117	
for stability—failed at 8 min	$(NO_3 + NO_2)$	0.120	99.17
on a 10-min KI test)			
	$x\ NO_3^-$	0.112	
		0.111	
$xNO_3^- + yNO_2^- = 0.120$		0.113	
		0.115	
$xNO_3^- = 0.112$		0.111	
Diff $yNO_2^- = 0.008$		0.112	
5-Nitrobarbituric acid	0.109	0.110	
$C_4H_3O_3N_2 \cdot NO_2 \cdot 3\,1/2H_2O$		0.096	
		0.103	
		0.115	
		0.115	
		0.108	99.08

TABLE 4—(Continued)

RESULTS OF NITRATE, NITRITE, OR NITRO DETERMINATION BY AAS

Sample	(ppm) Added	Found	Recovery (%)
1-Nitropropane $CH_3CH_2CH_2NO_2$	0.404	0.409	
		0.402	
		0.406	
		0.400	
		0.399	
		0.403	99.75
			Av 99.97

SUMMARY

An indirect method has been developed for the determination of nitro, nitrite, and nitrate groups by complexation with copper(I) neocuproine, extraction into methyl isobutyl ketone, and measurement of the complexed copper(I) by atomic absorption spectroscopy. This method depends on the oxidation of nitro and nitrite groups to nitrate by cerium(IV) sulfate or potassium permanganate. The nitrate is then determined as described above. Reduction of nitro and nitrite groups to elemental nitrogen by sulfamic acid permits the determination of nitrate in the presence of the other two types of compound.

REFERENCES

1. Belcher, R., and Wilson, C. L., "New Methods in Analytical Chemistry," 284 pp. Reinhold, New York, 1955.
2. Böttger, W., and Oesper, R. E., "New Methods of Volumetric Chemical Analysis," 268 pp. Van Nostrand, New York, 1938.
3. Branch, G. E. K., and Calvin, M., "Theory of Organic Chemistry, An Advanced Course," 523 pp. Prentice-Hall, Englewood Cliffs, N.J., 1945.
4. Collinson, W. J., and Boltz, D. F., Indirect spectrophotometric and atomic absorption spectrometric methods for determination of perchlorate. *Anal. Chem.* **40,** 1896–1898 (1968).
5. Gould, E. S., "Mechanism and Structure in Organic Chemistry," 790 pp. Holt, Rinehart and Winston, New York, 1959.
6. Jolly, W. L., "Chemistry of the Non-metals," 149 pp. Prentice-Hall, Englewood Cliffs, N.J., 1966.
7. Kumamaru, T., Tao, E., Okamoto, N., and Yamamoto, Y., A new method for the determination of nitrate ions by atomic absorption spectrophotometry. *Bull. Chem. Soc. Jap.* **38,** 2204 (1965).
8. Lundell, G. E. F., Hillebrand, W. F., Bright, H. A., and Hoffman, J. I., "Applied Inorganic Analysis," 1034 pp. Wiley, New York, 1953.
9. Pauling, L., "The Nature of the Chemical Bond," 540 pp. Cornell Univ. Press, Ithaca, N.Y., 1939.
10. Royals, E. E., "Advanced Organic Chemistry," 948 pp. Prentice-Hall, Englewood Cliffs, N.J., 1954.

11. Scott, W., "Standard Methods of Chemical Analysis," Vols. 1,2, 2617 pp. Van Nostrand, New York, 1953.

12. Wheland, G. W., "Advanced Organic Chemistry," 799 pp. Wiley, New York, 1949.

13. Whitman, C. L., Titrimetric determination of sulfamic acid. *Anal. Chem.* **29,** 1684–1685, (1957).

14. Wilson, C. L., and Wilson, D. W., "Comprehensive Analytical Chemistry," Vol. 1A, 577 pp. Elsevier, Amsterdam/New York, 1959.

MICROCHEMICAL JOURNAL 15, 409–414 (1970)

The Coulometric Microdetermination of Iodide After Oxygen Flask Combustion of Organic Iodine Compounds. The Use of Iodate, Biiodate, and Iodide as Primary Standards

G. E. SECOR AND L. M. WHITE

Western Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Albany, California 94710

Received October 23, 1969

INTRODUCTION

A convenient method for the microdetermination of chlorine, bromine, and iodine in organic compounds consists of the combustion of the compound in an oxygen filled flask, conversion of the halogen to halide, and the coulometric titration of the halide. For the precise coulometric determination of chloride, bromide, and iodide, an acidic standard solution of the specific halide to be titrated is used for conditioning the titrator electrodes and for determining the titration constant (mg of halide/second) (*1, 2, 5*). National Bureau of Standards (NBS) No. 145 2-iodobenzoic acid, after oxygen flask combustion, is recommended (*1*) for determining the iodide titration constant. Because this microchemical standard is no longer issued (*9*) by the NBS, an alternative iodide standard was sought. Inorganic standards (*1*) are used for determining the chloride and bromide constants. If an inorganic standard were also used for determining the iodide constant, the speed and simplicity of the determination for iodide would be increased. We have found that under favorable acidity conditions, suitable amounts of potassium iodate and biiodate can be quantitatively reduced to iodide by hydrazine sulfate, which is used in the absorbing solution (*1*) in the oxygen flask when combusting iodine or bromine compounds. This paper reports a comparison of the coulometric iodide titration constants using NBS 2-iodobenzoic acid, potassium iodate, biiodate, and iodide as standard sources of iodide.

EXPERIMENTAL METHODS

Apparatus

The oxygen flask combustion equipment, the coulometric titration assembly, and special glassware items were the same as previously described (*12*).

Reagents

Hydrazine sulfate (Eastman No. 575[1]). Prepare approximately 0.4 *N* aqueous KOH, 0.1 *N* HNO_3 + 10% glacial acetic acid, 0.6 *N* HNO_3 + 20% glacial acetic acid, and gelatin reagent according to directions (*1, 2*). Prepare the following reagents daily (*3*): In a glass-stoppered flask, dissolve, by magnetic stirring for 15–20 minutes, (a) hydrazine sulfate in 0.4 *N* KOH in the proportion of 0.2 g to 5 ml (for Procedure I), and (b) hydrazine sulfate in 0.1 *N* HNO_3 + 10% glacial acetic acid in the proportion of 0.2 g to 15 ml (for Procedure II).

Iodide Standards

NBS No. 145 2-iodobenzoic acid.

Potassium iodate, primary standard grade (Mallinckrodt No. 1093). Potassium iodate, ACS reagent grade, recrystallized three times from water. Both were dried 16 hours in air at 150°C (*7a*).

Potassium biiodate, primary standard grade (G. F. Smith No. 75). Potassium biiodate, ACS reagent grade, recrystallized three times from water. Both were dried 16 hours in air at 100°C (*7b, 8*).

Potassium iodide, ACS reagent grade (J. T. Baker No. 3164). Potassium iodide, ACS reagent grade, recrystallized one time under nitrogen from water. Both were dried 6 hours in air at 150°C (*11*).

After pulverizing the reagents in a mortar to break up lumps and large crystals and after drying, the inorganic standards were stored in all-glass containers in a dark desiccator.

Procedure

The iodide titration constant (mg of iodide/second) was determined by Procedures I, II, and III below for the compounds indicated. Sample weights of each compound were uniformly distributed over a weight range to yield approximately 2.5 to 5.1 mg of total iodide. All titrations were made at the "Medium" titration rate with the automatic chloride titrator according to the directions (*1*) for iodide solutions.

I. For determining the iodide titration constant with potassium iodate, biiodate, and iodide. Weigh the compound in a weighing tube by difference into a dry 15-ml volumetric flask. Add 5 ml of the hydrazine sulfate–0.4 *N* KOH reagent. Shake the flask gently until all the sample is completely dissolved, and gas formation has ceased. Add dropwise, with gentle shaking, 0.5 to 1 ml of a 5-ml aliquot of

[1] Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

0.6 *N* HNO_3 + 20% glacial acetic acid from a measuring pipet. When all the iodine color is gone (from the reduction of potassium iodate or biiodate), add, while gently shaking, the remainder of the 5-ml portion of 0.6 *N* HNO_3 + 20% glacial acetic acid reagent. After 5–10 minutes make to 15 ml with 0.1 *N* HNO_3 + 10% glacial acetic acid reagent. Mix well to degas the solution (invert about 20 times) releasing the pressure on the stopper occasionally. Wait 1 minute before aliquoting. Titrate three 4.00-ml aliquots each containing 0.2 ml of gelatin reagent. Average the three titration times, subtract the time for a similarly determined reagent blank, and calculate the iodide titration constant using the appropriate theoretical iodine percentage in Table 1.

$$\text{Iodide constant} = \frac{\text{theoretical \% iodine} \times \text{weight of total sample in mg}}{(\text{titration time} - \text{blank time}) \times 15/4 \times 100}.$$

II. For determining the iodide titration constant with potassium iodide. Weigh the potassium iodide in a weighing tube by difference into a dry 15-ml volumetric flask. Add about 10 ml of the hydrazine sulfate–0.1 *N* HNO_3 + 10% glacial acetic acid reagent and shake gently about 3 minutes until the potassium iodide is completely dissolved. Make to 15 ml with the same reagent and mix well. Titrate three 4.00-ml aliquots,

TABLE 1

COULOMETRIC IODIDE TITRATION CONSTANTS

Procedure	Compound	No. of detns	Iodide (mg/sec; av)	SD
I	KIO_3 (59.30% I)			
	primary std. grade	6	0.008829	0.0000102
	ACS rgt. grade [a]	6	0.008832	0.0000232
I	$KH(IO_3)_2$ (65.09% I)			
	primary std. grade	6	0.008839	0.0000127
	ACS rgt. grade [a]	6	0.008848	0.0000121
I	KI (76.45% I)			
	ACS rgt. grade [b]	6	0.008832	0.0000212
	ACS rgt. grade	6	0.008823	0.0000147
II	KI (76.45% I)			
	ACS rgt. grade [b]	5	0.008842	0.0000100
	ACS rgt. grade	6	0.008840	0.0000133
III	2-Iodobenzoic acid (51.17% I)			
	NBS No. 145	12	0.008841	0.0000305

[a] Recrystallized three times.

[b] Recrystallized one time.

each containing 0.2 ml of gelatin reagent. Average the three titration times, subtract the time for a similarly determined reagent blank, and calculate the iodide titration constant.

III. For determining the iodide titration constant with NBS 2-iodobenzoic acid. Weigh the sample onto a black flag wrapper and fold wrapper to enclose sample completely. Add 200 mg of solid hydrazine sulfate to 5 ml of 0.4 *N* KOH in the oxygen flask, with slight swirling, just prior to a combustion. Carry out as previously described (*12*) an oxygen flask combustion, 45-minute absorption, rinse, transfer, and volume adjustment to 15 ml. Mix well with occasional release of pressure on the stopper. Titrate three 4.00-ml aliquots, each containing 0.2 ml of gelatin reagent. Average the three titration times, subtract the time for a similarly determined combusted blank and calculate the iodide titration constant.

DISCUSSION AND RESULTS

The coulometric titration conditions were chosen to be used in combination with the oxygen flask combustion method (*1, 12*) for the microanalytical determination of iodine. Hydrazine sulfate is one of the reducing agents used routinely (*1*) to completely convert the iodine products formed in the combustion of organic compounds to iodide. The reaction between iodine and hydrazine (*4*),

$$N_2H_6SO_4 + 2I_2 = N_2 + H_2SO_4 + 4HI,$$

is rapid at a pH of 7–7.4 (*10*), which is the pH of the hydrazine sulfate–0.4 *N* KOH reagent used as an absorbing solution in the oxygen flask combustion method for NBS 2-iodobenzoic acid in Procedure III. In an acidic solution, hydrazine, when present in excess, will also reduce iodate to iodide with the intermediate formation of iodine (*4*); however, the reduction of the iodine formed may be slow, depending on the acidity (*6*). With an excess of hydrazine, the overall reaction is represented by the equation (*4*)

$$3N_2H_4 \cdot H_2SO_4 + 2KIO_3 = 3N_2 + 2KI + 6H_2O + 3H_2SO_4.$$

The conditions used in Procedure I for the reduction of potassium iodate or biiodate to iodide are satisfactory if care is taken not to acidify too rapidly the hydrazine sulfate–0.4 *N* KOH reagent, and not to complete the acidification until the iodine color disappears.

Procedure II corresponds in reagents to the simple standard solutions used for determining the chloride and bromide titration constants, with the addition of hydrazine sulfate to protect the iodide from air oxidation. It is not recommended for determining the titration constant with potassium iodate or biiodate because at the initial higher acidity they react

too vigorously with the hydrazine with possible loss of iodine; also, the iodine formed is reduced less rapidly to iodide than under the less acidic conditions present in the early stages of Procedure I.

Procedure III combines a standard oxygen flask combustion method for converting organic iodine to iodide with a coulometric titration finish. Although the inorganic iodine compounds can be carried through the entire combustion process as in Procedure III, it is not necessary and leads to slightly higher titration constants and standard deviations than in Procedures I and II. The quantitative recovery after oxygen flask combustion of iodide from potassium iodate, and of bromide from potassium bromate (unpublished data), indicates that organic iodate and bromate also may be amenable to analysis as the halide after oxygen flask combustion as was found previously for organic perchlorate (*12*).

Comparative results are given in Table 1 for the iodide titration constants for the inorganic compounds and for NBS 2-iodobenzoic acid. A variation of 0.00005 in the constant will change the apparent percentage of iodine in 5 mg of total sample by 0.3% absolute. None of the average constants for any of the inorganic compounds differs by as much as 0.000025 from that for NBS 2-iodobenzoic acid. As measured by the standard deviations, the determination of the constant for the inorganics is more precise than the constant for the NBS sample. The precision of Procedures I and II for the iodide constant with the inorganic compounds is comparable to that for the chloride and bromide constants with inorganic chloride and bromide standards.

Once the reduction to iodide is complete, the iodide concentration in the solutions prepared by Procedures I and II remains remarkably stable. Standard iodide solutions were prepared in 250-ml volumes in volumetric flasks from potassium iodate, biiodate, and iodide by Procedure I, and from potassium iodide by Procedure II and kept in a storage cabinet at 25°C protected from evaporation, light, laboratory fumes, and dust. The iodide titration constants remained essentially unchanged for at least 3 months.

The above results show that any of the above inorganic iodine compounds, when pure, can replace NBS 2-iodobenzoic acid for determining the coulometric titration constant. Potassium iodate is the preferred substitute because of its combined advantages of high equivalent weight, favorable solubility, and availability as a recognized primary standard of high purity (*7a*).

SUMMARY

Under the conditions presented, potassium iodate, biiodate, and iodide can satisfactorily replace NBS 2-iodobenzoic acid, which is no longer available, as a

standard source of iodide for determining the coulometric iodide titration constant. Use of these inorganic compounds as primary iodide standards increases the speed and simplicity of the coulometric titration finish to the oxygen flask combustion method for microanalytical determination of iodine without loss of precision or accuracy. Potassium iodate is the preferred replacement.

REFERENCES

1. Alford, W. C., and McCann, H. G., Determination of chlorine, bromine, and iodine in organic compounds. Reprint No. 159, American Instrument Co., Silver Spring, Maryland.
2. Aminco Instruction Manual No. 751-D, American Instrument Co., Silver Spring, Maryland, 1965.
3. Audrieth, L. F., and Ogg, B. A., "The Chemistry of Hydrazine," pp. 139–145, 154–155. Wiley, New York, 1951.
4. Bray, W. C., and Cuy, E. J., The oxidation of hydrazine. I. The volumetric analysis of hydrazine by the iodic acid, iodine, bromine, and hypochlorous acid methods. *J. Amer. Chem. Soc.* **46,** 858–875 (1924).
5. Cotlove, E., Determination of chloride in biological materials. *In* "Methods of Biochemical Analysis" (D. Glick, ed.), Vol 12, pp. 341, 383–386. Wiley (Interscience), New York, 1964.
6. Kolthoff, I. M., The volumetric analysis of hydrazine by the iodine, bromate, iodate and permanganate methods. *J. Amer. Chem. Soc.* **46,** 2009–2016 (1924).
7. Kolthoff, I. M., and Stenger, V. A., "Volumetric Analysis," Vol. 2, 2nd ed., (a) p. 88; (b) p. 100. Wiley (Interscience), New York, 1947.
8. Kolthoff, I. M., and Berk, L. H. van, The use of potassium bi-iodate as a standard substance in alkalimetric and iodimetric titrations. *J. Amer. Chem. Soc.* **48,** 2799–2801 (1926).
9. *Nat. Bur. Stand. (U.S.), Misc. Publ.* 260-Standard Reference Materials-Quarterly Insert Sheets, p. 9 (Oct. 1, 1966).
10. Panwar, K. S., Mathur, N. K., and Rao, S. P., Hydrazine as a titrimetric reagent in iodometry. *Anal. Chim. Acta* **24,** 541–545 (1961).
11. "Reagent Chemicals." American Chemical Society Specifications 1950, p. 276. Amer. Chem. Soc., Washington, D.C., 1950.
12. Secor, G. E., Ricci, B. A., and White, L. M., Microdetermination of perchlorate as chloride by oxygen flask combustion. *Microchem. J.* **13,** 273–278 (1968).

MICROCHEMICAL JOURNAL 15, 415–421 (1970)

Applications Involving the Iodide Ion

VI. Determination of Thallium(I) and Analysis of Its Mixtures with Some Metal Ions

H. KHALIFA AND Y. M. ISSA

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

Received September 27, 1969

INTRODUCTION

The quantitative determination of mercuric and iodide ions by titrating either of them with the others using a silver amalgam electrode over a wide range of pH, has recently created renewed interest in methods based on quantitative reduction of some anions and high valent cations (*10*), or formation of slightly soluble iodides, (*9–12*) with excess iodide, which is back-titrated with mercury(II). Such a titrimetric method has the advantages of being simple, rapid, and accurate, suitable for macro- and microanalysis, and preferable to instrumental techniques which are largely empirical and dependent for their calibration on the availability of standard substances, or gravimetric methods which are tedious and time consuming. The fact that thallium(I) iodide is very slightly soluble in water (*15*) (0.083 g/liter at 25°C) suggested the applicability of the above method to the determination of thallium(I).

Thallium(I) is determined gravimetrically as iodide or chromate which should stand 18 and at least 12 hours, respectively, before their separation (*8*). The excellent chromate method suffers interference from halides, reducing substances, lead, and mercury(I) (*2*). Cimerman and Selzer (*1*) used thionalide in a microgravimetric method for 3–6 mg with a maximum error of 0.4%. Mercaptobenzothiazole precipitates thallium(I) along with seven other cations (*3*) with no distinct advantage over thionalide.

Thallium(I) iodide is the basis of a titrimetric method with the end point detected by the adsorption indicator bromophenol blue (*16*), by the potentiometric method (*19*) or by the amperometric method. Potentiometric titration of thallium(I) with thioacetamide at 70–80°C in ammoniacal medium containing hydrazine, makes use of its precipitation as sulfide (*5*).

Among the various oxidation reactions for thallium(I) are recommended: (i) the bromate titration with the end point detected by methyl

orange (*6*) or potentiometrically (*7*); (ii) the iodate titration with the end point detected by the disappearance of violet color due to free iodine from CCl_4 or $CHCl_3$ (*14*) or potentiometrically; and (iii) cerium(IV) sulfate titration (*22*) with the end point detected by the yellow color of cerium(IV), by the use of ferroin (*17*), or potentiometrically.

Sill and Peterson (*20*) stated that the idometric method involving oxidation of thallium(I) with bromine, the excess of which is removed with phenol, and reduction of thallium(III) with iodide (*14*), involves less than 0.1% error.

Very recently (*18*), Rao reported a similar method to the present one, save for the fact that he discards the first few milliliters of filtrate containing excess iodide, prior to titrating a portion of it with Hg(II) using diphenylcarbazone to a violet end point. Under such condition the determination seems to lack quantitativeness and hence accuracy.

The present method affords a simple means of the accurate determination of 4 to 200 mg of thallium(I) with the least reagents [iodide and mercury(II)] and time of 30 minutes.

EXPERIMENTAL METHODS

The water used was always twice distilled from all glass equipment. The chemicals were all of the requisite purity. They were sulfates of thallium(I), nickel, chromium(III), manganese(II), copper(II), cobalt(II), and magnesium; mercuric nitrate; disodium salt of ethylenediaminetetraacetic acid (EDTA); chloride and hydroxide of ammonium; hexamine; hydroxide, thiosulfate, and citrate of sodium; Eriochrome black T, pyrocathechol violet (PCV), methylthymol blue (MTB), and murexide indicators.

Solutions

The 0.0101 and 0.0498 *M* Tl(I) solutions were prepared from AR Tl_2SO_4 and standardized gravimetrically as chromate and iodide (*8*). The 0.01 and 0.05 *M* mercury(II) solutions were prepared from a nitrate sample and standardized against EDTA volumetrically using methylthymol blue and potentiometrically using silver amalgam as the indicator electrode in hexamine at pH 9–10. The 0.0202 and 0.0966 *M* iodide solutions were prepared from KI and standardized potentiometrically against mercury(II) solutions. The 0.01 and 0.05 *M* EDTA solutions were prepared and standardized as previously mentioned. The sulfate of the above-mentioned cations were prepared by recommended procedures.

The titration system consisted of a Pyrex glass vessel fitted with a

glass cover with ground joints for the silver amalgam, the salt bridge with its other end immersed together with the tip of the saturated calomel electrode in saturated KCl and the tip of a 10 ml 1/50 graded burette; a magnetic stirrer No. 9986 type R H 12 Rührомag; a Scalamp galvanometer (W. G. Pye and Co. Ltd. Cambridge CAT No 7901/5) connected to a Pye student potentiometer.

Procedures

(A) For thallium(I) alone or in presence of cations which are indifferent towards iodide, add 5–15 ml of iodide to 1–10 ml of a hot Tl(I) solution in a 100-ml beaker under constant stirring; filter yellow TlI using 42 Whatman and wash thrice with water; titrate excess iodide with mercury(II) using the silver amalgam electrode.

(B) For Tl in presence of cations which react with iodide follow (A) after adding sufficient EDTA as masking agent prior to addition of iodide.

(C) For Tl in binary or ternary mixtures follow either (A) or (B) according to the type of cation involved.

(D) Determine Ni or Cu(II) in an identical binary mixture by back-titrating excess EDTA with mercury(II) and the silver amalgam in hexamine buffer pH 9–10; Zn by titration with EDTA in hexamine using MTB indicator; Mn(II) with EDTA in ammoniacal buffer, pH 10, using PCV (*21*).

(E) Determine copper(II) in ternary mixtures idometrically; total Cu and Ni, Cu and Cr(III) or Ni and Cr potentiometrically by back-titration with Hg(II); Ni in presence of Tl and Cr by back-titration with Hg(II) after masking Cr with 1 *M* citrate (0.4 ml for each milligram of Cr).

RESULTS AND DISCUSSION

Table 1 lists the results of determining thallium(I) alone or in presence of large amounts of a variety of cations, which show high accuracy and precision in almost all cases. In Tables 2 and 3 are recorded the results of analysis of 4 binary and 3 ternary mixtures. The data indicate that the procedures described for such an analysis are simple, rapid, and extremely reliable.

Comparison between the solubilities of TlI and HgI_2 of 1.68×10^{-4} and 8.8×10^{-7} mole/liter as computed, respectively, from the values 0.083 g/liter (*4*) and 0.004 g/liter (*20*) indicates that when excess iodide is back-titrated with Hg(II) in presence of TlI the latter will

TABLE 1

DETERMINATION OF MILLIGRAM AMOUNTS OF THALLIUM(I) [a]

No.	Tl(I) (mg)		Error (±%)	Titrant (mV/0.1 ml)
	Taken	Found		
1	203.55	201.43	1.04	424
2	142.48	141.06	1.00	435
3	122.13	120.91	1.00	429
4	101.77	100.55	1.2	428
5	81.42	80.93	0.60	424
6	61.06	60.70	0.60	407
7	50.88	50.52	0.72	436
8	40.71	40.95	0.6	416
9	30.53	30.65	0.4	444
10	20.35	20.35	—	445
11	20.35	20.41	0.3	391
12	20.35	20.41	0.3	371
13	20.35	20.41	0.3	420
14	20.35	20.41	0.3	390
15	20.35	20.41	0.3	385
16	20.35	20.55	1.0	320
17	20.35	20.55	1.0	335
18	20.35	20.19	0.8	154
19	20.66	20.68	0.1	247
20	16.53	16.51	0.12	262
21	10.33	10.37	0.39	220
22	6.20	6.14	0.98	241
23	4.13	4.11	0.49	238

[a] Nos. 1–18, 0.0966 *M* I^- × 0.0503 *M* Hg(II); 19–23; 0.02018 *M* I^- × 0.0101 *M* Hg(II). 11–18, Determined, respectively, in presence of 50; 100 mg of Mg; 16 mg of Zn; 33 mg of Zn + 50 mg of Mg; 50 mg of Cr(III) + 50 mg of Mn(II); 50 mg of Co(II) + 50 mg of Ni; 20 mg of each of Mg, Ni, Cr, Mn, and Co + 16 mg of Zn; and 20 mg of Cu(II) + 10 ml 0.05 *M* EDTA.

dissociate towards production of its ions, a fact which was experimentally verified, thus,

$$TlI + 3I^- + Hg^{2+} \rightarrow Tl^+ + HgI_4^{2-},$$
$$TlI + I^- + Hg^{2+} \rightarrow Tl^+ + HgI_2,$$
$$2TlI + Hg^{2+} \longrightarrow 2Tl^+ + HgI_2.$$

It becomes evident that in order to obtain accurate results the easily filterable yellow TlI should be separated before back-titrating excess iodide.

TABLE 2

ANALYSIS OF BINARY MIXTURES

Tl(I) (mg)		Error	Metal (mg)		Error
Taken	Found	(±%)	Taken	Found	(±%)
101.77	100.14	1.6	6.32 Cu	6.35	0.47
61.06	60.46	0.98	12.64	12.64	—
20.35	20.19	0.8	31.61	31.36	0.8
101.77	100.59	1.16	9.25 Mn	9.21	0.43
61.06	60.70	0.6	13.88	13.81	0.43
20.35	20.51	0.8	18.50	18.48	0.1
20.66	20.66	—	16.48 Ni	16.48	—
6.20	6.14	0.98	10.99	11.03	0.36
10.33	10.37	0.39	6.86	6.85	0.14
20.66	20.64	0.1	6.54 Zn	6.51	0.4
10.33	10.40	0.7	13.08	13.02	0.4
6.20	6.25	0.8	16.35	16.25	0.62

TABLE 3

ANALYSIS OF TERNARY MIXTURES

Tl(I) (mg)		M (mg)		M (mg)	
Taken	Found	Taken	Found	Taken	Found
10.33	10.40	6.32 Cu	6.32	5.49 Ni	5.49
10.33	10.40	6.32 Cu	6.29	5.66 Cr	5.65
16.53	10.64	5.49 Ni	5.49	5.66 Cr	5.72

The end point potential breaks are of excellent order of magnitude averaging 400 and 240 mV/0.1 ml of 0.05 and 0.01 *M* Hg(II) respectively. With such breaks the end points are determined with high accuracy and precision (Table 1). With EDTA used to mask copper(II) (Table 1, No. 18) by forming a complex which renders copper unavailable for normal action with iodide, it is observed that the end point potential break is lowered to 154 mV/0.1 ml of 0.05 *M* Hg(II). This is in harmony with our previous investigation (*11*) that breaks detecting iodide decrease with increasing amount of EDTA or CDTA, which was attributed to a decrease in the concentration of free mercuric ions in the vicinity of the end point, as they are majorly imprisoned in the stable mercury–EDTA or CDTA complex. However, the potential break under such conditions is large enough to permit detection of the end point accurately with but 0.8% error.

SUMMARY

A new reliable potentiometric method for thallium(I), based on its precipitation as the slightly soluble iodide and back-titration of the excess iodide after separation of the precipitate, with mercury(II) using silver amalgam as the indicator electrode is given. By its aid 4 to 200 mg of thallium are accurately determined in pure solutions, in presence of a cluster of cations which are indifferent towards iodide or in binary and ternary mixtures. End point potential breaks averaged 400 and 240 mV per 0.1 ml of 0.05 and 0.01 *M* Hg(II), respectively. 30 to 40 minutes are sufficient for a single determination.

REFERENCES

1. Cimerman, C., and Selzer, G., Microgravimetric determination of thallium with thionalide. *Anal. Chim. Acta* **15,** 213–217 (1956).
2. Cited in Kolthoff, p. 58.
3. Cited in Kolthoff, p. 60.
4. Cited in Kolthoff, p. 63.
5. Cited in Kolthoff, p. 63.
6. Cited in Kolthoff, p. 64.
7. Cited in Kolthoff, p. 64.
8. Cited in Kolthoff, p. 92.
9. Khalifa, H., and Ateya, B., Applications involving the iodide ion. I. A new potentiometric method for the micro- and semimicrodetermination of silver. *Microchem. J.* **12,** 440–446 (1967).
10. Khalifa, H., and Ateya, B., Applications involving the iodide ion. II. Microdetermination of oxidizing agents use of potassium iodate and periodate as primary standards for mercury(II). *Microchem. J.* **13,** 147–154 (1968).
11. Khalifa, H., and Ateya, B., Applications involving the iodide ion. III. Determination of iodide in presence of some complexing agents.; analysis of binary and ternary mixtures of silver with some metal ions. *Microchem. J.* **13,** 664–670 (1968).
12. Khalifa, H., and Kotry, M. F., Applications involving the iodide ion. IV. Potentiometric micro- and semimicrodetermination of palladium analysis of mixtures. *Microchem. J.* **13,** 705–711 (1968).
13. Kohlrausch, F., and Rose, F., "Comprehensive treatise on inorganic and theoretical Chemistry" (J. W. Mellor, ed.), Vol. 4, p. 911. Longmans, Green, New York 1946.
14. Kolthoff, I. M., and Belcher, R., "Volumetric Analysis," Vol. 3. Interscience, New York, 1957.
15. Kolthoff, I. M., and Elving, P. J., "Treatise on analytical Chemistry," Pt. 2, Vol. 2, p. 12, Wiley (Interscience), New York, 1963.
16. Mehrotra R. C., Tetrabromophenolsulphonphthaline as adsorption indicator. I. Volumetric estimation of Thallium. *Anal. Chim. Acta* **3,** 73–77 (1949).
17. Rao, K. B., and Rao, G. J., Cerimetric determination of thallium(I): *Fresenius' Z. Anal. Chem.* **168,** 83 (1959).
18. Roa, A. L. J., Indirect mercurimetric determination of Thallium via iodide precipitation. *Fresenius' Z. Anal. Chem.* **235,** 180–181 (1968).
19. Rienacker, G., Jerschkewitz, H. G., and Lang, K. "Thallium" *In* "Handbuch

der analytischen Chemie" (W. Frezenlus, and G. Jander, eds.), 2nd. ed., Pt. 3, Vol. III aB/III b, pp. 81–140, 157–162. Springer-Verlag, Berlin/New York, 1956.

20. Sill, C. W., and Peterson, H. E., Iodometric semimicrodetermination of thallium in ores and flue dusts. *Anal. Chem.* **21,** 1268 (1949).

21. Welcher, F. J., "The Analytical Uses of Ethylenediaminetetraacetic Acid," p. 220. Van Nostrand, Princeton, N. J., 1957.

22. Willard, H. H., and Young, P., Ceric sulfate as a volumetric oxidizing agent. X. The determination of thallium. *J. Amer. Chem. Soc.* **52,** 36–42 (1930).

MICROCHEMICAL JOURNAL 15, 422–427 (1970)

Submicrogram Determination of Manganese with Other Elements by Polarography [1]

V. FANO [2]

Istituto di Fisica dell'Università, Parma, Italy

Received October 24, 1969

INTRODUCTION

Recently the polarography method with hanging mercury drop electrode (HMDE) has become more important as a method of analysis of ultrapure substances and semiconductors (*6*), whose intrinsic properties are often dependent on the content of impurities even below 1 ppm. Furthermore, the number of elements which can detected by this method is increasing. Lately, the method of determination has been discussed for a few transition elements, such as: Fe in 0.5 *M* of Na citrate (pH 6.3) (*4*) and in 2 *M* KSCN (*1*); Co and Ni in ammonium buffer solution with $2 \times 10^{-4}\%$ of dimethylglyoxina (*7*) or in a supporting electrolyte of KCl, KNO_3 or KSCN (*5*).

However no method for the detection of Mn has been reported. This is due mostly to the fact that the potential of preelectrolysis is quite negative. This paper describes a method for the determination of Mn to a concentration of $10^{-6}\%$ which has been developed in this laboratory. The possibility of detecting Mn^{2+} in the presence of Cu, Pb, Cd, Zn, Ni, Fe, and Co has been studied. This method, therefore, is a valid one either for fast checks of background impurities in ultrapure materials, or for a precise determination of Mn used as doping of semiconductors and insulators such as NaCl.

EXPERIMENTAL RESULTS

The measurements have been performed with the polarograph previously described (*3*). The preparation of the solutions and the measurement technique are described in (*2*). Every measurements has been repeated at least three times. The standard deviation is less than 0.3. All potentials are given in reference to the saturated calomel electrode.

[1] This research has been sponsored in part by Contratto CNR Fisica Applicata No. 115/0300/5142.

[2] Gruppo Nazionale Struttura della Materia (GNSM) del Consiglio Nazionale delle Ricerche.

The peak of Mn occurs in a supporting electrolyte of alkali-chlorides. The behavior of Mn^{2+} in a NaCl solutions has been studied in detail. In an 0.1 *N* supporting electrolyte of NaCl a large anodic peak of Mn occurs at the potential −1.47 V. By using a more concentrated solution (1 *N* NaCl), the decrease in sensitivity is minimal. The preelectrolysis potential was −1.75 V. For preelectrolysis potentials closer to zero, the height of the peak decreases. The scan rate chosen was 0.35 V/minute. At a lower scan rate, the height and the width of the peak decreases; at a higher scan rate, instead, the height increases a little (Table 1a), while the width enlarges considerably. The temperature was maintained at 25 ± 0.1°C. In increasing the temperature up to 50°C, the peak height increases linearly by about 2.5%/°C. For all measurements the radius of the mercury drop was 3.95×10^{-2} cm. The sensitivity increases for radii up to 3.5 times greater than the one given. For greater values of the radii, the peak disfigures because of the considerable increase of the charging current. A linear dependence of the height of the peak on the preelectrolysis time and Mn^{2+} concentration has been observed (Table 1b and c).

The possibility of the determination of Mn in the presence of some bivalent cations (Cu, Pb, Cd, Zn) has been studied. It has been noted that within the ratios of concentrations studied, (Table 2), Cu, Pb, Cd,

TABLE 1

Mn Peak Height as a Function of Scan Rate, Preelectrolysis Time, Mn^{2+} Concentration

a. Scan rate [a]					
Scan rate (V/min):	0.13	0.21	0.35	0.42	0.87
Peak height (cm)	4	5	9.1	9.3	12
b. Preelectrolysis [b]					
Preelectrolysis time (min):	5	10	15	20	
Peak height (cm)	9.1	18.3	27.4	36	
c. Mn^{2+} conc [c]					
Mn^{2+} conc (μg/40 ml of 0.1*N* NaCl):	1	5	10		
Peak height (cm)	9.1	44.7	89		

[a] Mn height peak as a function of scan rate: preelectrolysis time, 5 min; Mn concentration, 1 μg/40 ml 0.1 *N* NaCl.

[b] Mn height peak as function of preelectrolysis time: Mn^{2+} concentration same as in *a*.

[c] Mn height peak as a function of Mn^{2+} concentration; preelectrolysis time same as in *a*.

TABLE 2

DETERMINATION OF Cu, Pb, Cd, Zn, Ni, Mn AT DIFFERENT CONCENTRATIONS [a]

Conc (μg/40 ml of 0.1 *N* NaCl)						Peak height (cm)					
Mn	Ni	Cu	Pb	Cd	Zn	Mn	Ni	Cu	Pb	Cd	Zn
1	1	1	1	1	1	9.2	5.8	5.5	5	15.8	19.8
1	5	5	5	5	5	9.3	28	29	25	75.5	96.5
5	1	1	1	1	1	45.5	5.9	5.8	5	15.8	19.7
10	1	1	1	1	1	92.5	5.7	5.7	5.1	15.9	19.8

[a] Preelectrolysis time, 5 min; Scan rate, 0.35 V/min.

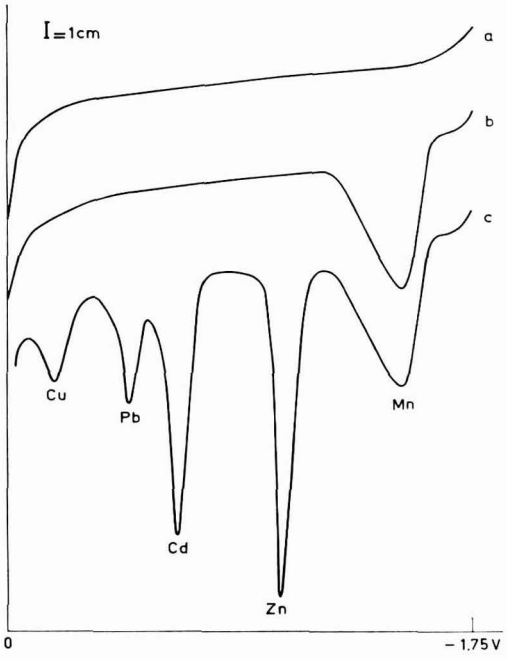


FIG. 1. Polarograms: current–voltage curve of (a) 0.1 *N* NaCl solution; (b) 1 μg of Mn^{2+} in 40 ml of 0.1 *N* NaCl solution; (c) 1 μg of Cu^{2+}, Pb^{2+}, Cd^{2+}, Zn^{2+}, Mn^{2+} in 40 ml of 0.1 *N* NaCl solution. Preelectrolysis time, 5 min; scan rate, 0.35 V/min.

Zn, and Mn can be detected simultaneously, and the height of the peak of Mn does not depend on the concentration of the other cations. In Fig. 1 a typical polarogram of Cu, Pb, Cd, Zn, and Mn is shown.

Behavior of Mn in Presence of Transition Elements: Ni, Fe, Co

In a supporting electrolyte of 0.1 *N* NaCl, Ni shows an anodic peak at a potential between that of Cu and Pb. In the concentration ratios

studied, it has been observed that the presence of Ni does not influence the determination of Mn, and both cations can be detected simultaneously (Table 2). Cations such as Cd^{2+} and Zn^{2+}, which show anodic peaks at potentials much different from that of Ni, can be detected simultaneously with Mn and Ni. The polarograms of Ni, Cd, Zn, and Mn are shown in Fig. 2. If Cu and Pb are present, the curve of Ni overlaps those of Cu and Pb, and these three cations cannot be detected at the same time. In any case, even if Cu, Ni, and Pb are present, Cd, Zn, Mn can be detected simultaneously.

In the presence of Fe^{2+}, the height of the Mn peak, with reference to the branch of the curve further away from zero, is proportional to the concentration of Mn. By increasing the concentration of Fe^{2+} (Fig. 2),

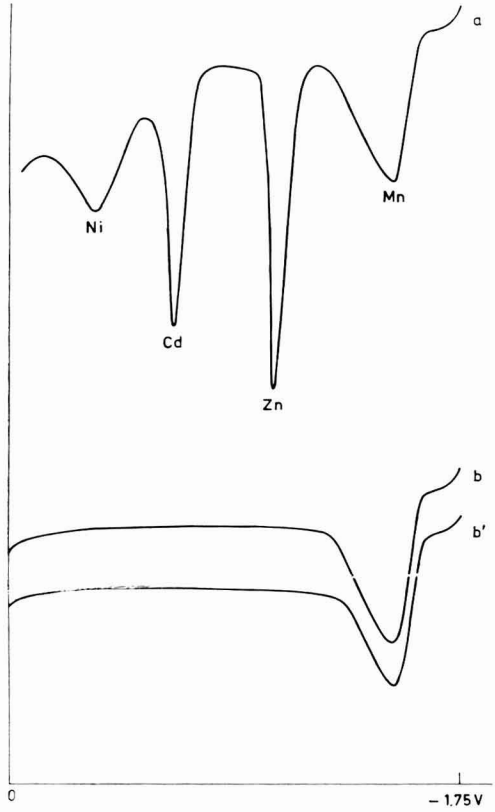


FIG. 2. Polarograms: current–voltage curve 1 μg of Ni^{2+}, Cd^{2+}, Zn^{2+}, Mn^{2+} in 40 ml of 0.1 *N* NaCl solution; (b) 1 μg of Mn^{+2}, Fe^{2+} in 40 ml of 0.1 *N* NaCl solution; (b′) 1 μg of Mn^{2+} and 20 μg of Fe^{2+} in 40 ml of 0.1 *N* NaCl solution. Preelectrolysis time and scan rate same as in Fig. 1.

the branch of the curve closer to zero decreases, and finally disappears at concentration ratio Mn:Fe of about 1:50.

Co, on a supporting electrolyte of NaCl or KCl, shows more than one anodic peak, depending on its concentration. Mn, in the presence of Co^{2+} at a concentration of the same order of magnitude, or greater, is no longer detectable. Instead of the peaks of Co and Mn, only one curve can be seen at a potential close to zero.

CONCLUSIONS

Conditions have been found for detecting submicrogram amounts of Mn^{2+} to a concentration of the order of magnitude of 10^{-6}%. Cu, Pb, Cd, Zn, and Ni do not influence the height of the peaks of Mn, and they can be detected together. However, if Ni is present with Cu or Pb, or Cu and Pb together, the curve of Ni overlaps that of Cu and Pb, because then anodic potentials are fairly close: −0.17 V for Cu; −0.30 V for Ni; −0.46 V for Pb. In this simultaneous detection becomes difficult. Yet Cu and Pb can be detected by performing the electrodeposition at a potential less negative than that of Ni, for instance at −0.9 V. Then if a polarogram is made of Cu, Ni, and Pb together, the Ni concentration can be calculated on the basis of the difference between these two polarograms.

In the presence of Fe^{2+}, the amount of Mn can be determined with sufficient accuracy to a concentration Mn:Fe of about 1:50. Instead, in the presence of the same amount or greater of Co^{2+}, the formation of intermetallic compounds between Co and Mn renders more difficult the exact determination of Mn, because only one peak appears at a potential close to zero.

ACKNOWLEDGMENTS

Thanks are due to Miss Battaglioli for the accuracy of our measurements.

REFERENCES

1. Buffie, J., Monnier, D., and Haerdi, W., Submicrogram determination of iron by inverse polarography. *Chimia* **21,** 578–81 (1967).
2. Fano, V., Trace Impurities distribution in potassium chloride and sodium chloride powers and single crystals. *Microchem. J.* **14,** 511–518 (1969).
3. Fano, V., and Scalvini, M., A versatile automatic polarograph with hanging mercury drop electrode. *Microchem. J.* **15,** 97–102 (1970).
4. Kowalski, Z., and Zarebski, J., Simultaneous determination of iron and titanium in raw materials and ceramics by alternating current polarography. *Chem. Anal.* (*Warsaw*) **13,** 55–63 (1968).
5. Markova, I. V., and Sinyakova, S. I., Determination of nanogram amounts of Co and Ni by stripping analysis. *Zh. Anal. Khim.* **23,** 1023–1027 (1968).

6. Sinyakova, S. I., Contemporary state and development of a polarographic method for determining trace impurities in pure and semiconductor substances. *Tr. Kom. Anal. Khim., Acad. Nauk. SSSR* **16,** 219–233 (1968).
7. Vinogradova, E. N., and Prokhorova, G. V., Rapid polarographic determination of microgram amounts of Ni and Co present together in natural H_2O and other materials. *Zh. Anal. Khim.* **23,** 711–715 (1968).

MICROCHEMICAL JOURNAL 15, 428–433 (1970)

Detection of Various Alicyclic Compounds Via the Komarowsky Reaction

HARVEY W. YUROW AND SAMUEL SASS

Chemical Research Laboratory, Edgewood Arsenal, Maryland 21010

Received October 27, 1969

INTRODUCTION

In contrast to aromatic compounds, cycloalkyl compounds are less amenable to spot test reactions.[1] One test that has been reported to give colors with various alicyclics is the Komarowsky reaction which involves condensation with aromatic aldehydes in strong acid solution. This reaction has been used for the identification of a number of alkenes, alcohols, and ketones as well as several unsaturated bicyclic compounds (*8*) and cyclohexanol (*1*). However no systematic study has been made on the alicyclic series.

The aim of this work was to thoroughly study the Komarowsky reaction with respect to various cycloalkyl and cycloalkylaryl compounds for purposes of differentiation and to obtain some insight into mechanisms.

MATERIALS AND METHODS

Reagents

The compounds used were practical or reagent grade, available from commercial sources. Cyclobutanol was obtained from Chemical Samples Co. (Columbus, Ohio). Concentrated stock solutions were prepared in methanol.

Procedure

The cycloalkyl derivatives in methanol (50 μl) and aldehydes in methanol (200 μl) were treated in a test tube with 2 ml of water; then 4 ml of concentrated sulfuric acid were added dropwise, followed by heating for 15 minutes at 100°C. Final concentrations were: substrate, 6.7×10^{-5} *M* (except cyclopentanone with *p*-hydroxybenzaldehyde, 3.3×10^{-5} *M*); and aldehyde, 1.0×10^{-2} *M*.

[1] A recent paper has appeared on the colorimetric determination of cycloalkanols by condensation with phenols in strong acid solution: A. J. Kalb and S. Erlich-Rogozinsky, *Microchem. J.* **14,** 478 (1969).

Spectrophotometric curves were obtained with the Carey model 10 recording spectrophotometer. Absorbances were measured on the Bausch and Lomb Spectronic 20 colorimeter and a reagent blank subtracted.

RESULTS

Table 1 shows the absorbances at λ_{max} for a variety of cycloalkanols with benzaldehyde and a number of *p*-substituted benzaldehydes.

Table 2 shows the absorbance and λ_{max} values for a number of cycloalkyl derivatives, with both *p*-hydroxybenzaldehyde and *p*-chlorobenzaldehyde.

TABLE 1

ABSORBANCES FOR CYCLOALKANOLS AND VARIOUS *p*-SUBSTITUTED BENZALDEHYDES

p-Substituted benzaldehyde	Substituent *I* value[a]	Net absorbance and λ_{max} for cycloalkanol			
		Cyclo-butanol	Cyclo-pentanol	Cyclo-hexanol	Cyclo-octanol
Hydroxy	−0.36	0.510 (600)	1.00 (590)	1.84 (620)	0.720 (520)
Methoxy	−0.27	0.570 (570)	0.940 (600)	1.41 (630)	0.830 (565)
Methyl	−0.17	0.940 (580)	0.930 (570)	0.890 (510)	0.420 (510)
Isopropyl	−0.15	0.580 (585)	0.480 (570)	0.590 (510)	0.340 (450)
Benzaldehyde	0.00	0.500 (550)	0.520 (490)	0.540 (480)	0.270 (440)
Fluoro	+0.06	0.630 (560)	0.440 (505)	0.990 (540)	0.400 (500)
Chloro	+0.23	0.890 (570)	0.230 (455)	0.530 (540)	0.350 (520)

[a] H. H. Jaffé, *Chem. Rev.* **53**, 222 (1953).

TABLE 2

ABSORBANCES FOR VARIOUS CYCLOALKYL DERIVATIVES WITH *μ*-HYDROXYBENZALDEHYDE AND *p*-CHLOROBENZALDEHYDE

Cycloalkyl derivative	Net absorbance and λ_{max}	
	p-Hydroxybenzaldehyde	*p*-Chlorobenzaldehyde
Cyclopropyl carbinol	0.650 (570)	0.760 (570)
Cyclobutylphenyl glycolic acid	1.11 (565)	0.770 (560)
Cyclobutylphenylketone	1.19 (590)	0.390 (580)
Cyclopentanone	1.31 (560)	1.54 (515)
Cyclopentene	0.470 (570)	0.120 (560)
Cyclohexanone	0.970 (550)	0.580 (490)
Cyclohexene	0.980 (630)	0.360 (440)
1-Methylcyclohexanol	0.650 (530)	0.400 (560)
2-Methylcyclohexanol	0.620 (530)	0.370 (560)
Cyclohexylmethanol	0.640 (530)	0.480 (560)
Cyclodecanol	0.320 (560)	0.160 (460)
Cyclododecanol	0.400 (565)	0.210 (460)

Weak or negative tests with *p*-hydroxybenzaldehyde were given by cyclopropylphenyl glycolic acid and ketone; cyclobutanone; cyclopentylphenyl glycolic acid and ketone; and cyclohexylphenyl glycolic acid and ketone. Dicyclohexylcarbodiimide gave a test similar to cyclohexanol, and 3-buten-1-ol gave one similar to cyclobutanol. Among the alkanols, strong tests were given by 2-butanol, 2-methyl-1-propanol, 2-methyl-2-propanol, 1-pentanol, and 1-hexanol. Weak tests were given by 1-butanol, 1-propanol, and 2-propanol.

By elimination of the heating step and rapid cooling of the solution 30 seconds after addition of sulfuric acid, cyclobutanol, cyclopentanol, and cyclohexanol could be differentiated with *p*-chlorobenzaldehyde. The colors obtained were light magenta, orange-pink and yellow-green, respectively; and the fluorescence colors (350 mμ excitation) were bright pink, pale blue, and light orange, respectively.

Cyclobutanol was also tested with a number of halogenated benzaldehydes in addition to the *p*-fluoro and *p*-chloro compounds. The following were examined: *o* and *m*-fluoro, *o* and *m*-chloro, *p*-bromo, 2,4-dichloro, and 3,4-dichloro. In all cases, either the color was weaker than for the *p*-fluoro- and the *p*-chlorobenzaldehyde, or else it was relatively unstable on heating.

DISCUSSION

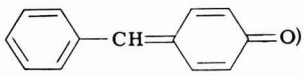
The Komarowsky reaction is a general one involving condensation of phenols, alcohols, ketones or unsaturated compounds, with aromatic aldehydes in strong acid solution (*8*). The mechanism has not yet been elucidated but several proposals have been advanced. The basic questions involved are: In what form does the substrate condense with the aldehyde, and what type of structure is responsible for the final color? With respect to the first question: Von Fellenberg (*8*) proposed dehydration of alcohols to olefines which condense with electrophilic aromatic aldehydes (*1*); Duke (*4*) postulated electrophilic carbonium ions which condense with the aromatic aldehyde on the aldehyde moiety, followed by oxidative cleavage to give a ketone or aldehyde that in turn can give colored aldol condensation products with the aromatic aldehyde. Since the cycloalkanols and cycloalkenes tested gave almost identical spectra, and the cycloalkanols and cycloalkyl ketones gave different ones, Fellenberg's mechanism seems more reasonable. However, Duke's postulation of intermediate carbonium ions may have some bearing on the test. Numerous studies have been made on decomposition of alcohols in strong acid solution, and mechanisms have been postulated which

involve carbonium ion intermediates undergoing various inter- and intramolecular condensation reactions, rearrangements, and cyclization (*3*). The stability of the carbonium ion is frequently of importance here, but in the Komarowsky test it does not appear to be so.

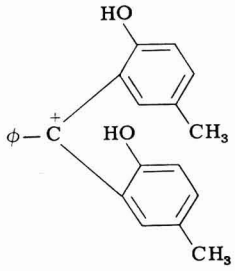
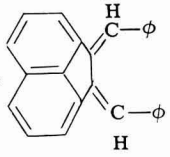
Thus, in the alkanol series, strong tests were given by *p*-hydroxybenzaldehyde with 2-butanol, 2-methyl-1-propanol, and 2-methyl-2-propanol which formed moderately stable carbonium ions; and by 1-pentanol and 1-hexanol which gave strong tests but did not form relatively stable ions. Weak tests were given by 1-propanol and 1-butanol (unstable carbonium ions) and by 2-propanol (a moderately stable carbonium ion). Similarly, in the cycloalkanol series, cyclohexanol gave results comparable to cyclobutanol and cyclopentanol although it formed the least stable carbonium ion of the three. The compounds 1-methylcyclohexanol, 2-methylcyclohexanol and cyclohexylmethanol gave almost identical absorbances and spectra with *p*-hydoxybenzaldehyde although they would be expected to differ widely in carbonium ion stability. Therefore it is more likely that it is the aromatic aldehyde rather than the carbonium ion that is the electrophilic species in the condensation.

From the types of compounds giving the Komarowsky reaction (*8*), the test seems to be applicable to active hydrogen or active methylene compounds in general. It may be pertinent that butanols in strong acid form alkylated cyclopentenyl cations (*3*) that, if not completely alkylated, could give rise to active methylene compounds. At present the structures of the intermediates from cycloalkanols, that condense with aromatic aldehydes in strong acid solution, must remain speculative.

The second problem is the structures of the colored products formed in the aldehyde condensations in strong acid. Feigl has proposed *p*-quinoid compounds (*6*) that would necessitate aromatic aldehydes having para groups that can convert to quinoid moieties (e.g. hydroxy) or substrates such as phenols with the para position unblocked

(to give, e.g. ). Farmilo and Genest (*5*) pointed

out that intense colors are sometimes formed with phenols that have blocked *para* positions. They postulated triarylmethane-type dye formation (*5*), involving condensation of the aromatic aldehyde at the *ortho*

positions to the activating group, to give a triarylmethane that is readily oxidized to a carbinol and that converts to a carbonium ion in strong acid (e.g. ). De Fazi has proposed fulvene formation (*2*) for condensation of aromatic aldehydes with active methylene compounds. (e.g. acenaphthene, ).

Examination of the behavior of the cyloalkanols with *p*-chlorobenzaldehyde would tend to indicate that, unless the chloro group can be removed during the reaction (which seems unlikely), the *para* position is blocked by a ring deactivating group. Thus both Feigl's and Farmilo and Genest's mechanisms do not appear to be relevant. The possibility of fulvene formation would necessitate that the substrate condensing with the aldehyde have a considerable degree of resonance in order to account for the intense colors obtained.

The use of *p*-chlorobenzaldehyde allows differentiation among the C_4, C_5, and C_6 cycloalkanols. Also, cyclobutanol differs from the other cycloalkanols in that colors are generally more, rather than less, intense with benzaldehydes having ring deactivating *para* substituents. Why this is so, is not known at present. The fulvene reaction would involve the change, $sp^3 \rightarrow sp^2$ for the cycloalkyl ring. This reaction is strongly disfavored in the cyclobutyl compounds because of considerable ring strain; less so, in the cyclohexyl derivative (bond opposition); and favored in cyclopentyl and cyclooctyl compounds. However, in discussions of the cyclobutyl group, it should be remembered that the cyclobutyl cation is in equilibrium with the cyclopropyl methyl cation and the 3-butenyl cation (*7*), the parent compounds of which gave similar reactions with *p*-chlorobenzaldehyde. Consequently, this system is quite complicated. The unique behavior of the cyclobutyl ring is also shown in the intense color given by cyclobutylphenyl glycolic acid and ketone in contrast to their cyclopropyl, cyclopentyl and cyclohexyl homologs.

SUMMARY

A considerable number of cycloalkanols, cycloakyl ketones, and cycloalkenes have been found to give colors on condensation with aromatic aldehydes in strong sulfuric acid. Cyclobutyl and cyclobutylphenyl compounds behave in a qualitatively different manner in these tests from the other cycloalkyl derivatives so that some degree of differentiation is possible.

REFERENCES

1. Dal Nogare, S., and Mitchell, J., Some applications of the Komarowsky reaction. *Anal. Chem.* **25,** 1376 (1953).
2. De Fazi, R., Una Nuova Reazione delle Aldeidi. *Gazz. Chim. Ital.* **46,** 334 (1916).
3. Deno, N. C., Carbonium ions. *Prog. Phys. Org. Chem.* **2,** 141–144 (1964).
4. Duke, F. R., The Komarowsky Reaction. *Anal. Chem.* **19,** 661 (1947).
5. Farmilo, C. G., and Genest, K., Alkaloids and related bases: Identification. *In* "Toxicology: Mechanisms and Analytical Methods." (C. P. Stewart and S. Stolman, eds.) Vol. 2, pp. 247–250. Academic Press, New York, 1961.
6. Feigl, F., "Spot Tests in Organic Analysis," 5th ed., pp. 133–134, Elsevier, Amsterdam, 1956.
7. Roberts, J. H., and Mazor, R. H., Small Ring Compounds. IV. Interconversion Reaction of Cyclobutyl, Cyclopropylcarbinyl and Alkylcarbinyl Derivatives. *J. Amer. Chem. Soc.* **73,** 2509 (1951).
8. Von Fellenberg, T., Über die Farbenreaktion nach Komarowsky. *Chem. Ztg.* **34,** 791 (1910).

MICROCHEMICAL JOURNAL 15, 434–437 (1970)

Tripositive Copper as a Titrant: Determination of Some Sugars

P. K. JAISWAL

Department of Chemistry, M.M.M.V. Bhat Par Rani, Deoria, India

Received August 9, 1969

INTRODUCTION

Myrbäck (*1*) studied the oxidation of aldoses by hypoiodite in various alkaline media. Nakata (*2*) determined sugars by anthrone. Some methods such as colorimetric method (*3,4*) partition chromatography (*5*), and paper chromatography (*6*) have been developed. In the present investigation, some sugars (lyxose, ribose, allose, altrose, gulose, and sorbose) were oxidized; and the use of Cu(III) as an oxidant was studied in detail.

An excess of Cu(III) was added to the substrate compounds. The oxidation was slow at room temperature. Complete oxidation of the substrate compound took place when the solution mixture was boiled. It was oxidized to carbon dioxide and water. Probably the following reaction appears to take place.

$$\underset{\text{(pentose)}}{C_5H_{10}O_5} + 10[O] \rightarrow 5CO_2 + 5H_2O$$

$$\underset{\text{(hexose)}}{C_6H_{12}O_6} + 12[O] \rightarrow 6CO_2 + 6H_2O$$

One mole of pentose and hexose require 20 and 24 equivalents of the oxidant, respectively, for complete oxidation.

EXPERIMENTAL METHODS

Reagents. All the chemicals used were of reagent grade. Cu(III) was prepared and standardized as described earlier (*7*).

Procedure. An aliquot (see Tables 1–6) was added to an excess of Cu(III) solution. The mixture was heated on the hot plate, when it was necessary for complete oxidation, and unconsumed Cu(III) was determined by addition of known excess of arsenite solution. The remaining arsenite solution was titrated with iodine in bicarbonate medium. A blank was also run. The equivalence in terms of Cu(III) for 1 g mole of the compound was calculated. The observations with different sugars are given in Tables 1–6. The excess of Cu(III) was determined after the copper(III)–sugar mixture had (a) stood for 0 minutes, (b) stood for 15 minutes, and (c) been boiled and cooled.

TABLE 1

ESTIMATION OF LYXOSE

4.0×10^{-2} *M* Cu(III) (ml)	1.20×10^{-3} *M* Lyxose (ml)	Equivalents of Cu(III) consumed
2.0	2.0	9.50[a]
2.0	2.0	13.40[b]
2.0	2.0	20.00[c]
2.0	2.5	20.10[c]
2.0	1.0	20.05[c]

[a] The Cu(III)–sugar stood for 0 min; [b] stood for 15 min; and [c] had been boiled and cooled.

TABLE 2

ESTIMATION OF RIBOSE

4.0×10^{-2} *M* Cu(III) (ml)	1.30×10^{-3} *M* Ribose (ml)	Equivalents of Cu(III) consumed
2.0	2.0	10.20[a]
2.0	2.0	14.50[b]
2.0	2.0	20.00[c]
2.0	2.5	20.04[c]
2.0	2.8	20.03[c]

TABLE 3

ESTIMATION OF ALLOSE

4.0×10^{-2} *M* Cu(III) (ml)	1.00×10^{-3} *M* Allose (ml)	Equivalents of Cu(III) consumed
3.0	2.5	8.60[a]
3.0	2.5	12.00[b]
3.0	2.5	24.00[c]
3.0	3.0	24.00[c]
3.0	1.5	24.01[c]

TABLE 4

ESTIMATION OF ALTROSE

4.0×10^{-2} *M* Cu(III) (ml)	1.05×10^{-3} *M* Altrose (ml)	Equivalents of Cu(III) consumed
3.0	2.5	9.50[a]
3.0	2.5	12.90[b]
3.0	2.5	24.10[c]
3.0	3.0	24.06[c]
3.0	2.0	24.00[c]

TABLE 5

ESTIMATION OF GULOSE

4.0×10^{-2} *M* Cu(III) (ml)	4.70×10^{-3} *M* Gulose (ml)	Equivalents of Cu(III) consumed
8.0	1.0	11.2[a]
8.0	1.0	13.0[b]
8.0	1.0	24.0[c]
8.0	1.5	24.05[c]
8.0	0.5	24.04[c]

TABLE 6

ESTIMATION OF SORBOSE

4.0×10^{-2} *M* Cu(III) (ml)	4.80×10^{-3} *M* Sorbose (ml)	Equivalents of Cu(III) consumed
8.0	1.0	11.6[a]
8.0	1.0	12.9[b]
8.0	1.0	24.01[c]
8.0	0.7	24.02[c]
8.0	0.5	24.02[c]

DISCUSSION

Tables 1–6 clearly show that the sugars are oxidized to carbon dioxide and water when heated with an excess of copper(III) solution. Lyxose and ribose, which are pentoses, require 20 equivalents/mole; the hexoses allose, altrose, gulose, sorbose consume 24 equivalents. They were estimated on this basis. The consumption of less copper(III) at room temperature by these sugars indicates that their oxidation at ordinary temperature is incomplete.

ACKNOWLEDGMENTS

The author expresses his sincere thanks to Dr. K. L. Yadava and Km. Sarla. Prakash, and Rakesh Jaiswal.

REFERENCES

1. Myrbäck, K., The oxidation of aldoses by hypoiodite. *Sv. Kem. Tidskr.* **51,** 179–192, 206–217 (1939).
2. Nakata, T., Determination of mono- and disaccharide by anthrone. *Tokyo Jikeikai Ika Daigaku Zasshi* **72,** 464–470 (1957).
3. Plumel, M., Colorimetric microanalysis of sugars. *Ann. Biol. Clin.* (*Paris*) **9,** 307–317 (1951).
4. Bolliger, A., Color reactions for sugars. Identification and determination of monosaccharides with thymol, HCl, and $FeCl_3$. *J. Proc. Roy. Soc. N. S. W.* **77,** 109–115 (1944).

5. Hirst, E. L., Hough, L., and Jones, J. K. N., Quantitative analysis of mixtures of sugars by the method of partition chromatography. Separation and determination of methylated aldoses. *J. Chem. Soc., London* **1949,** 928–933.
6. Stoll, A., and Rüegger, A., Separation and estimation of sugars by paper chromatography of their *p*-nitrophenylhydrazones. *Helv. Physiol. Pharmacol. Acta* **10,** 385–394 (1952).
7. Chandra, S., and Yadava, K. L., Oxidation of some carbonyl compounds with Cu(III). *Microchem. J.* **13,** 491–499 (1968).

MICROCHEMICAL JOURNAL 15, 438–440 (1970)

Determination of the Minimal Amount of Antigen Detected by the Electroprecipitin Test on Cellulose Acetate [1]

FREDERICK A. ZYDECK

Department of Comparative Medicine, Wayne State University, School of Medicine, Detroit, Michigan 48207

Received October 30, 1969

Previous studies comparing the electroprecipitin test (*4*) on cellulose acetate (*5*) to the agar gel technique of double diffusion in one dimension for detecting precipitin reactions indicated that in instances when high titered antiserum was utilized undiluted, the electroprecipitin test may be more sensitive in detecting minute amounts of antigen (*6*). However, due to the system employed, it was impossible to quantitate amounts of antigen utilized and therefore no conclusion could be reached as to the minimal amount of antigen detected by the electroprecipitin test.

This study reports the quantitative comparison of the electroprecipitin test to the precipitin technique of double diffusion in one dimension in agar gel, utilizing a crystallized egg albumin–antiegg albumin system.

MATERIALS AND METHODS

Antigen and antiserum. Crystalline egg albumin (5x)[2] was dissolved in saline and mixed with complete Freunds adjuvant [3] to yield a final concentration 12 mg/ml. One ml of this preparation was then injected subcutaneously and into the foot pads of albino rabbits. Three weeks after initial injection, a booster injection was given in the same manner. Three weeks later blood was collected via venipuncture, the serum was separated and stored frozen until tested. For testing purposes, dilution of antigen or antiserum were made in saline. In antigen test determinations, a serial 10-fold dilution was used.

Precipitin tests. The electroprecipitin test on cellulose acetate was performed as previously described (*5, 6*). A Veronal barbital buffer,[2] pH 8.6, ionic strength 0.05, was used in all electroprecipitin tests.

[1] This work was supported in part by PHS Grant FR00437-01A1 from the Animal Resources Branch of the Division of Research Facilities and Resources, National Institutes of Health, U.S.

[2] Nutritional Biochemical Corporation; Cleveland, Ohio.

[3] Difco Laboratories; Detroit, Michigan.

Cellulose acetate electrophoretic support strips [4] were presoaked for 30 minutes prior to using, then blotted and placed in the electrophoretic chamber. A 5-μ drop of antiserum was placed 1 inch from the cathode, and a current of 250 V, 5 to 7 mA, was passed for 30 minutes. The current was shut off, and the antigen (approx 5μl), were applied with a fire-polished capillary tube (0.5 to 0.9-mm i.d.) as a straight line immediately behind (on the cathode side) of the point of antiserum application. Current was restored for 20 minutes. At the end of that time, the strip was transferred to 0.85% saline solution and rinsed for 5 to 10 minutes, with occasional movement of the saline solution. The strip was then dried and placed in Ponceau S stain [5] for 5 minutes. The strip was then rinsed twice in 5% acetic acid and once in tap water. After drying, the strips were examined for a precipitin band by means of illumination from behind. The reference standard test was the agar gel technique of double diffusion in one dimension (*3*). The gel interphase between antigen and antiserum was 0.6% agar [6] with 1% sodium azide added.

RESULTS

As determined by both the electroprecipitin and agar gel technique, the egg albumin–antiegg albumin system revealed one distinct precipitin reaction. Utilizing a predetermined optimal concentration of 0.01 mg/ml of albumin, the antiserum to be used in all subsequent tests titered to 1:256. The minimal amount of antigen detected in agar gel, was 0.001 mg/ml. While the minimal amount of antigen detected by the electroprecipitin test was 0.00001 mg/ml (Table 1).

[4] Sepraphore III. Gelman Instrument Co.; Ann Arbor, Michigan.
[5] Harleco, Hartman-Leddon Company; Philadelphia, Pennsylvania.
[6] Noble Agar, Difco Laboratories; Detroit, Michigan.

TABLE 1

TEST TO COMPARE MINIMAL AMOUNT OF ANTIGEN NECESSARY TO YIELD VISIBLE PRECIPITATE: ANTISERUM CONSTANT AT 1:256

Antigen conc (mg/ml)	Gel diffusion	Electroprecipitin
0.1	+	+
0.01	+	+
0.001	+	+
0.0001	−	+
0.00001	−	+
0.000001	−	−

DISCUSSION

In comparison with an agar gel technique, considered to be one of the most sensitive for quantitative precipitin reactions (*1*), the electroprecipitin test utilizing undiluted antisera can detect antigen in lesser amount. This is attributed to the forceful movement of all available antigen into the antibody-containing gamma globulin portion of the antiserum.

As the electroprecipitin test requires but 0.005 ml of either reactant (as compared to 0.01 ml in agar gel) to yield visible reactions and reactions are visualized in less than 2 hours (compared to 24 to 48 hours in gel), conclusions drawn previously are substantiated. That is, the electroprecipitin test should be of value where high titered antiserum is available in detecting minute amounts of antigen where speed is essential.

Although the test was not compared directly to the technique of electroimmunodiffusion (EID) (*2*), it appears to be at least as sensitive, if not more sensitive, than results reported in the literature. Studies are currently underway to determine these sensitivities.

CONCLUSION

In examining a crystalline egg albumin–antiegg albumin system, the electroprecipitin test detected antigen at a concentration of 0.00001 mg/ml. As suggested previously the test appears more sensitive than gel diffusion (where high titer antiserum is available) in detecting minute amounts of antigen via the precipitin reaction.

REFERENCES

1. Finger, I., and Kabat, E. A., A comparison of human antisera to purified diptheria, a toxoid with antisera to other purified antigens by quantitative precipitin and gel diffusion techniques. *J. Exp. Med.* **108,** 453–474 (1958).
2. Merrill, D., Hartley, T. F., and Clamon, H. N., Electroimmunodiffusion: A simple, rapid method for quantitation of immunoglobulins in dilute biological fluids. *J. Lab. Clin. Med.* **69**(11), 151–159 (1967).
3. Preer, J. F., A quantitative study of a technique of double diffusion in agar. *J. Immunol.* **77,** 52–60 (1956).
4. Watson, D., and Whinfrey, H., An electroprecipitin test. *Lancet* **2,** 1375 (1958).
5. Zydeck, F. A., Muirhead, E. E., and Schneider, H., A rapid technique for detecting multiple antigen–antibody precipitin reactions. *Nature (London)* **205,** 189–190 (1965).
6. Zydeck, F. A., Muirhead, E. E., and Schneider, H., Electrophoretic precipitin test on cellulose acetate. *Amer. J. Clin. Pathol.* **45,** 3 (1966).

MICROCHEMICAL JOURNAL 15, 441–445 (1970)

Spectrophotometric Determination of Silicon in the Presence of Germanium

F. A. SORRENTINO, NANCY J. WITIAK, AND J. PAUL [1]

Chemistry Department, University of Bridgeport, Bridgeport, Connecticut 06602

Received November 19, 1969

During the spectrophotometric determination of silicon as the yellow silicomolybdic acid (*1*) or as the silicoheteropoly blue (*2, 3*) germanium interferes by formation of the yellow germanomolybdic acid (*4, 5*) or the germanoheteropoly blue (*6*). Investigations on the possible elimination of germanium by selective solvent extraction of germanomolybdic acid or the germano-heteropoly blue have hitherto proved unsuccessful because of the general nonselectivity of the extracting solvents.

Buchanan (*7*), and Dennis and Johnson (*8*) reported the separation of germanium from other interferences by the formation and distillation of germanium tetrachloride. The method has the disadvantage of being time consuming. Germanium was also removed by precipitation as the sulfide (*9*) and as magnesium germanate (*10*). Davies and Morgan (*11*) reported the precipitation of germanium with tannin in the presence of sulfuric acid. The procedure was later modified by Holness (*12*) who reported quantitative precipitation of germanium with tannin from oxalate solutions, while Weissler (*13*) reported on the superiority of the tannic acid method of precipitation of germanium over other methods of precipitation.

Since silicon is not precipitated by tannic acid, there seemed the possibility of removing germanium interference during the spectrophotometric determination of silicon as the heteropoly blue, by precipitation with a solution of tannic acid, provided that any interferences caused by an excess of tannic acid on the development of the silicoheteropoly blue color could also be removed during the course of the analysis. This paper describes a procedure for the spectrophotometric determination of silicon in the presence of germanium, by firstly eliminating germanium interference by precipitation with tannic acid, followed by removal of excess tannic acid by solvent extraction with isoamyl alcohol, and the subsequent formation, and selective solvent extraction of the yellow

[1] To Whom Inquiries should be addressed

silicomolybdic acid with 2-ethyl hexanol. The silicomolybdic acid is then reduced in the solvent phase to the silicoheteropoly blue.

MATERIALS AND METHODS

Reagents

All reagents were of Analar grade. Distilled water was used throughout. All reactions were carried out at room temperature.

Silicate solutions are prepared by fusing 0.100 g of pure precipitated silica with 1.5 g of anhydrous sodium carbonate in a platinum crucible, and dissolving the melt in 100 ml of water in a polythene beaker. Prepare further solutions of soluble silica from it by dilution.

Germanate solutions are prepared by fusing 0.100 g of germanium dioxide with about 0.5 g of anhydrous sodium carbonate in a platinum crucible and dissolving the melt in 100 ml of water in polythene beaker. Prepare further solutions from it by dilution.

1-Amino-2-naphthol-4-sulfonic acid reagent is prepared by dissolving 1-amino-2-naphthol-4-sulfonic acid (0.20 g), sodium sulfite heptahydrate (2.4 g) and sodium metabisulfite (12.0 g) in water and diluting to 100 ml. Store in a dark bottle and prepare freshly weekly.

Tannic acid solution (10% w/v)—freshly prepared before use.

Hydrochloric acid (2 *N*).

Ammonium molybdate solution (10% w/v of the tetrahydrate salt).

Perchloric acid (70%) sp gr 1.6.

Isoamyl alcohol.

2-Ethyl hexanol.

Procedure

Pipette 4.0-ml portions of the test solutions into a small beaker, add 4.0 ml of hydrochloric acid and mix. Add 2.0 ml of tannic acid solution, mix and let stand for 30 minutes. Then filter into a 50-ml separatory funnel and extract with 10.0 ml of isoamyl alcohol by shaking for 30 seconds. Pipette 2.0 ml of the aqueous filtrate into another separatory funnel and add 2.0 ml of ammonium molybdate solution. Mix and allow to stand for 10 minutes. Then add 2.0 ml of perchloric acid (70%) and, after mixing, extract the yellow silicomolybdic acid with 10 ml of 2-ethyl hexanol by shaking for 30 seconds. Carefully withdraw the aqueous layer, and to the solvent phase, add 2.0 ml of perchloric acid (70%) and 1.0 ml of 1-amino-2-naphthol-4-sulfonic acid reagent. Shake for about 20 seconds and allow to stand for 1 hour for maximum color development. Read at 690 mμ against a similarly treated and extracted blank.

RESULTS AND DISCUSSION

The procedure as outlined for the direct reduction of silicomolybdic acid in the solvent phase in the presence of perchloric acid, gave a straight-line calibration graph over the concentration range of 0–20 μg/ml of soluble silica. For the efficient and homogeneous reduction of the silicomolybdic acid in the solvent phase, the addition of perchloric acid was essential. It was found that 2.0 ml of perchloric acid (70%) was sufficient to allow homogeneous reduction of the silicomolybdic acid in isooctyl alcohol by the amino-naphthol-sulfonic acid reagent. A similar procedure for the direct reduction of phophomolybdic acid in isobutyl acetate has also been reported (*14*).

The results in Table 1 show that germanium is completely precipitated by 10% tannic acid in amounts of up to 80 μg of GeO_2, while amounts larger than this quantity are incompletely precipitated. Substantially similar results are obtained when a 20% solution of tannic acid is used as the precipitating agent and when the standing time allowed for precipitation is increased to 1–4 hours. The results in Table 2 confirm that

TABLE 1

PRECIPITATION OF GERMANIUM WITH TANNIC ACID; SILICON ABSENT

GeO_2 present (μg)	GeO_2 found in filtrate after precipitation (μg)
20	0
40	0
60	0
80	0
120	<5
160	10
240	15

TABLE 2

EFFECT OF TANNIC ACID ON SILICON DETERMINATION; GERMANIUM ABSENT

SiO_2 present (μg/2 ml)	SiO_2 found (μg/2 ml)
10	10
16	16
20	20
24	24
30	30
40	40

silicon is not precipitated by tannic acid, and at the same time demonstrate that soluble silica is not extracted by isoamyl alcohol during the removal of excess tannic acid by solvent extraction with this reagent. To complete this study, the recoveries of silicon in the presence of germanium from mixtures containing the two elements were determined by the procedure. The results of these experiments recorded in Table 3 demonstrate that this method of silicon determination in the presence of germanium is simple, accurate, and reproducible when the amounts of germanium present as interference does not exceed 120 μg of GeO_2.

TABLE 3

DETERMINATION OF SILICON IN THE PRESENCE OF GERMANIUM

	SiO_2 in 2.0-ml aliquot of filtrate (μg)	
GeO_2 present (μg)	Present	Found
10	6, 24	6, 24
20	4, 12, 18	4, 12, 18
40	2, 12	2, 12
60	8, 12	8, 13
80	8, 16	8, 16
120	8, 16	9, 18

SUMMARY

A simple, accurate, reliable, and reasonably rapid method for the determination of silicon in the presence of germanium is reported. The method involves the precipitation of germanium with 10% tannic acid and the solvent extraction of excess tannic acid by isoamyl alcohol. The silicomolybdic acid is then formed in the aqueous phase, and selectively extracted with isooctyl alcohol. It is then reduced directly in the solvent phase by 1-amino-2-naphthol-4-sulfonic acid in the presence of perchloric acid.

REFERENCES

1. Chalmers, R. T., and Sinclair, A. G., Analytical applications of -heteropoly acids. Part I. *Anal. Chim. Acta* **33,** 384 (1965).
2. Straub, F. C., and Grabowski, H. A., Photometric determination of silica, *Ind. Eng. Chem., Anal. Ed.* **16,** 574 (1944).
3. Paul, J., Simultaneous determination of phosphorus and silcon in the presence of each other. *Anal. Chim. Acta* **23,** 178 (1960).
4. Alimaim, I. P., and Ivanov-Emin, B. N., Determination of germanium. Mikrochemie **21,** 1 (1936).
5. Kitson, R. E., and Mellon, M. G., Colorimetric determination of germanium as molybdodigermanic acid. *Ind. Eng. Chem., Anal. Ed.* **16,** 128 (1944).
6. Krause, H. H., and Johnson, O. H., Analytical methods for germanium. *Anal. Chem.* **25,** 134 (1953).

7. Buchanan, G. H., Occurrence of germanium in zinc material. *Ind. Eng. Chem.* **8,** 585 (1916).
8. Dennis, L. M., and Johnson, E. B., Germanium extraction from germanium bearing zinc oxide. Direct preparation of germanium dioxide free from arsenic. *J. Amer. Chem. Soc.* **45,** 1380 (1923).
9. Johnson, E. B., and Dennis, L. M., The determination of germanium. Part X. *J. Amer. Chem. Soc.* **47,** 790 (1925).
10. Muller, J. H., A new method for the gravimetric determination of germanium. *J. Amer. Chem. Soc.* **44,** 2493 (1922).
11. Davies, G. R., and Morgan, G., The gravimetric determination of germanium. *Analyst* (*London*) **63,** 388 (1938).
12. Holness, H., The precipitation of germanium by tannin. *Anal. Chim. Acta* **2,** 254 (1948).
13. Weissler, A., Determination of germanium in steel. *Ind. Eng. Chem., Anal. Ed.* **16,** 311 (1944).
14. Paul, J., Simultaneous determination of arsenic and phosphorus. *Mikrochim. Acta* **5–6,** 830 (1965).

MICROCHEMICAL JOURNAL 15, 446–451 (1970)

Simultaneous Determination of Arsenic, Germanium, Phosphorus, and Silicon

F. A. SORRENTINO [1] AND J. PAUL [2]

Chemistry Department, University of Bridgeport, Bridgeport, Connecticut 06602

Received December 19, 1969

Methods have been reported for the simultaneous determination of phosphorus and silicon (*5*), arsenic and phosphorus (*6*), arsenic, phosphorus, and silicon (*7*) and arsenic, germanium, and phosphorus, as their heteropoly blues (*8*). However, no method has been reported hitherto, for the simultaneous determination of all four elements in the presence of each other. The difficulty encountered in such a multiple simultaneous determination is the lack of an efficient, selective method of separation of germanium and silicon from each other as either their heteropoly acids or heteropoly blues. Preliminary investigations on the selective solvent extraction of germano- and silicoheteropoly acids, and also, of their heteropoly blues utilizing a variety of polar oxygen-containing solvents, and mixtures of such solvents, at varying acid concentrations, failed to give a sufficiently selective method for the quantitative separation of germanium and silicon from each other.

Cluley (*2*) reported a highly sensitive method of determination of germanium based on the phenylfluorone spot test of Gillis *et al.* (*3*). His investigations showed that phosphorus and arsenic did not interfere in the procedure and arsenic interference was appreciable only at high concentrations. However, interference from molybdenum was most marked. Luke and Campbell (*4*) modified the phenylfluorone procedure by reducing the analysis time from 30 to 5 minutes by adjusting the pH of the solution to 3.1 before the addition of the phenylfluorone reagent, while Burton and Riley (*1*) successfully used the procedure for the determination of germanium in sea water.

Since molybdenum interferes in the phenylfluorone method of germanium determination but arsenic, phosphorus, and silicon do not substantially interfere, the possibility of firstly determining germanium as the phenylfluorone complex, when the former is present in a mixture containing arsenic, phosphorus, and silicon, was considered with a view

[1] Taken from a thesis submitted in partial fulfillment of the M.S. degree

[2] To whom all inquiries should be addressed

to effecting a simultaneous determination of germanium, arsenic, phosphorus, and silicon in the presence of each other, by selective solvent extraction of the germanium phenylfluorone complex and unreacted phenylfluorone reagent before the formation of the arseno-, phospho-, and silicomolybdic acids in the remaining aqueous phase. Arsenic, phosphorus, and silicon may then be determined individually by a procedure previously reported (*7*).

MATERIALS AND METHODS

Reagents

All reagents were of Analar Grade. Distilled water showing no detectable amount of germanium, silicon, phosphorus, or arsenic was used throughout. All reactions were carried out at room temperature.

Phosphate and Arsenate Solutions

Prepare stock solutions of phosphorus or arsenic by dissolving 0.100 g of disodium hydrogen phosphate (anhydrous) or disodium hydrogen arsenate (heptahydrate) in water and diluting to 100 ml. Prepare further solutions from these by dilution.

Silicate Solutions

Prepare solutions of soluble silica by fusing 0.100 g of pure precipitated silica with about 1.5 g of anhydrous sodium carbonate in a platinum crucible, and dissolving the melt in 100 ml of water in a polythene beaker. Prepare further solutions of soluble silica from it by dilution.

Germanate Solutions

Prepare stock solutions of germanium by fusing 0.100 g of germanium dioxide with about 0.5 g of anhydrous sodium carbonate in a platinum crucible and dissolving the melt in 100 ml of water in a polythene beaker. Prepare further solutions from it by dilution.

1-Amino-2-naphthol-4-sulfonic Acid Reagent

Dissolve 0.20 g of 1-amino-2-naphthol-4-sulfonic acid, 2.4 g of sodium sulfite (heptahydrate) and 12.0 g of sodium metabisulfite in water and dilute to 100 ml. Filter and store in a dark bottle. Prepare freshly weekly.

Buffer Solution (pH 5.0)

Dissolve 225 g of sodium acetate trihydrate in about 250 ml of water by heating. Transfer to a 500-ml volumetric flask containing 120 ml of

glacial acetic acid and, after cooling, make up to mark with water and mix.

Phenylfluorone reagent, 0.01% solution of 2,3,7-trihydroxy-9-phenyl-6-fluorone in 95% ethanol containing 1 ml of concentrated hydrochloric acid.

Hydrochloric acid, 10% (v/v).
Ammonium molybdate solution, 5% (w/v).
Perchloric acid, 70% sp gr 1.6.
Hydrazine sulfate solution, 1% (w/v).
Sulfuric acid solution, 25% (v/v).
Citric acid solution, 10% (w/v).
Isobutyl acetate.
Isoamyl alcohol.

Germanium Determination

Pipette 2.0 ml of the test solution into a 50-ml separation funnel, add 0.6 ml of sulfuric acid and mix. Then add 2.0 ml of the buffer solution followed by 2.0 ml of the phenylfluorone reagent. Mix and let stand for 4 minutes for maximum color development and then add 3.4 ml of hydrochloric acid solution to redissolve any precipitated sodium acetate. Read immediately at 525 mμ against a similarly treated blank.

Arsenic and Phosphorus Determination

After measuring the absorbance of the germanium phenylfluorone complex, return the solution to the separatory funnel and extract with 10.0 ml of isoamyl alcohol by shaking for 20 seconds. Allow the layers to separate and then withdraw the aqueous layer. Pipette a 4.0-ml aliquot of this aqueous solution into another separatory funnel. Add 1.0 ml of perchloric acid (70%), mix, and allow to stand for 15 minutes for complete polymerization of the soluble silica. Then add 2.0 ml of ammonium molybdate solution, mix, and allow to stand for 10 minutes for the complete formation of the arseno- and phosphomolybdic acids. Add 10.0 ml of isobutyl acetate, shake for 30 seconds, and proceed according to the method already described for the simultaneous determination of arsenic and phosphorus (*6*).

Silicon Determination

Pipette another 4.0 ml aliquot of the aqueous solution remaining after the extraction of the germanium phenylfluorone complex with isoamyl alcohol, into another separating funnel. Add 2.0 ml of ammonium molybdate solution, mix, and allow to stand for 10 minutes for the complete formation of the arseno-, phospho-, and silicomolybdic

acids. Then proceed to determine silicon by the procedure previously described (*7*) involving the selective destruction of the arseno- and phosphomolybdic acids with citric acid and reduction of the remaining silicomolybdic acid to the silicoheteropoly blue with 1-amino-2-naphthol-4-sulfonic acid.

RESULTS AND DISCUSSION

The procedure as outlined for the determination of germanium by the phenylfluorone method gave a straight-line calibration graph at 525 mμ over the concentration range of 0–5 μg/ml. Greater sensitivity (0–1.5 μg/ml) may be obtained by measuring the absorbance of the complex at 515 mμ. Straight-line calibration graphs were also obtained for phosphorus, after direct reduction of the phosphomolybdic acid in isobutyl acetate, and for arsenic and silicon after reduction of their heteropoly acids to their corresponding heteropoly blue.

The results in Table 1 demonstrate that excellent recoveries of germanium are obtained by the phenylfluorone method of germanium determination in the presence of relatively high concentration of arsenic, phosphorus, and silicon. Table 2 is a composite table showing recoveries of arsenic, phosphorus, and silicon in the presence of phenylfluorone reagent, the latter being removed by selective solvent extraction with isoamyl alcohol before the addition of ammonium molybdate to form the arseno-, phospho-, and silicoheteropoly acids. These recovery experiments demonstrate that an excess of the phenylfluorone reagent does not interfere with the subsequent determination of arsenic, phosphorus, and silicon through complexation of phenylfluorone with molybdenum, because of the complete elimination of unreacted phenylfluorone by solvent extraction with isoamyl alcohol.

To complete these studies, germanium, phosphorus, and silicon were determined in the presence of each other in recovery experiments. The

TABLE 1

RECOVERY OF GERMANIUM IN THE PRESENCE OF ARSENIC, PHOSPHORUS, AND SILICON

GeO_2 present (μg)	Phosphate present (μg)	Arsenate present (μg)	SiO_2 present (μg)	GeO_2 recovered (μg)
1	100	100	60	1
3	100	100	60	3
4	50	50	30	4
5	100	100	60	5
8	50	50	30	8
9	100	100	60	9

results of these experiments, recorded in Table 3, demonstrate that using this procedure of simultaneous determination, excellent recoveries of all four elements over a wide range of concentration are obtained. The method is simple, rapid, accurate, and reproducible.

Investigations are presently in progress to effect an alternative method of simultaneous determination of arsenic, germanium, phosphorus, and silicon based on a procedure for the selective solvent extraction of silicon from arsenic instead of the present method of selective destruction of the arseno- and phosphomolybdic acids by citric acid, and the acid polymerization of soluble silica.

TABLE 2

RECOVERY OF ARSENIC, PHOSPHORUS, AND SILICON IN THE PRESENCE OF PHENYLFLUORONE

The test solutions were extracted with isoamyl alcohol before the addition of ammonium molybdate.

Arsenate (μg)		Phosphate (μg)		SiO_2 (μg)	
Present	Found	Present	Found	Present	Found
8	6	8	8	8	8
16	17	16	16	24	24
32	30	40	40	32	32
40	40	80	79	40	40
64	63	120	120	—	—

TABLE 3

SIMULTANEOUS DETERMINATION OF ARSENIC, GERMANIUM, PHOSPHORUS, AND SILICON

	GeO_2 (μg)		Phosphate (μg)		Arsenate (μg)		SiO_2 (μg)	
Mixtures	Added	Found	Added	Found	Added	Found	Added	Found
A	1	1	8	9	8	6	32	32
B	3	3	80	79	80	79	8	8
C	4	4	40	39	40	39	24	24
D	5	5	60	61	32	30	40	39
E	6	6	16	16	16	17	40	40
F	8	8	120	120	64	63	8	8
G	9	9	40	39	8	6	8	8

SUMMARY

A simple, rapid, accurate, and reliable method of simultaneous determination of arsenic, germanium, phospohrus, and silicon is reported. The method involves first, the determination of germanium as the phenylfluorone complex and its

selective extraction with isoamyl alcohol. Arsenic and phosphorus are determined in the remaining aqueous phase by selective formation of the arseno- and phosphomolybdic acids after polymerization of soluble silica with perchloric acid followed by the selective extraction of the phosphomolybdic acid in the presence of arsenomolybdic acid by isobutyl acetate. Silicon is a determined by a differential procedure after selective destruction of arseno- and phosphomolybdic acids in the presence of silicomolybdic acid. Arsenic, silicon, and phosphorus are determined as their heteropoly blues, the latter after direct reduction of the phosphomolybdic acid in isobutyl acetate.

REFERENCES

1. Burton, J. D., and Riley, J. P., The Spectrophotometric determination of germanium with phenylfluorone. *Mikrochim. Acta* **1959,** 586–591.
2. Cluley, J. H., The Determination of Germanium. Part II. Absorptiometric determination with phenylfluorone. *Analyst (London)* **76,** 523–530 (1951).
3. Gillis, J., Hoste, J., and Clays, A., A specific reagent for germanium: Phenylfluorone. *Anal. Chim. Acta* **1,** 302–308 (1947).
4. Luke, C. L., and Campbell, M. E., Photometric determination of germanium with phenylfluorone. *Anal. Chem.* **28,** 1273–1276 (1956).
5. Paul, J., Simultaneous determination of phosphorus and silicon in the presence of each other. *Anal. Chim. Acta* **23,** 178–182 (1960).
6. Paul, J., Simultaneous determination of arsenic and phosphorus. *Mikrochim. Acta* **1965,** 830–835.
7. Paul, J., Simultaneous determination of arsenic, phosphorus and silicon. *Mikrochim. Acta* **1965,** 836–841.
8. Paul, J., Simultaneous determination of phosphorus, arsenic and germanium and the separation of silicon and arsenic by solvent extraction. *Anal. Chim. Acta* **35,** 200–205 (1966).

MICROCHEMICAL JOURNAL 15, 452–458 (1970)

Semimicrodetermination of Oxalate with a Lead-Specific Electrode [1]

WALTER SELIG

Lawrence Radiation Laboratory, University of California
Livermore, California 94550

Received February 1, 1970

INTRODUCTION

Oxalate ion is usually determined by redox methods with titrants such as permanganate, bromate, vanadate, iodate, and ceric ion (*2*). It has been determined potentiometrically by titration with silver nitrate (*9*) and recently by means of a calcium-ion specific electrode (*4*). A lead-ion specific electrode has recently become commercially available. It has been used for the potentiometric determination of sulfate (*5*) and for sulfur in organic compounds (*8*).

This paper describes the use of the lead-specific electrode for the direct potentiometric titration of semimicro amounts of oxalate in 40% *p*-dioxane. Satisfactory titrations are possible between pH 3.5 and 10.5. Anions forming insoluble lead salts interfere. Formates, acetates, propionates, and phthalates can be present at levels of 24, 18, 19, and 3 times the level of oxalate, respectively, without interfering.

MATERIALS AND METHODS

Equipment and Reagents

pH meter, Corning Model 12 or similar instrument capable of scale expansion.

Lead-ion activity electrode, Orion Model 94-82.

Reference electrode, double junction, Orion Model 90-02, outer chamber filled with 1 *M* sodium nitrate solution.

Buret, 10-ml automatic, graduated in 0.05-ml divisions, with attached 1-liter reservoir.

Magnetic stirrer with cooling plate.

Lead perchlorate, 0.01 *M*, adjusted to pH 4.4 with dilute perchloric acid.

1,4-Dioxane, analytical reagent.

[1] Work performed under the auspices of the U.S. Atomic Energy Commission.

Procedure

Dissolve a sample containing 1–25 mg of oxalate in 30 ml of distilled water. Adjust the pH to between 4.5 and 9.5 with dilute sodium hydroxide or perchloric acid, using a pH meter. Add 20 ml of 1,4-dioxane and titrate potentiometrically with 0.01 *M* lead perchlorate with a lead-ion activity electrode and a double-junction reference electrode. Monitor the emf on the expanded scale of a pH meter. As the end point is approached (indicated by increasing potential jumps) add the titrant in 0.10-ml increments or use an automatic titrator. Determine the end point either from a titration curve or by calculation. (*3*)

Standardize the titrant against oxalate of known purity such as oxalic acid dihydrate. The titer of 0.01 *M* lead perchlorate is 0.88 mg of oxalate/ml.

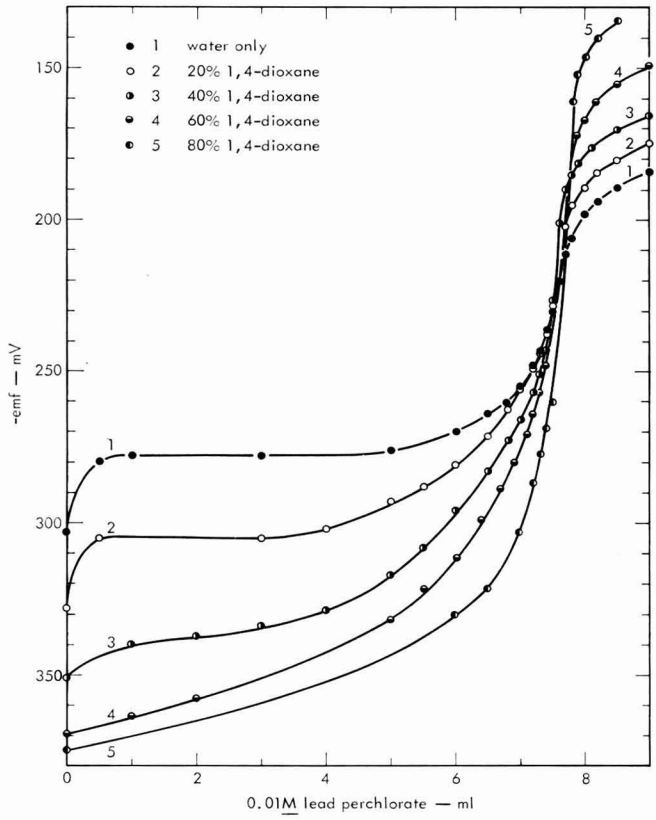


FIG. 1. Potentiometric titration of 10.12 mg of oxalic acid with 0.01 *M* lead perchlorate at pH 5.

Deposits may occasionally accumulate on the sensing element of the lead electrode, making its response sluggish. They can be removed with tissue paper or, if required, by No. 3/0 emery polishing paper.

RESULTS AND DISCUSSION

The determination of oxalate with a specific-ion electrode was recently reported by Mukherji (*4*), who used a liquid ion-exchange calcium electrode with standard calcium chloride titrant at pH 7–11. According to Ross (*6*) the use of liquid ion-exchange electrodes for the determination of heavy metals is now obsolete due to the recent development of solid-state membrane electrodes, which in almost all applications show better selectivity than the liquid systems.

Ross and Frant (*5*) have used a lead-selective electrode for potentiometric titrations of sulfate with lead perchlorate in 50% 1,4-dioxane.

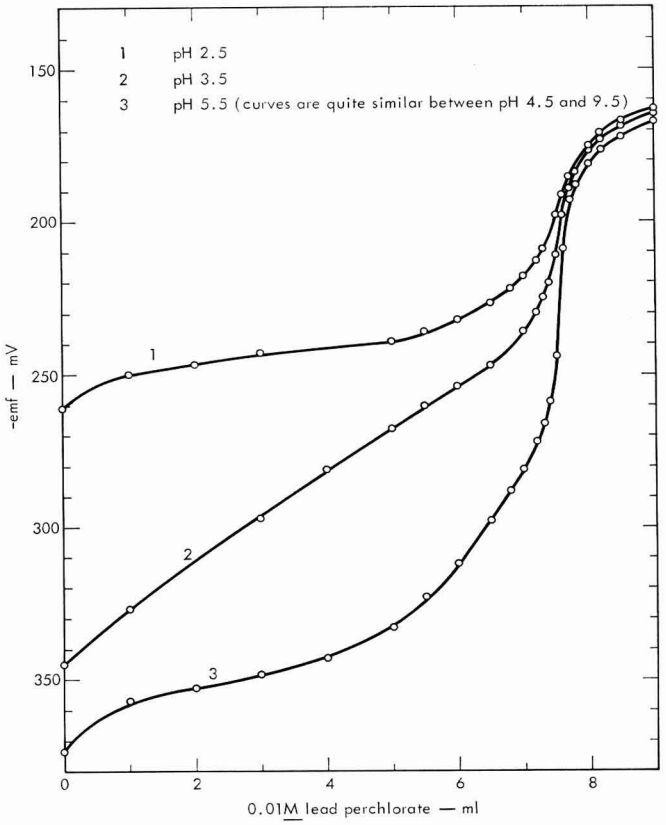


FIG. 2. Potentiometric titration of 10.12 mg of oxalic acid in 40% dioxane at various pH values.

Similarly, the author (*8*) has used this electrode in 60% dioxane for the determination of sulfur (as sulfate) in organic compounds. In an aqueous medium the solubility of lead sulfate is sufficiently large ($K_{sp} = 1.06 \times 10^{-8}$ at 18°C) to suppress the potential breaks near the end point. Lead oxalate, however, is less soluble than the sulfate ($K_{sp} = 2.74 \times 10^{-11}$ at 18°C) and oxalate can therefore be titrated in aqueous solution as shown in Fig. 1. Figure 1 also shows the effect of an increasingly nonaqueous medium on the titration curve. The potential breaks are larger in a partially nonaqueous medium. If the dioxane content exceeds 40%, equilibrium potentials are established at a sluggish rate and considerable drifting of potentials is observed. We have therefore used a 40% dioxane medium in our experiments.

The effect of pH on the titration of oxalate in 40% dioxane is shown in Fig. 2. Satisfactory titration curves are obtained between pH 3.5 and 9.5. At lower pH the potential breaks near the end point are quite

TABLE 1

RECOVERY OF OXALIC ACID [a]

Taken (mg)	Recovered (mg)	Recovery (%)	Av (%)
1.012	1.010	99.84	
2.024	2.023	99.94	
3.036	3.027	99.71	
	3.037	100.02	99.87
5.060	5.042	99.65	
	5.045	99.71	99.68
7.084	7.116	100.45	
	7.074	99.85	
	7.112	100.39	100.23
10.120	10.136	100.15	
	10.131	100.11	100.13
15.180	15.180	100.00	
	15.182	100.01	100.01
20.240	20.315	100.37	
	20.284	100.22	100.30
25.300	25.368	100.27	
	25.369	100.27	100.27

[a] Oxalic acid dihydrate, standardized against 10.120 mg, titrated with 0.01 *M* lead perchlorate.

small. Beyond pH 10.5 precipitation of other lead salts such as basic lead carbonate, $Pb_3(CO_3)_2(OH)_2$ (as determined from X-ray powder patterns), occurs. Although any pH between 3.5 and 9.5 is practical we have performed our experiments at pH 6–7.

Unlike the titration of fluoride with lanthanum(III) using a fluoride-

TABLE 2

ANALYSIS OF SOME OXALATES

Compound	Amount taken (mg)	Oxalate found (%)	Difference (%)
$K_2C_2O_4 \cdot H_2O$ [a] (calc 47.77%)	7.530	47.74	−0.03
	7.530	47.78	+0.01
	7.530	47.77	0.00
$Na_2C_2O_4$ [b] (calc 65.69%)	5.478	65.71	+0.02
	5.478	65.73	+0.04
	5.478	65.68	−0.01

[a] Baker Analyzed Reagent.

[b] Baker & Adamson, Primary Standard.

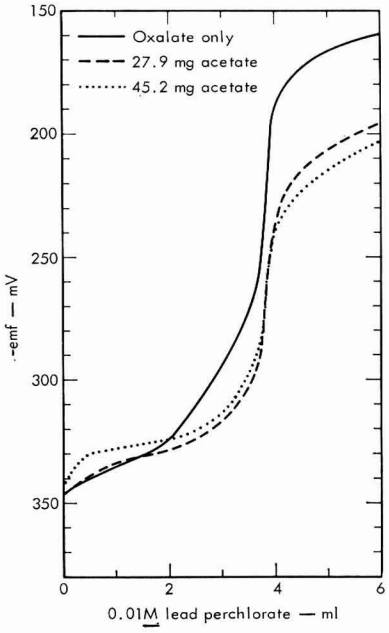


FIG. 3. Potentiometric titration of 3.60 mg of oxalate in the presence of acetate.

ion specific electrode (*1*, *7*) and the titration of sulfate with a lead-ion specific electrode (*8*) the titration of oxalate with lead perchlorate is stoichiometric over the range investigated, as shown in Table 1. The average recovery of oxalic acid over the 1–25-mg range was 100.06% with a standard deviation of 0.25. For less than 5 mg of oxalic acid, increments of 0.05 ml of titrant were added near the end point; for larger amounts the increments were 0.1 ml. The results for two other oxalates are shown in Table 2. The standard deviation of the absolute error was 0.02; the absolute errors were less than 0.05%.

The effect of formate, acetate, propionate, and phthalate on the recovery of oxalate was investigated. In each case, as the amount of these ions increased, the potential breaks near the end point decreased and equilibrium potentials were established at an increasingly slower rate. Figure 3 shows this effect for acetate. Table 3 shows the recovery

TABLE 3

RECOVERY OF OXALIC ACID IN THE PRESENCE OF OTHER IONS

5.15 mg oxalic acid dihydrate taken (= 3.60 mg oxalate).

	Amount added (mg)	Oxalic acid found (mg)	Recovery (%)	Ion:oxalate ratio
Acetate [a]	4.4	5.159	100.18	1.22
	18.4	5.152	100.04	5.11
	27.9	5.141	99.83	7.76
	35.7	5.148	99.96	9.91
	45.2	5.140	99.81	12.56
	65.0	5.134	99.69	18.06
Formate [b]	9.1	5.140	99.81	2.53
	21.6	5.148	99.97	6.00
	35.0	5.158	100.16	9.72
	52.2	5.156	100.11	14.50
	69.8	5.152	100.04	19.39
	86.5	5.149	99.98	24.03
Propionate [c]	13.2	5.157	100.13	3.65
	28.9	5.158	100.16	8.03
	48.3	5.154	100.07	13.40
	69.5	5.152	100.04	19.29
Phthalate [d]	5.9	5.167	100.33	1.65
	8.4	5.172	100.42	2.32
	11.8	5.167	100.33	3.28

[a] As sodium acetate dihydrate.
[b] As sodium formate.
[c] As sodium propionate.
[d] As potassium acid phthalate.

of 3.6 mg of oxalate (as oxalic acid dihydrate) in the presence of these ions. Satisfactory recoveries were obtained in the presence of 18 times as much acetate, 24 times as much formate, and 19 times as much propionate as oxalate. Phthalate interfered at levels greater than 3 times the amount of oxalate. Any anions forming insoluble lead salts will interfere in this determination. Strong complexing agents of lead, such as citrate, also interfere.

SUMMARY

A procedure for the semimicrodetermination of oxalate is described. Oxalate is titrated potentiometrically with standard lead perchlorate in 40% *p*-dioxane solution at pH 3.5 to 10.5. A lead-ion specific electrode in conjunction with an expanded-scale pH meter is used to monitor the emf. Anions forming insoluble lead salts interfere. An excess of formate, acetate, propionate, and phthalate does not interfere.

REFERENCES

1. Harzdorf, C., Verwendung einer fluoridspezifischen Elektrode bei automatisch-potentiometrischen Titrationen. *Z. Anal. Chem.* **245,** 67 (1969).
2. Kolthoff, I. M., and Belcher, R., "Volumetric Analysis," Vol. 3, Intersicence, New York, 1957.
3. Lingane, J. J., "Electroanalytical Chemistry," 2nd ed., p. 91. Interscience, New York, 1958.
4. Mukherji, A. K., Determination of calcium and oxalate with an ion-exchange electrode. *Anal. Chim. Acta* **40,** 354–356 (1968).
5. Ross, J. W., Jr., and Frant, M. S., Potentiometric titrations of sulfate using an ion-selective lead electrode. *Anal. Chem.* **41,** 967–969 (1969).
6. Ross, J. W., Jr., Solid-state and liquid membrane ion-selective electrodes. *In* "Symposium on ion-exchange electrodes, Gaithersburg, Maryland, January 30-31, 1969," Nat. Bur. Stand., Washington, D.C., 1969.
7. Selig, W., Potentiometric micro and semimicro determination of fluorine in organic compounds. *Z. Anal. Chem.* **249,** 30–34 (1970).
8. Selig, W., Micro and semimicro determination of sulfur in organic compounds by potentiometric titration with lead perchlorate. *Mikrochim. Acta* **1970,** 168–175.
9. Shchigol, M. B., and Birnbaum, S. M., Potentiometric determination of oxalate and chloride in ammoniacal solution. *Zavod. Lab.* **14,** 1427–1429 (1948).

MICROCHEMICAL JOURNAL 15, 459–460 (1970)

Microdetermination of Glycolic Acid with Guanidine Carbonate as a Titrant

A. K. SAXENA

Chemistry Department, University of Allahabad, Allahabad, India

Received September 29, 1969

Carboxylic acids are most simply determined by titration with standard alkali (*2*) in aqueous medium. In the present paper, a new method is described for the determination of glycolic acid in microchemical quantities, in which it is titrated with a standard guanidine carbonate (*3*) solution, using bromcresol purple as indicator. The end point occurs when the color of the solution changes from yellow to faint purple. In the earlier publication (*1*) from this laboratory a new method was suggested for the determination of this acid by titration with a microcosmic salt solution.

EXPERIMENTAL METHODS

Reagents used. Glycolic acid (Riedel 57%), guanidine carbonate (Fluka) and bromcresol purple (B.D.H.).

Procedure. To a given solution of acid, add some distilled water to raise its volume to about 15 ml, followed by 1 or 2 drops of 0.1% solution of bromcresol purple indicator. The solution is yellow at this point. Now titrate it with a standardized guanidine carbonate solution until the yellow color is completely discharged and the solution is a faint purple.

RESULTS

The results are given in Table 1; glycolic acid was estimated over a range of 0.380–0.038 mg. The results are concordant and precise.

TABLE 1

MICRODETERMINATION OF GLYCOLIC ACID

Sample no.	Vol of soln (ml)		Glycolic acid (mg)		
	0.001 *M* Glycolic acid taken	Guanidine carbonate used 0.001 *M*	Found	Theoretical value	Error (mg)
1	5.00	5.04	0.383	0.380	0.003
2	3.00	3.00	0.228	0.228	0.000
3	1.00	0.98	0.074	0.076	0.002
4	0.50	0.50	0.038	0.038	0.000

SUMMARY

The glycolic acid was determined in micro quantities, with guanidine carbonate solution using bromcresol purple as indicator. Estimates were carried out in the range of 0.380—0.038 mg with a maximum error of ±0.003 mg.

ACKNOWLEDGMENT

The author is grateful to Drs. B. B. L. Saxena and M. N. Srivastava for their kind guidance and to U. G. C. (Govt. of India) for providing financial assistance.

REFERENCES

1. Saxena, A. K., Use of microcosmic salt as a new titrant for the microdetermination of acetic and glycolic acid. *Michrochem. J.* 14, 361 (1969).
2. Siggia, S., "Quantitative Organic Analysis Via Functional Groups," p. 41. Wiley, New York, 1958.
3. Welcher, F. J., "Organic Analytical Reagents," Vol. 2, p. 390. Van Nostrand, New York, 1955.

MICROCHEMICAL JOURNAL 15, 461–469 (1970)

Coulometric Microdetermination of Oxygen in Organic Compounds

KAN-ICHI NAKAMURA

Central Research Laboratories of Sankyo Co., Ltd., Tokyo, Japan

MASA-AKI NISHIMURA AND TETSUO MITSUI

Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Japan

Received May 19, 1970

INTRODUCTION

The principle of semimicrodetermination of oxygen was first described by Schütze (*1*). The microdetermination based on Schütze's method was introduced by Zimmerman (*2*). The first important modification was made by Unterzaucher (*3*). Wherein the temperature of the carbon reducing zone was elevated to 1120 ± 5°C to complete the conversion of oxygen into carbon monoxide. The direct oxygen determination has become more readily available since Oita and Conway (*4*) lowered the reduction temperature from 1120 to 900° by the addition of platinum to the carbon. Many supplementary reports have also been made.

In recent years, the coulometric determination of hydrogen in organic compounds has become available using a Pt–P_2O_5 electrolytic cell. The cell was devised by Keidel (*5*) and modified by Barendrecht (*6*). Anisimova and Klimova (*7*) compared these hygrometers and noted that the Keidel cell gave high value owing to the recombination of hydrogen and oxygen generated through the electrolysis of the water. Haber *et al.* (*10*) reported a coulometric method for the simultaneous determination of carbon and hydrogen using a lithium hydroxide converter.

In this paper, the direct oxygen determination of organic compounds using a modified Keidel cell combined with LiOH converter will be discussed.

MATERIALS AND METHODS

The method involves pyrolysis of the compound in a stream of nitrogen over heated platinized carbon whereby all the oxygen in the pyrolysis products is converted to carbon monoxide. This is then oxidized to carbon dioxide by heated copper oxides. The oxygen content of the sample is determined as water in an electrolytic hygrometer after the carbon

dioxide has been converted to an equivalent amount of water with a lithium hydroxide.

The flow scheme of the apparatus is shown in Fig. 1. Nitrogen, at a flow rate of 15 ml/min, is passed through a purification tube (T-1) filled with reduced copper which is maintained at 500°C, then dried by passing through a tube (A-1) containing Anhydrone and P_2O_5–silica gel before entering the pyrolysis tube via a side arm.

A plunger and a pyrolysis tube employed are the same as described in the previous report (*8*). The pyrolysis tube, 12 mm i.d. (T-2), is filled with granular carbon, platinized granular carbon and reduced copper wire. The temperature distribution is shown in Fig. 2.

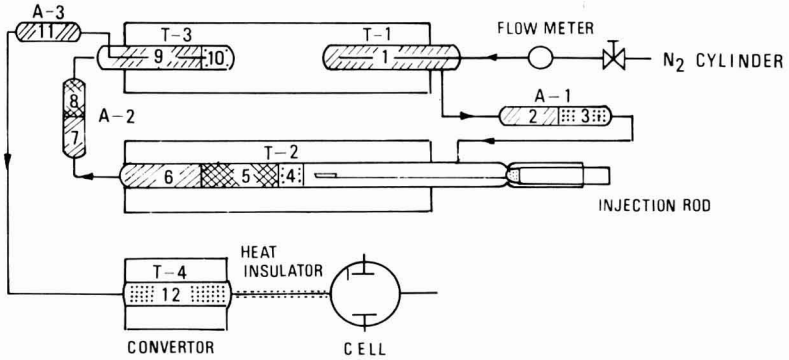


FIG 1. Flow diagram of apparatus: (1) Reduced Cu wire; (2) Anhydrone; (3) P_2O_5–silica gel; (4) Carbon granules; (5) Pt–Carbon granules; Reduced Cu wire; (7) H_2SO_4–silica gel; Carbofix(NaOH granules); (9) CuO wire; (10) Sulfix-(Ag–Co_3O_4 granules); (11) Anhydrone; and (12) LiOH fine powder.

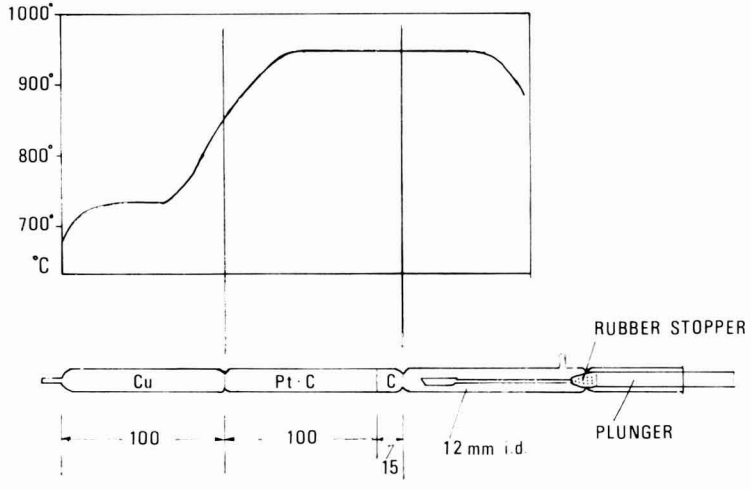


FIG. 2. Pyrolysis tube and temperature distribution.

A scrubber (A-2) is charged with H_2SO_4–silica gel and Carbofix (NaOH granules) to remove the other interfering gases. An oxidizing tube (T-3) is filled with copper oxide wire and Sulfix (Ag–Co_3O_4) to oxidize carbon monoxide to carbon dioxide.

A lithium hydroxide converter (T-4) consists of a Pyrex tube, 9 mm i.d. and 75 mm in length, charged with 6 g of finely powdered lithium hydroxide. The charged tube is heated at 210 ± 5°C for the complete conversion of carbon dioxide to water.

An electrolytic hygrometer is constructed from three Teflon rods on which the two platinum wires are coiled. They are made according to the following procedure: Two platinum wires, 0.3 mm in diameter and 100 cm in length, are spirally coiled on a Teflon rod, 5 mm in diameter and 70 mm long. Between these wires two polypropylene yarns, 0.18 mm in diameter, are coiled to space the two platinum wires uniformly and closely to each other as shown in Fig. 3. The cells are coated with a solution of phosphoric acid in acetone. After removal of the acetone, they are set in a glass mantle, 16 mm i.d., using rubber O-ring seals. The carrier gas is introduced from the center of the three sensing cells. The hygrometer is conditioned by electrolyzing the acid to dryness before use and is operated at 30 V from a constant voltage supply.

A 30-V dc power supply and a coulomb counter are arranged as shown in Fig. 4. The coulomb counter is a digital integrating frequency

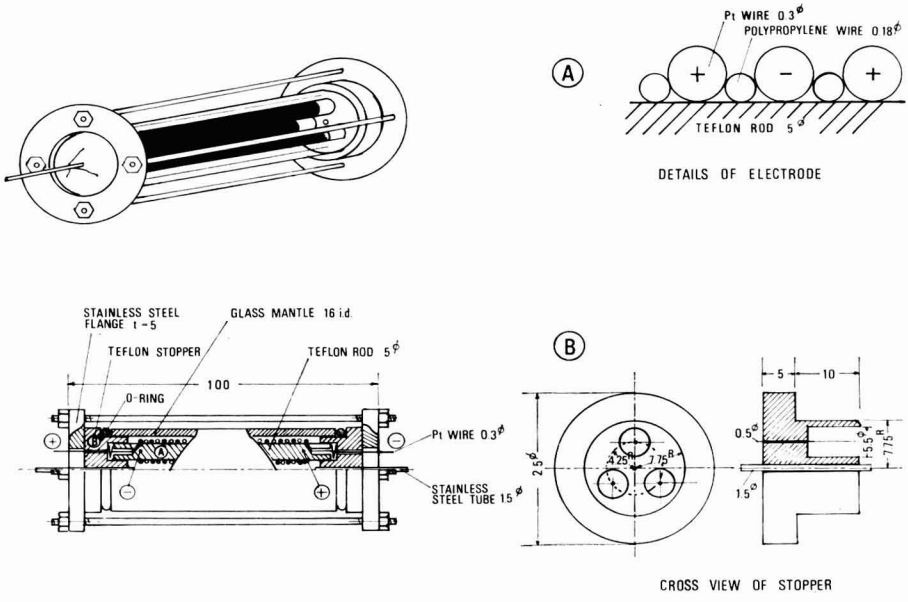


FIG. 3. Pt–P_2O_5 electrolytic cell (mm in unit).

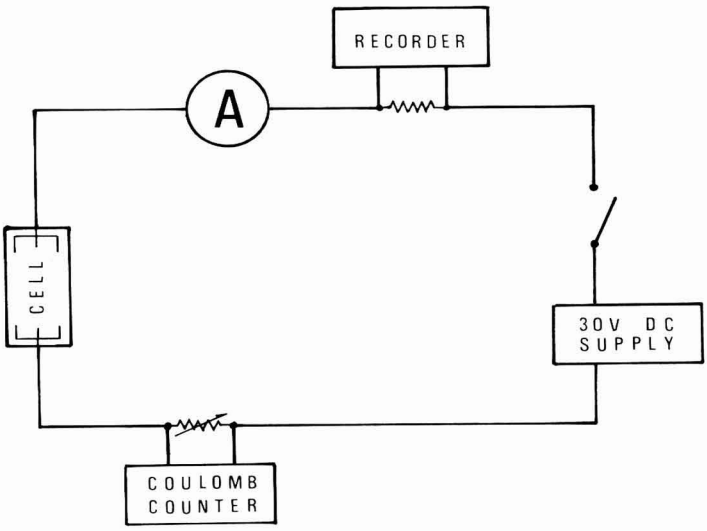


FIG. 4. electrical scheme.

counter from Shimadzu Seisakusho Co., Ltd., which can be read out from 0.01 to 99.99 coulombs. A recorder is used for the observation of electrolysis. A full scale 100 mA of ammeter, JIS C1102 CLASS 0.5, is used for terminating the electrolysis.

Preparation of Carbon and Platinized Carbon

Carbon. 20–24 mesh granular carbon is prepared by decomposing sucrose at 900°C in a nitrogen atmosphere.

Platinized carbon. A mixture of 3 g of granular carbon and 6 g of ammonium chloroplatinate is heated at 500–600°C in a stream of nitrogen. Thus prepared carbon and platinized carbon are packed into a pyrolysis tube and heated at 900°C in an atmosphere of hydrogen to activate the reagents.

PROCEDURE

The carrier gas flow is turned up to 100 ml/min using a needle valve, then the plunger is withdrawn immediately. After 5 minutes, a platinum boat, containing a sample, is placed in a rod cavity. The rod is placed at the mouth of the pyrolysis tube, and the carrier gas flow (100 ml/min) is continued for 15 seconds to expel all of the air that entered the tube during the insertion of the sample. Then the rod is pushed into the tube until a rubber stopper of the plunger plug fits tightly at a constriction of the tube. The carrier gas flow is then decreased to 15 ml/min. Ten minutes after the insertion of the plunger, the electrolysis of the Pt–P_2O_5

cell is set in operation. When the electrolysis current decreases to 10.0 mA, the cell is switched off and the electrolysis time required is measured. A blank value is also measured every 15 seconds, using an empty platinum boat for 22 minutes from insertion of the plunger.

Every day prior to the analysis of a sample and blank measurements, the cell must be conditioned by pyrolysis of an organic compound, such as 3 mg of sucrose.

CALCULATION AND RESULTS

The percentage of oxygen in organic compound is calculated from the equation:

Sanzone 6-3-70—35587 Acad. Press-Micro 10 on 12 No. 362 gal. 1 p. 21

$$0\% = 0.08291 \times \frac{\text{no. of coulombs (found - blank)}}{\text{sample wt (mg)}} \times 100.$$

Results on standard samples are shown in Tables 1, 2, 3, 4, and 5.

DISCUSSION

The newly designed electrolytic hygrometer has certain merits: It is easy to make and to maintain, and there is no problem of the recombination of generated hydrogen and oxygen after electrolysis of water. Using polypropylene yarn of the appropriate diameter as a spacer for

TABLE 1

DETERMINATION OF OXYGEN BY COULOMETRY

Sample: sucrose ($C_{12}H_{22}O_{11}$); theory: O(%) = 51.42%.

No.	Sample wt (mg)	Found			Δ% from theory
		Coulombs	O(mg)	O(%)	
1	3.361	20.60	1.708	50.82	−0.60
2	2.963	18.20	1.509	50.93	−0.49
3	3.472	21.16	1.754	50.53	−0.89
4	3.110	19.12	1.585	50.97	−0.45
5	3.156	19.89	1.649	52.25	+0.83
6	3.347	20.76	1.721	51.43	+0.01
7	2.927	17.80	1.476	50.42	−1.00
8	2.813	17.74	1.471	52.29	+0.87
9	3.161	19.51	1.616	51.17	−0.25
10	3.179	19.40	1.608	50.60	−0.82
11	2.812	17.67	1.465	52.10	+0.68
Mean				51.23	−0.19
SD				0.695	

TABLE 2

DETERMINATION OF OXYGEN BY COULOMETRY

Sample: Benzoic acid ($C_7H_6O_2$); theory: O(%) = 26.20%.

No.	Sample wt (mg)	Found			Δ% from theory
		Coulombs	O(mg)	O(%)	
1	3.330	10.32	0.8556	25.69	−0.51
2	3.609	11.52	0.9551	26.47	+0.27
3	4.037	12.74	1.056	26.16	−0.04
4	3.504	12.01	0.9128	26.05	−0.15
5	3.923	12.34	1.023	26.08	−0.12
6	3.873	12.09	1.002	25.88	−0.32
7	3.790	12.10	1.003	26.47	+0.27
8	3.324	10.64	0.8822	26.54	+0.34
9	3.294	10.37	0.8598	26.10	−0.10
10	3.726	11.58	0.9601	25.77	−0.43
11	3.416	10.69	0.8863	25.95	−0.25
Mean				26.11	−0.09
SD				0.287	

TABLE 3

DETERMINATION OF OXYGEN BY COULOMETRY

Sample: 2,4-dichlorophenoxy acetic acid ($C_8H_6O_3Cl_2$); theory: O(%) = 21.72%.

No.	Sample wt (mg)	Found			Δ% from theory
		Coulombs	O(mg)	O(%)	
1	3.658	9.62	0.7976	21.80	+0.08
2	3.853	10.30	0.8540	22.16	+0.44
3	4.498	11.67	0.9676	21.51	−0.21
4	4.145	10.66	0.8838	21.32	−0.40
5	4.155	10.85	0.8996	21.65	−0.07
6	4.278	11.36	0.9419	22.02	+0.30
7	4.290	11.16	0.9253	21.57	−0.15
8	4.266	11.23	0.9311	21.83	+0.09
9	3.948	10.22	0.8473	21.46	−0.24
10	4.135	11.05	0.9162	22.16	+0.44
11	4.318	11.49	0.9526	22.06	+0.34
Mean				21.78	+0.06
SD				0.293	

TABLE 4

DETERMINATION OF OXYGEN BY COULOMETRY

Sample: acetone-2, 4-dinitrophenylhydrazone ($C_9H_{10}O_4N_4$); theory: O(%) = 26.87%.

No.	Sample wt (mg)	Found			Δ% from theory
		Coulombs	O(mg)	O(%)	
1	3.454	11.34	0.9402	27.22	+0.35
2	3.485	11.30	0.9369	26.88	+0.01
3	3.138	10.27	0.8515	27.13	+0.26
4	3.058	9.73	0.8067	26.38	−0.49
5	3.585	11.44	0.9485	26.46	−0.41
6	3.089	10.05	0.8332	26.97	+0.10
7	3.266	10.36	0.8589	26.30	−0.57
8	3.251	10.89	0.9029	27.77	+0.90
9	3.190	10.34	0.8573	26.87	Nil
10	3.193	10.34	0.8573	26.85	−0.02
11	3.344	10.65	0.8830	26.41	−0.46
Mean				26.84	−0.03
SD				0.287	

TABLE 5

DETERMINATION OF OXYGEN BY COULOMETRY

Sample: sulfonal ($C_9H_{16}O_4S_2$); theory: O(%) = 28.03%.

No.	Sample wt (mg)	Found			Δ% from theory
		Coulombs	O(mg)	O(%)	
1	3.239	10.72	0.8888	27.44	−0.59
2	3.009	10.15	0.8415	27.97	−0.06
3	3.269	10.92	0.9054	27.70	−0.33
4	3.290	11.12	0.9220	28.02	−0.01
5	3.480	11.68	0.9684	27.83	−0.20
6	3.466	11.70	0.9700	27.99	−0.04
7	3.351	11.37	0.9427	28.13	+0.10
8	3.384	11.43	0.9477	28.00	−0.03
9	3.139	10.67	0.8846	28.18	+0.15
10	3.059	10.34	0.8573	28.03	Nil
11	3.464	11.64	0.9651	27.86	−0.17
Mean				27.92	−0.11
SD				0.208	

the two platinum wires, favorable separation of the two poles could be chosen. The total area of the electrodes is 28 cm^2.

The internal cell resistance is varied from 375 to 15000 ohms by the ratio of phosphoric acid to phosphorus pentoxide. The electrolysis current is maximum at 80 mA and decreases to about 2 mA at 30 V. The cell current during an analysis is shown in Fig. 5. A direct current source of 30 V is sufficient from a constant dc supply; higher voltages gave faster responses, but the electrode supports of Teflon and polypropylene are destroyed above 60 V.

The cell coated phosphorus pentoxide plays a double role as a desiccant and electrolyte. To perform uniform electrolysis and to obtain a constant blank value, the electrolysis had to be undertaken 10 minutes after the insertion of a sample. During this period, the absorbed water spread widely over the electrodes as phosphoric acid.

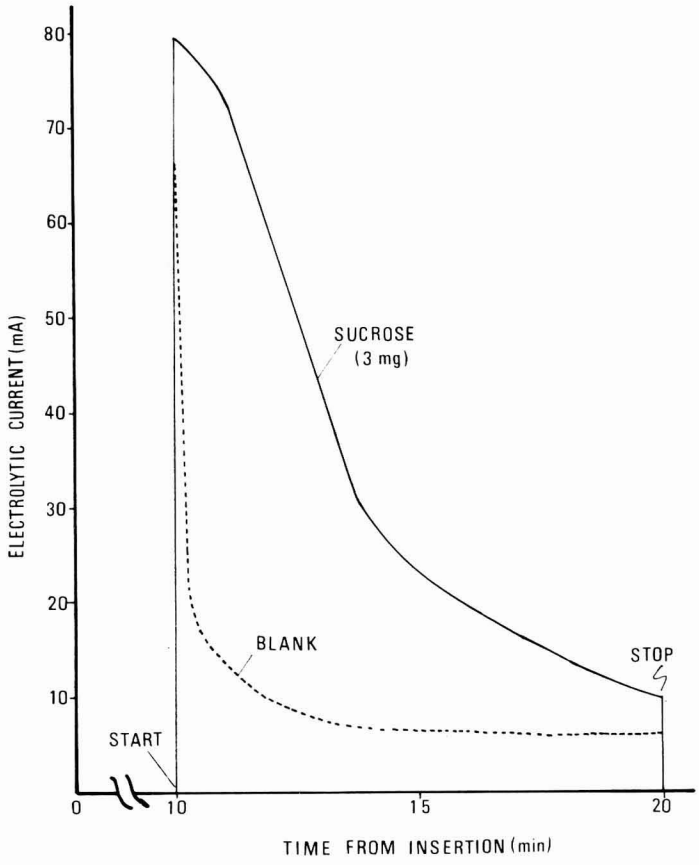


FIG. 5. Cell current during an analysis.

To shorten the analysis time, the end point of the electrolysis is set at 10.0 mA. The analysis time varies from 18 to 22 min according to the sample size and the oxygen content, so that the blank value must be measured every 15 seconds for 18 to 22 min, using the same plunger after a constant cooling period (5 min).

SUMMARY

Application of coulometry to the microdetermination of oxygen in organic substances using a newly designed Pt–P_2O_5 electrolytic cell has been investigated. A sample is pyrolyzed in a nitrogen stream, the gaseous products are passed over carbon, platinized carbon, and reduced copper. The resulting carbon monoxide is oxidized to carbon dioxide by copper oxide. Then the carbon dioxide is converted into water by passing through lithium hydroxide. The water vapor is absorbed onto the Pt–P_2O_5 electrolytic cell and electrolyzed. By measuring the number of coulombs required to electrolyze the water, the oxygen content is calculated. Several standard samples have been analyzed and the standard deviations of 11 analyses are within 0.2–0.6%.

ACKNOWLEDGMENT

We are thankful to Dr. Genshun Sunagawa of Sankyo Central Research Laboratories for his encouragement in this work.

REFERENCES

1. Schütze, M., A new method for the direct determination of oxygen in organic substances. *Z. Anal. Chem.* **118,** 245 (1939).
2. Zimmerman, W., The direct microdetermination of oxygen in organic substances. *Z. Anal. Chem.* **118,** 258 (1939).
3. Unterzaucher, J., The microdetermination of oxygen. *Chem. Ber.* **73,** 391 (1940).
4. Oita, I. J. and Conway, H. S., Direct determination of oxygen in organic substances: Modified Schütze method. *Anal. Chem.* **26,** 600 (1954).
5. Keidel, F. A., Determination of water by direct amperometric measurement. *Anal. Chem.* **31,** 2043 (1959).
6. Barendrecht, E., Determination of traces of water in gases by means of a modified Keidel electrolytic hygrometer. *Anal. Chim. Acta* **25,** 402 (1961).
7. Anisimova, G. F. and Klimova, V. A., On the coulometric ending of the carbon and hydrogen determination in organic compounds. *Zh. Analit. Khim.* **23,** 411 (1968).
8. Mitsui, T., Rapid nitrogen analyzer., *Kagaku No Ryoiki* **21,** 1, Al (1967).
9. Hamer, W. J. and Craig, D. N., The value of the Faraday. *J. Electrochem. Soc.* **111,** 1434 (1964).
10. Haber, H. S., Bude, D. A., Buck, R. P., and Gardiner, K. W., Carbon and hydrogen analysis by coulometric electrolysis of water. *Anal. Chem.* **37,** 116 (1965).

MICROCHEMICAL JOURNAL **15,** 470–474 (1970)

Microdetermination of the Hydrazine Function: A New Gasometric Method Based on Oxidation with Benzoquinone

S. S. M. HASSAN AND M. T. M. ZAKI

Research Microanalytical Labs., Department of Chemistry, Faculty of Science, A'in Shams University, Cairo, U.A.R.

Received November 3, 1969

INTRODUCTION

Despite the difficulties that have been encountered in determining the hydrazine function, titrimetric (*3*), potentiometric (*7*), colorimetric (*13*), and coulometric (*12*) procedures are available under carefully controlled conditions. Oxidimetric methods based on the use of iodine, bromine (*2*), permanganate, bromate, iodate (*6*), ferricyanide, ceric salts (*5*), cupric ion (*4*), chloramines (*11*), and hypochlorous acid (*2*) were described, but they suffer from lack of specificity. Moreover, ammonia or ammonium ion interfere with many of these oxidants.

Few macrogasometric methods have also been proposed based on reactions with potassium ferricyanide (*9*), potassium iodate (*8*), and copper sulfate (*10*), whereby the volume of nitrogen liberated is measured. In general, oxidation with inorganic substances does not always proceed directly to nitrogen but may give a variety of products as ammonia and/or hydrazoic acid (*1*).

The present work was undertaken to develop a new rapid and convenient microgasometric method suitable for determining the hydrazine function in structurally different organic and inorganic compounds. It was found that *p*-benzoquinone oxidizes the hydrazine quantitatively, in basic media to elemental nitrogen. This reaction is studied and a general procedure is developed. Apart from the high selectivity and simplicity of the reaction, it has the advantage, over the inorganic oxidants, that the ammonium ion does not interfere with oxidation.

MATERIALS AND METHOD

Apparatus

A simple apparatus is used which consists of a reaction vessel (10 × 2 cm) provided with a small glass funnel and two side arms. One arm,

which leads to a bubbler, is connected to a Dewar flask containing solid carbon dioxide. The other arm is connected to a micronitrometer (1.5-ml capacity with 0.01-ml divisions). All connections are polythene tubing.

Reagents

All reagents are of analytical grade unless otherwise specified. The hydrazine samples used are: hydrazine oxalate, hydrazine picrate, hydrazine–copper(II) double sulfate, hydrazine cobalt(II) double sulfate, and dihydrazinate–cobalt(II) thiocyanate. These were prepared by the present authors according to standard methods [cf. Ref. (*1*) pp. 172, 177, 184]. Commercial grades of hydrazine sulfate, phenylhydrazine hydrochloride, *p*-nitrophenylhydrazine, and 2, 4-dinitrophenylhydrazine are also employed. All the above mentioned samples were purified and provided as test samples of purity not less than 99.5% (confirmed by Dumas method).

Procedure

Introduce 3–5 mg of the hydrazine sample into the reaction vessel, and add about 10–20 mg of previously sublimed *p*-benzoquinone. Displace the air with carbon dioxide for about 5 minutes until no air bubbles are collected in the nitrometer, then introduce about 3 ml of 5% disodium hydrogen phosphate solution through the small funnel. Gently heat the reaction mixture for 5 minutes using a microburner. Sweep the decomposition gases (for 5 minutes at the rate of 100 bubbles/min). Collect the nitrogen gas produced over 50% potassium hydroxide solution in a micronitrometer. Carry out a blank experiment under identical conditions.

RESULTS AND DISCUSSION

Nature of the Reaction

When a representative hydrazine sample (e.g., hydrazine sulfate) is treated with *p*-benzoquinone, in basic media, 1 mole of nitrogen is instantaneously liberated per mole of the hydrazine sample. At room temperature the nitrogen recovery is 90% of the theoretical, slight warming is necessary for the completion of the reaction.

Reaction of unsubstituted hydrazines with benzoquinone can be represented as follows:

With aryl hydrazines (e.g., phenylhydrazine) 1 mole of nitrogen is also

liberated per mole of the sample and a hydroxyl group replaces the hydrazine function in the aryl moiety. The minimum quinone:hydrazine mole ratio required for quantitative reaction is 2:1. With 1:1 and 1:2 quinone:hydrazine mole ratio, about 50 and 25% of the required nitrogen is recovered, respectively.

Oxidation of hydrazine with benzoquinone depends not only on the nature of the sample used but also on the reaction medium. Different media were studied to determine the most suitable conditions. In acidic media (i.e., 1 *N* hydrochloric acid or 6 *N* sulfuric acid), 60–70% of the required hydrazine–nitrogen is recovered. Quantitative oxidation is obtained in basic media as sodium acetate, sodium carbonate, and disodium hydrogen phosphate solutions.

Determination of Hydrazines

A wide variety of hydrazine samples including hydrazine salts, substituted and unsubstituted aryl hydrazines, hydrazine–metal double salts, and dihydrazinate–metal complexes are oxidized with *p*-benzoquinone in different basic media.

The results obtained (cf. Table 1) show that in 5% sodium acetate solution, the total average recovery of the hydrazine–nitrogen is 99.97%, the mean absolute error is ±0.12% and the maximum absolute error is ±0.29%. However, substituted and unsubstituted aryl hydrazines give low recoveries varying between 45 and 85%. In a solution of 5% sodium carbonate the total average recovery is 99.22%, the mean absolute error is ±0.18% and the maximum absolute error being ±0.35%. Dihydrazinate–metal complexes and disubstituted aryl hydrazines failed to be oxidized quantitatively into nitrogen under these conditions; about 65–85% of the required nitrogen is recovered. In 5% disodium hydrogen phosphate solution, an average recovery of 99.66% and a mean absolute error of ±0.09% are obtained, the maximum absolute error being ±0.15%. Of all the samples analyzed, only dinitrophenylhydrazine gave values approximately 15% below the anticipated value. It is generally convenient to determine hydrazines by oxidation with benzoquinone in disodium hydrogen phosphate solution.

Determination of hydrazines in the presence of ammonia is of interest, not only because ammonia may be an impurity in hydrazine but also because ammonia is formed in many reactions of hydrazine, as a by-product. Microdetermination of typical hydrazine samples in the presence of ammonium chloride (3–50 mg) shows results within ±0.1% of that obtained in its absence. These results indicate that ammonium salts do not interfere with the present analytical procedure.

TABLE 1

MICROGASOMETRIC DETERMINATION OF SOME HYDRAZINE SAMPLES BY OXIDATION WITH BENZOQUINONE IN DIFFERENT BASIC MEDIA

Sample	Hydrazine–nitrogen (%)			
	Calc	Found		
		CH_3COONa	Na_2CO_3	Na_2HPO_4
Hydrazine sulfate	21.71	21.42	21.83	21.66
$N_2H_4 \cdot H_2SO_4$		21.57	21.66	21.60
Hydrazine oxalate	22.95	23.20	22.60	22.83
$N_2H_4 \cdot C_2O_4H_2$		23.14	22.62	22.80
Hydrazine picrate	10.73	10.66	10.63	10.81
$N_2H_4 \cdot C_6H_3N_3O_7$		10.59	10.56	10.76
Phenylhydrazine hydrochloride	19.36	15.70	19.06	19.26
$C_6H_5NHNH_2 \cdot HCl$		16.02	19.33	19.21
p-Nitrophenylhydrazine	18.30	15.02	18.28	18.19
$C_6H_4(NO_2)$–$NHNH_2$		14.52	18.16	18.20
2, 4-Dinitrophenylhydrazine	14.14	6.17	9.53	12.07
$C_6H_3(NO_2)_2$–$NHNH_2$		6.51	10.21	11.90
Hydrazine–copper(II) double sulfate	17.42	17.56	17.29	17.38
$(N_2H_5)_2SO_4 \cdot CuSO_4$		17.49	17.41	17.47
Hydrazine–cobalt(II) double sulfate	17.67	17.73	17.66	17.61
$(N_2H_5)_2SO_4 \cdot CoSO_4$		17.65	17.35	17.53
Dihydrazinate–cobalt(II) thiocyanate $2N_2H_4 \cdot Co(CSN)_2$	23.05	23.06	19.12	22.95
		23.09	19.33	22.92

SUMMARY

An accurate, specific, and selective new gasometric method for the microdetermination of the hydrazine function in structurally different organic and inorganic substances is described. It is based on the oxidation of the hydrazine group to elemental nitrogen with p-benzoquinone in 5% disodium hydrogen phosphate solution. The method is simple, reproducible, and accurate to $\pm 0.2\%$.

REFERENCES

1. Audrieth, L., and Ogg, B., "The Chemistry of Hydrazine," pp. 153–162. Wiley, New York, 1951.
2. Bray, W., and Cuy, E., The oxidation of hydrazine. I. The volumetric analysis of hydrazine by the iodic acid, iodine, bromine and hypochlorous acid methods. *J. Amer. Chem. Soc.* **46**, 858–875 (1924).

3. Browne, A., and Shetterly, F., On the oxidation of hydrazine. IV. *J. Amer. Chem. Soc.* **31,** 783–799 (1909).
4. Britton, H., and Clissold, E., A potentiometric study of the oxidation of phenylhydrazine by cupric ions, including the titration of Fehling's solution with phenyl hydrazine. *J. Chem. Soc., London* **1942,** 528–531.
5. Dernbach, C., and Mehlig, J., Determination of hydrazine. A rapid ferricyanide–ceriometric method, *Ind. Eng. Chem., Anal. Ed.* **14,** 58–60 (1942).
6. Kolthoff, M., Volumetric analysis of hydrazine by iodine, bromate, iodate and permanaganate methods. *J. Amer. Chem. Soc.* **46,** 2009–2016 (1924).
7. McBride, W., Henry, R., and Skolnik, S., Potentiometric analytical methods for hydrazino compounds. *Anal. Chem.* **23,** 890–893 (1951).
8. McKennis, M., Weatherby, J., and Dellis, E., Gasometric determination of hydrazine and its derivatives. *Anal. Chem.* **30,** 499–502 (1958).
9. Rây, P., and Sen, H., Action of hydrazine and hydroxylamine on ferricyanide and new methods for the determination of hydrazine and ferricyanide, *Z. Anorg. Chem.* **76,** 380–386 (1912).
10. Siggia, S., and Lohr, L., Determination of organic hydrazines. *Anal. Chem.* **21,** 1202–1203 (1949).
11. Singh, B., and Rehmann, A., Potentiometric studies in oxidation–reduction reactions. Part VIII. Oxidation with chloramine-T. *J. Indian. Chem. Soc.* **17,** 169–172 (1940).
12. Szebellédy, L., and Somogyi, Z., Coulometric analysis as methods of percision. V. Determination of hydrazine. *Z. Anal. Chem.* **112,** 391–395 (1938).
13. Watt, G., and Chrisp, J., A spectrophotometric method for the determination of hydrazine. *Anal. Chem.* **24,** 2006–2008 (1952).

MICROCHEMICAL JOURNAL **15,** 475–480 (1970)

A Microchemical Study of the Photodegradation of 4,4′-Bis(diethylamino)benzophenone Oxime on Silica Gel

E. J. POZIOMEK

Physical Research Laboratory, Edgewood Arsenal, Maryland 21010

AND

A. F. FENTIMAN AND R. H. POIRIER

Battelle Memorial Institute, Columbus, Ohio 43201

Received November 4, 1969

INTRODUCTION

Although 4,4′-bis(diethylamino)benzophenone oxime(I) has been reported to be a detector reagent for certain organophosphorous compounds (*1*), its usefulness in detection is limited because of its susceptibility to photodegradation. The photochemical decomposition of I is easily induced with ultraviolet light in the presence or absence of air. This paper describes a microchemical study of the photoinduced decomposition of I on a silica gel thin layer support. The photodegradation products include 4,4′-bis(diethylamino) benzophenone (II), 4,4′-bis-(diethylamino)benzanilide (III), *N*-(*p*-ethylaminophenyl)-*p*-diethylaminobenzamide (IV), and 4,4′-bis(diethylamino)benzophenone imine (V).

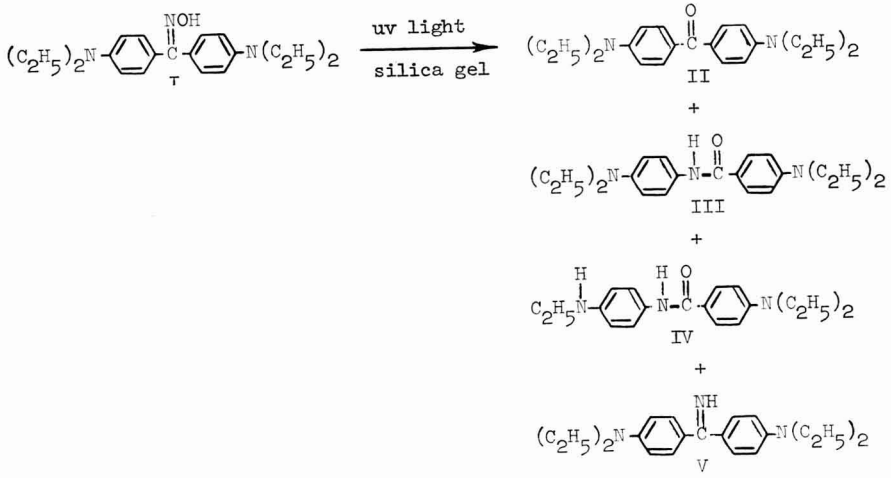
METHODS AND MATERIALS

Compounds

4,4′-Bis(diethylamino)benzophenone oxime (I) and 4,4′-bis(diethylamino)benzanilide (III). These were prepared according to procedures described previously (*2*). Compound I was twice recrystallized from ethanol/benzene followed by washing with ethanol, which removed any residual yellow color left by the mother liquor. When dry, the crystals were well formed and perfectly white, mp 194–196°C; reported (*2*) 200–201°C. III, mp 137–139°C; reported (*2*) 140–141°C.

4,4′-Bis(diethylamino)benzophenone (II). This compound was obtained commercially and was recrystallized several times from ethanol, mp 95.0–95.8°C; reported (*3*) 95–96°C.

4,4′-Bis(diethylamino)benzophenone imine hydrochloride (V·HCl).



In 10 ml of tetrahydrofuran (THF) was placed 0.16 g of lithium wire and to this was added 2.6 g of *p*-bromo-*N*, *N*-diethylaniline in 10 ml of THF. After 2.5 hours of refluxing, most of the lithium wire was consumed. Two g of 4-diethylaminobenzonitrile in 10 ml of THF were added in 10 minutes to the hot organolithium solution. The mixture was refluxed an additional 0.5 hour, then cooled in ice and treated with 1.2 g of ammonium chloride in 3.0 ml of water. The entire hydrolysis mixture was chromatographed on 100 g of alumina using THF as an eluant. The yellow band that separated was collected and the THF was evaporated to leave a yellow oil, which appeared to be a mixture of the ketone and ketimine hydrochloride. The oil was triturated with water and the water-oil combination was filtered. The water was then evaporated leaving a small amount of yellow solid. Infrared, ultraviolet, and mass spectra of this solid appeared to be consistent with that expected for V·HCl; uv $\lambda max_{MeOH} m\mu(\varepsilon)$, 437(ca. 45,000), 371(ca. 19,000), 253(ca. 9400), 304(low), and 316(low); ir (neat) 1600, 1415, 1360, 1275, 1200, and 1150 cm^{-1}. Mass spectrum (*m/e*) 323, 308, 264, 251, 220, 178, 162.

Photolysis.

Compound I in benzene (1.0–4.0 $\mu g/\mu l$) was deposited as a series of spots on one end of a 20-cm Eastman Chromatogram sheet K301R. A total of approximately 500 μg was spotted. After the benzene had evaporated the residue was irradiated for 3 hours with a high pressure broad spectrum mercury lamp with maximum output at 300 $m\mu$. After development of the plate with 5% methanol in diethyl ether, several spots were eluted. The part of the sheet containing spots corresponding

to $R_f > 0.5$ was cut away. The part of the sheet containing the original spots was redeveloped but now with 35% methanol in diethyl ether to give another series of spots. Each spot was scraped from both halves of the sheet and the products were extracted from the adsorbent with methanol. Ultraviolet absorption spectra were determined on the methanol solutions; mass spectra were determined on residues of the same solutions.

Product identification

Product identification was based primarily on comparisons of spectroscopic properties with those of authentic samples (Table 1). The compounds in Table 1 are listed in a decreasing order of their mobility on the thin layer support, Compound I being most mobile.

Compound V apparently separated as an acid salt, the acid of which could stem from impurities or even the carbon dioxide of the atmosphere. In fact the ketimine free base forms of both V and auramine (the *N*-methyl analog of V) are both colorless but turn yellow on bubbling carbon dioxide through their solutions. Ultraviolet irradiation of 4,4′-bis(dimethylamino)benzophenone oxime on silica gel and isolation of the yellow product led to its identification as auramine. It has been noted that both auramine and V turn yellow on exposure to the atmosphere (*4*). The mass spectra of V and V·HCl both show parent peaks at 323 corresponding to the free base. This is not unusual, for other amine salts tend to lose the acid moiety under mass spectrometric conditions.

TABLE 1

ULTRAVIOLET AND MASS SPECTRAL DATA OF PHOTODEGRADATION PRODUCTS

		Absorption maximum [mμ (MeOH)]		Parent peak *m/e*	
Compound	Amount	Product	Authentic sample	Product	Authentic sample
I	Negligible	—	308	339	339
II	Minor [a]	375	375	324	324
III	Major [b]	323	323	339	339
IV	Trace	323	—	311	311 [d]
V	Trace [c]	437	437	323	323

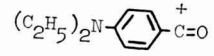
[a] Yield, approximately 15–30%.

[b] Estimated yield 30–60%.

[c] Compound V was present in quantity less than II but more than IV.

[d] Calc mol wt.

The structure of IV was assigned on the the basis of its long wavelength ultraviolet absorption band (which occurs at the same wavelength as that of III) and the presence of *m/e* parent and base peaks of 311 and 176, respectively. The base peak corresponds to a molecular formula of $C_{11}H_{14}NO$. This fragment which was given structure VI, also

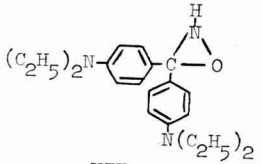


VI

arises from the fragmentation of the benzanilide, III. Hence, the loss of the ethylene necessary to produce IV must have occurred at the nitrogen attached to the *N*-phenyl ring of the benzanilide. In order to obtain more evidence for the structural assignment, compound IV was treated with acetyl chloride in pyridine and the mass spectrum of the product was determined. Although the product was contaminated with acetyl chloride–pyridine complex, the mass spectrum clearly exhibited the presence of material with a mass of 353, which corresponds to the *N*-acetyl derivative of IV. As expected, fragment VI with a mass peak at 176 was detected.

DISCUSSION

There is considerable current interest in the photochemistry of oximes for purposes of synthesis and mechanism study. Examples are given in Table 2. The formation of III as a major product in the photorearrangement of I proceeds undoubtedly through an oxaziridine intermediate (VII).



VII

Evidence has been obtained for the formation of oxaziridines during the irradiation of several oximes (*6, 10*), however, these were not stable enough to isolate. Similarly, we have not been able to isolate VII.

Certain oxaziridines have been shown to photofragment with the formation of ketones and imines (*12*). Compounds II and V may also form from VII but another logical source of the imine V is from a homolytic cleavage of the N–O bond. The formation of benzophenone imine during the irradiation of benzophenone hydrazone is seen as resulting from a nitrogen–nitrogen cleavage (*13*).

TABLE 2

REPORTED PHOTOCHEMICAL REACTIONS OF OXIMES

Starting material	Products	Conditions	Ref.
Syn-isonicotinaldehyde oxime	Anti-isomer	Acetone, 5°C	(5)
Benzaldoxime	Benzamide	Acetic acid	(6, 7)
Cyclohexanone oxime	Caprolactam (major) Cyclohexanone	Methanol/N_2	(8)
Cyclohexanone oxime	Caproamide (major) Cyclohexanone	Isopropanol/N_2	(9)
1, 1-Dimethyl-2-naphthalenone oxime	1, 1-Dimethyl-2-naphthalenone (major) 1, 1-Dimethyl-3, 4-dihydro-2-naphthalenone Lactam	Alcohol	(10)
Cholestanone oxime	Cholestanone (major) Cholestane	Benzene	(11)
p-Methoxyacetophenone oxime	*N*-(*p*-Anisyl)acetamide (major) *N*-Methylanisamide (major) *p*-Anisamide	Cyclohexane–acetic acid	(6)

The deethylation of I to IV occurs thermally as well as under the conditions of the experiment described here, for IV was detected among the products from I heated at 205°C for several hours.

ACKNOWLEDGMENT

The authors are indebted to R. L. Folz, Battelle-Columbus Laboratories, who determined the mass spectra.

REFERENCES

1. Kramer, D. N., Morin, R. D., and Poirier, R. H., Detection of chemical warfare agents (G agents). *U. S.* 2,926,072 Feb. 23, 1960.
2. Morin, R. D., Warner, J. S., and Poirier, R. H., Preparation and characterization of 4,4′-bis(dimethylamino) and 4,4′-bis(diethylamino)benzophenone oxime. *J. Org. Chem.* **21,** 616 (1956).
3. Rodd, E. H., ed., "Chemistry of Carbon Compounds," Vol. 3B, p. 1069. Elsevier, Amsterdam/New York, 1956.
4. Lynch, D. F. J., and Reid, J. D., The ketonimine dyestuffs and their derivatives. *J. Amer. Chem. Soc.* **55,** 2515 (1933).
5. Poziomek, E. J., Photochemical Isomerization. Synthesis of anti-isonicotinaldehyde oxime derivatives. *J. Pharm. Sci.* **54,** 333 (1965).
6. Izawa, H., De Mayo, P., and Tabata, T., The photochemical Beckmann rearrangement. *Can. J. Chem.* **47,** 51 (1969).

7. Amin, J. H., and De Mayo, P., The irradiation of aryl aldoximes. *Tetrahedron Lett.* No. **24,** 1585 (1963).
8. Taylor, R. T., Douek, M., and Just, G., Photolysis of oximes. *Tetrahedron Lett.* No. **34,** 4143 (1966).
9. Just, G., and Ng, L. S., Photochemistry of oximes. II. On the photo-induced formation of cycloalkanones and alkanoic acid amides from cycloalkanone oximes. *Can. J. Chem.* **46,** 3381 (1968).
10. Oine, T., and Mukai, T., Evidence for the formation of oxaziridines during the irradiation of oximes. *Tetrahedron Lett.* No. **3,** 157 (1969).
11. Vermès, J. P., and Beugelmans, R., Etudes de reactions photochimiques. VI. Oximes et acetates d'oximes. *Tetrahedron Lett. No.* **25,** 2091 (1969).
12. Splitter, J. S., and Calvin, M., Irradiation of 3-substituted-2-phenyloxaziridines—direct evidence for phenylnitrene. *Tetrahedron Lett.* No. **12,** 1445 (1968).
13. Binkley, R. W., and Kushner, A. S., The photochemistry of hydrazones: A photochemical Wolf-Kishner reduction. 158th ACS Nat. Meet., Division of Org. Chem., New York, 1969.

MICROCHEMICAL JOURNAL 15, 481–497 (1970)

An Automatic Microdetermination of Carbon, Hydrogen, and Nitrogen in Organic Compounds Using Data Printing System

KEIICHIRO HOZUMI

Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan

AND

OSAMU TSUJI AND HIDEYA KUSHIMA

Research and Development Division, Yanagimoto Mfg. Co. Ltd., Kyoto, Japan

Received June 23, 1970

INTRODUCTION

During the past decade numerous reports have appeared in automatic instrumentation for simultaneous microdetermination of carbon, hydrogen, and nitrogen in organic compounds using thermal conductivity detector which measured the quantities of carbon dioxide, water, and nitrogen resulted from the combustion of the organic samples (*5*). Most of those approaches have been based upon the gas chromatographic principle consisting of an instantaneous pyrolysis of the sample weighed in ultramicroscale, a chromatographic separation of the three combustion products, and sequential time integrations or measuring other parameters of their chromatograms (*4, 6, 9, 10, 12, 15, 17–19*). Attractive features of the gas chromatographic method have been summarized as rapid operation with electronic finish up, simple construction of the apparatus, and reliability to the established technique in gas chromatography.

Another type of approach was introduced by Simon and co-workers (*14*) and Clerc *et al.* (*1, 2*) using the differential thermal conductometry for measuring the individual component of the combustion gas mixture which had been collected in a vacuum pipet. The method had advantages that the samples could be rapidly weighed by microbalance, the combustion could be completed during the period of withdrawing the combustion gas into the vacuum pipet, and finally the concentration of individual component in the gas pipet was an accurately linear parameter of the quantity of the component.

Automatic instrumentations based upon the latter method have been

extensively developed by Condon (*3*) and also by Hozumi (*7*), both eliminating the vacuum system which required serious care of air tightness throughout the apparatus. Operations of the apparatus are automatically controlled and proceeded by preset programmers except charging the samples and reading the recorder charts. Additional basic investigations on the detector system and a statistical study of the error functions have been carried out by Hozumi and Tamura (*8, 16*), and Shimizu and Hozumi (*13*).

Further improvements have been successively made by the present authors using an automatic sample charger, a double action pump, and finally a data printing system. The analyses can be carried out full-automatically with the new instrumentation except the weighing of samples and occasional manual samples chargings for liquid or volatile solid materials. The double action pump withdraws the combustion gas and at the same time pushes out the previously drawn gas towards the detectors so that it has enabled the sequential operations at every 7.5 minutes that is twice as fast as the single action pump previously proposed by Hozumi (*7*). The data printing system involving an analog-digital converter and a printing mechanism provides time for the analysts to weigh the samples required for such fast analytical cycles.

MATERIALS AND METHODS

A schematic drawing of the instrumentation is illustrated in Fig. 1. A carrier gas of helium with a flow rate of approximately 180 ml/minute is fed to a side arm of a quartz combustion tube via a three-way solenoid valve A and is consistently overflowing from the mouth of the combustion tube preventing an inward diffusion of air. An oxygen flow of 10 ml/min is mixed with the helium via solenoid valve B during the combustions of organic sample. The combustion tube is filled with cupric oxide kept at 900°C by a cylindrical electric furnace and is followed by the reduction tube filled with reduced copper kept at 500°C. A cylinder pump with a capacity of 150 ml is constructed symmetrically in order to withdraw and to push out the combustion gas alternately at both sides of the piston which moves between an exactly reproduced stroke of 10 cm during a period of 5 minutes. Four solenoid valves C, D, E, F are provided for making varied flow paths around the pump system.

A detector system involves a series of three pairs of differential thermal conductometers which measure the individual concentrations of water, carbon dioxide, and nitrogen in the combustion gas fed from the pump. The output signals by the above conductometries are picked up successively by an automatic switching mechanism and are transmitted to a digital printing system for recording.

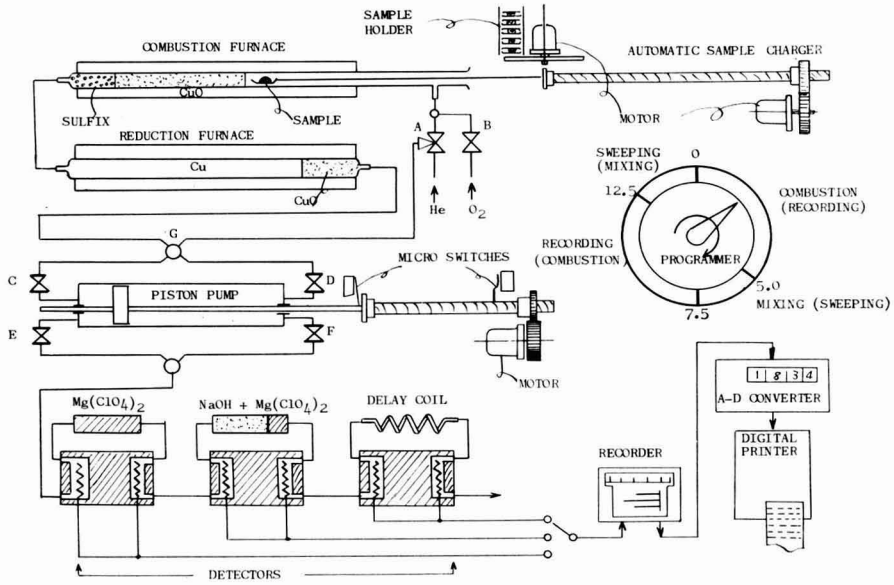


FIG. 1. Schematic diagram of the instrumentation.

All the solenoid valves, pump, detector system, and data printing system are operated under control of a preset programmer consisting of a set of rotary disk type cams and microswitches. One round of the programmer which requires 15 minutes is indicated in Fig. 1.

An attachment of automatic sample charger is provided for numbers of nonvolatile solid samples which have been taken in small quartz capsules and are successively charged into the combustion tube by a quartz spoon driven mechanically.

Full assembly of the apparatus is illustrated in Fig. 2.

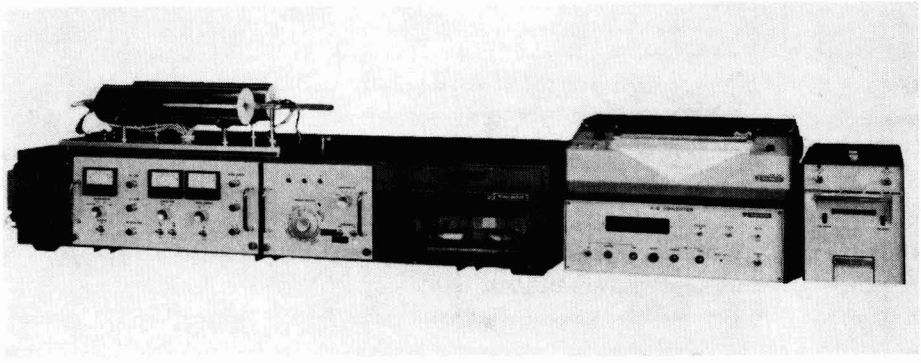


FIG. 2. Full assembly of the apparatus.

Combustion System

The combustion tube with a length of 55 cm and an outside diameter of 13 mm is packed mainly with cupric oxide and with a short section of Sulfix (granular mixture of silver particles dispersed in cobalto–cobaltic oxide; Kishida Chemicals Co., Osaka, Japan) (*11*) as illustrated in Fig. 3. The side arm is situated 15 cm from the mouth of the combustion tube and an empty space with a length of 13 cm is provided in the hot zone of the combustion furnace where the quartz spoon with a sample boat is inserted instantaneously. The maximum temperature at the middle part of the combustion furnace is kept at 900°C, while the Sulfix section is kept between 700–400°C by an end effect.

The reduction tube filled with reduced copper has a length of 38 cm, and an outside diameter of 13 mm, being kept at the maximum temperature of 500°C by the same type furnace as mentioned above. The input powers of the two furnaces are regulated by silicone controlled rectifiers changing the phase angles with variable resisters. A stainless steel pipe connecting between the combustion tube and the reduction tube is wound by a heating tape preventing a temporary condensation of water formed during the combustion of hydrogen rich organic compounds. A 70-cm stainless steel pipe with an inside diameter of 1 mm is provided to connect between the end of the reduction tube and the pump system in order to have approximately the same flow resistance as the flow path of the detector system.

Pump System

A symmetric construction of the stainless steel cylinder with two flat lids at both ends is provided having two openings on either lid as illustrated in Fig. 4. The well polished inside wall of the cylinder fits with

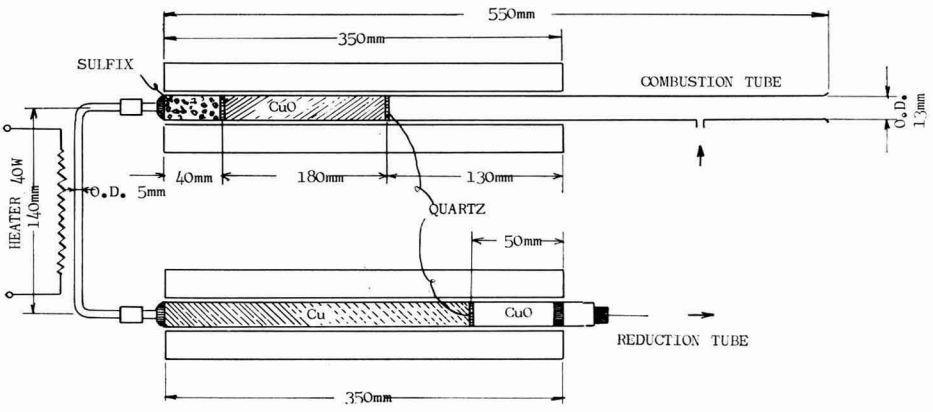


FIG. 3. Combustion system.

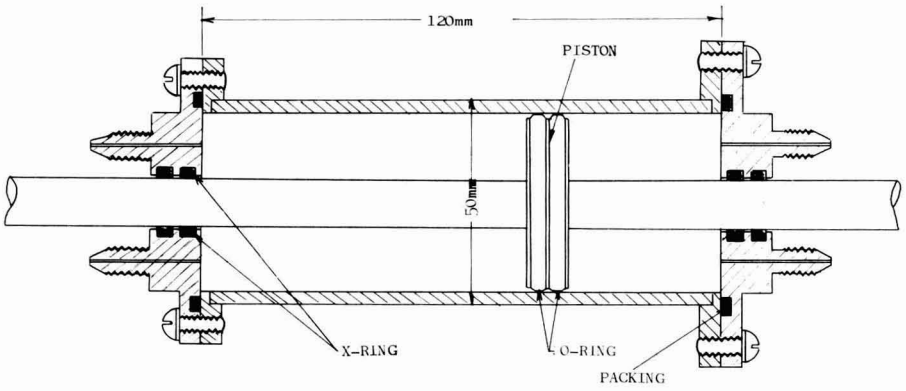


FIG. 4. Cross section of the piston pump.

a stainless steel piston having O-rings which smoothly moves with a piston shaft. The shaft is also kept air tight by X-rings fixed in the two lids. All the O-rings and the X-rings are lubricated by the minimum amounts of high vacuum silicone grease which will last at least during a period of 1 year. The maximum capacities of the both sides of the piston are adjusted as precisely the same as possible at approximately 150 ml which reproduce well by stop microswitches.

The piston shaft is driven by a 5-W synchronous motor via a free joint so that the moving speed is stabilized with an accuracy of the frequency of AC source, thus ensuring a constant flow rate of the carrier gas through the detector system.

The pump is installed in an air oven kept at 50°C with a precision of less than ± 0.05°C by means of a proportional temperature regulator and a motor fan. The air oven also admits all other temperature sensitive parts mentioned below.

Detector System

The three differential thermal conductivity cells involving stainless steel katharometers and individual 25-Ω tungsten filaments are mounted on a heavy wall aluminum plate which has been supported in the air oven. The attached bridge circuits are also wired in the air oven to have the electric dimensions perfectly stabilized and at the same time to shelter from the electrostatic noise. Three variable DC power suppliers are provided to energize the bridge circuits setting the supplying current at the certain values to obtain proper output signals for the data printing system. The bridge currents are normally suggested at 85 mA for the water and carbon dioxide detectors while at 150 mA for the nitrogen detector.

The absorption tubes for water and carbon dioxide are made of glass

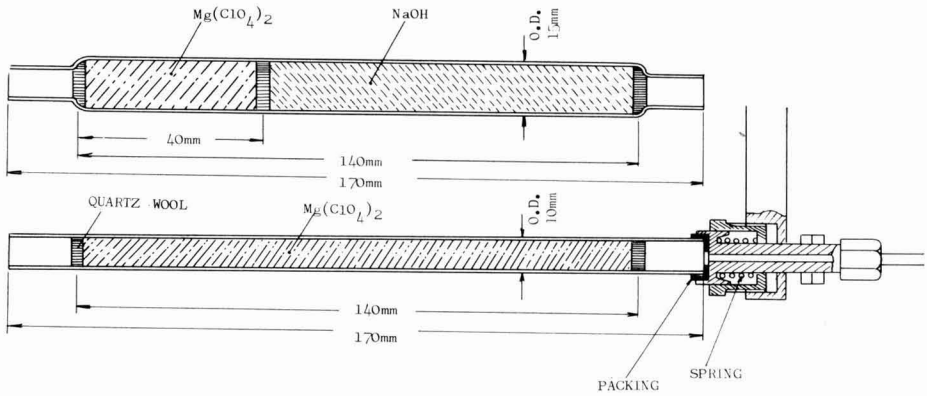


FIG. 5. Absorption tubes for water and carbon dioxide.

and their dimensions with filling reagents are illustrated in Fig. 5. Either tube is simply clamped between two metal joints by a coil spring using packing sheets of synthetic rubber. The tubes are supported on the outside wall of the air oven so that they are kept fairly constant at around 30°C. The delay coil which has an inside volume of approximately 150 ml and a total length of 2 m is divided into two coil blocks and is installed in the remaining space of the air oven. The inside view of the air oven is illustrated in Fig. 6.

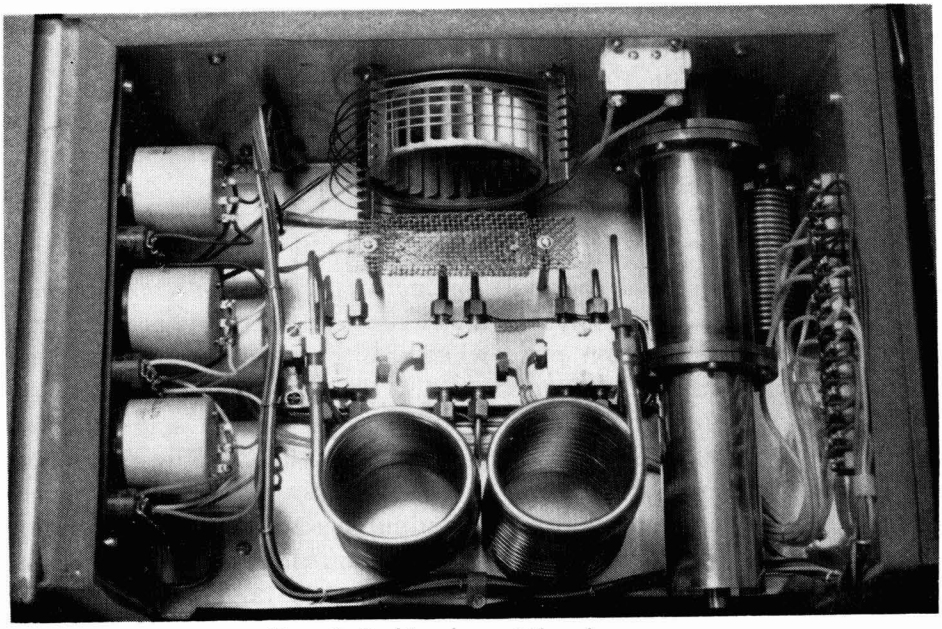


FIG. 6. Inside view of the air oven.

Data Printing System

The recorder with a sensitivity of 1 mV and a full span of 25 cm picks up the output signals sequentially from the water, carbon dioxide, and nitrogen detectors, that is, a disk-type cam in the programmer successively turns on three microswitches connecting each bridge circuit to the recorder during a period of 15 seconds, the same period of intermission being programmed between signal to signal.

The recorder installs a dual type helicalohm with two 1 kΩ resistances, one of which works as an ordinary balancing potentiometer and the other generates DC voltage proportional to the rotary angle of the helicalohm by applying 2.5 V to both end terminals. Amplification of nearly 70 dB has been easily achieved by this method.

The amplified signals are fed into an electonic analog-digital converter where the signals are interpreted into values of four numbers. These digital signals with the decimal code are further transferred to a mechanical printer which prints out the data on a paper strip. The recorder chart is normally stopped except for monitoring the output signals of the detector system. A block diagram of the data printing system is illustrated in Fig. 7.

Automatic Sample Charger

Analyses of nonvolatile solid compounds weighed in quartz capsules are conveniently carried out using the automatic sample charger. That is, a short quartz tubing with dimensions illustrated in Fig. 8 is loosely

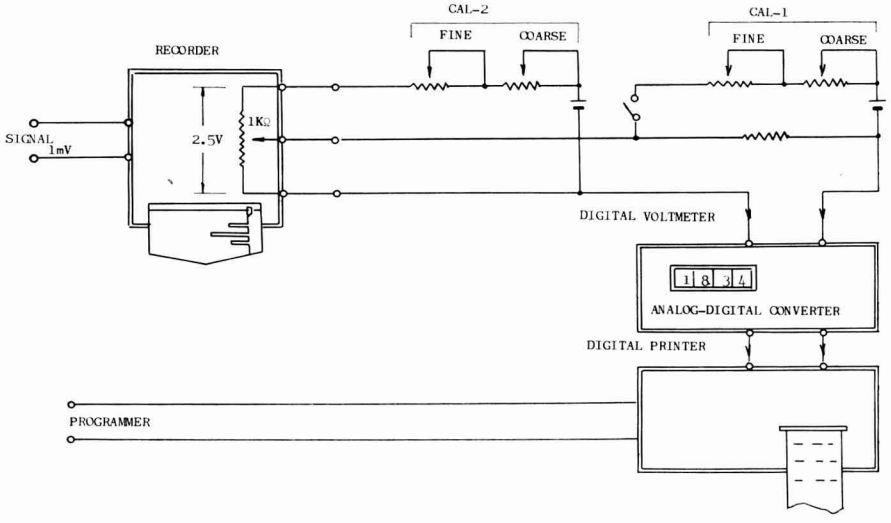


FIG. 7. Data printing system.

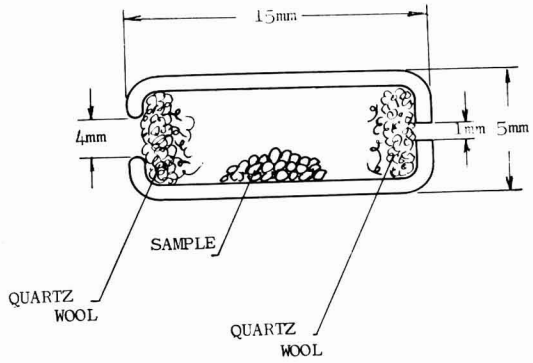


FIG. 8. Quartz capsule.

packed by quartz wool at the bottom and is precisely weighed by microbalance. A 2.5–3-mg portion of organic sample is then taken and reweighed. Quartz wool is finally packed at the open end to fix the organic sample. All the quartz tubings and the quartz wool must be preliminarily red-heated by a Bunsen burner to prevent any organic contamination.

Twelve capsules thus prepared are set in a sample chamber of the automatic sample charger which is illustrated in Fig. 9. The sample chamber is always filled with helium exhausted from the combustion tube in order to purge the air in the capsules.

When the programmer starts the "Combustion" cycle, the first capsule drops into the quartz spoon and the quartz spoon rapidly proceeds into

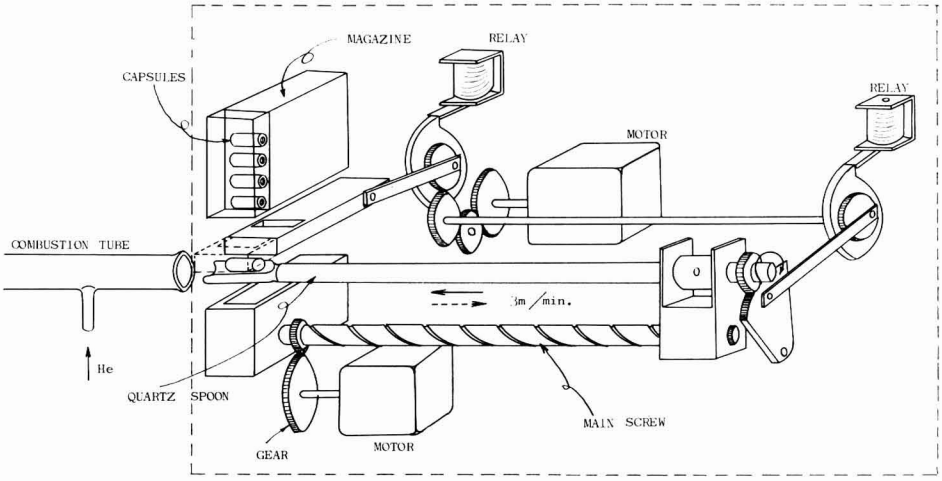


FIG. 9. Automatic sample charger.

the combustion tube with a traveling speed of 3 m/min until the sample reaches to a depth of 10 cm in the hot zone. When the "Combustion" cycle is finished, the quartz spoon is drawn back to the automatic sample charger and the capsule is cast from the quartz spoon by a rotary action. Analyses of the remaining eleven samples are automatically carried out with the same program.

A volatile solid sample can be also weighed in thee capsulee but it must be immediately charged in the quartz spoon to be burned. A liquid sample weighed in a glass capillary is placed in a empty quartz tubing and is directly charged in the quartz spoon.

Procedure

The helium carrier of 180 ml/min is fed to the combustion system and all the switches except for the solenoid valve B are turned on. The oxygen flow of 10 ml/min is fed when the reduction furnace will reach to the temperature of 400°C. The output signals are monitored by the strip chart recorder until the signals are settled after approximately 1 hour. The digital printer is then energized to print out the base signals on the paper strip.

The quartz capsules with 2.5–3 mg of nonvolatile organic samples are set in the automatic sample charger and are successively introduced into the combustion tube being subjected to the programmer. Opening the valves C, F and closing the valves D, E, the water, carbon dioxide, and nitrogen formed in the combustion system are withdrawn into the left side chamber of the piston pump (chamber I) until it reaches to the final capacity of 150 ml. After the "Combustion" cycle during 5 minutes, the three components in the chamber I are mixed homogeneously with helium by diffusion for 2.5 minutes. During the "Mixing" cycle, the valve D is opened and the three way solenoid valve A changes the flow path of the helium to the three way juncture G where the helium is divided into approximately two equal flows. One flows towards the combustion system reversely and the other flows through the right side chamber of the piston pump (chamber II) and the detector system.

The pump starts again by the reverse rotation of the motor opening the valves D, E and closing the valves C, F. The combustion gas is pushed out towards the series of differential thermal conductometers where the components of water, carbon dioxide, and nitrogen are sequentially absorbed or retained in the absorption tubes and the delay coil. The three output signals from the conductometers attain steady state within 2 minutes, so that the programmer picks up the three signals between 3–4 minutes after the pump has started moving.

Notice that during the period of "Recording" cycle for chamber I, chamber II is withdrawing the combustion gas of the next sample. Therefore the remaining 2.5 minutes in the programmer's one round is used for purging in chamber I and at the same time for homogenizing the gas in chamber II. Sequential analyses allow the charging of the samples and also the printing of data at every 7.5 minutes.

CALCULATION

The sensitivities of the three differential thermal conductometers should be preliminarily estimated at the given operative conditions of the apparatus. It must be emphasized that the combustion gases are withdrawn into chamber I and II alternately, which are not perfectly equalized in their capacities. Therefore the sensitivities should be estimated for either chamber I or II.

Several standard organic samples such as acetanilide and antipyrine are successively burned and chamber I and II are marked on the signals of the printer as illustrated in Fig. 10. The magnitudes of the response signals are calculated from the base signals obtained before and after the sequential analyses. Every response signal is treated in the previously described manner (*7*) to obtain the sensitivities of the three differential thermal conductometers. Two mean values of the sensitivities thus obtained for both chambers I and II are usable in the analyses of unknown samples.

Calculations for the unknown samples have been also described in the previous paper (*7*). Several functions necessary for the calculations are practically invariable unless the operative conditions of the apparatus are changed. Estimation of the sensitivities, however, must be retouched at certain intervals because of a small drift of the output voltage of the DC power supplier. The phenomenon is probably due to the change of average room temperature which affects the physical properties of the semiconductors mounted in the DC power supplier. It is therefore advised to install the apparatus in an air-conditioned laboratory.

It appears more practical to weigh a few standard samples in a series of 12 quartz capsules and check the sensitivities of the detectors. This provides the additional advantage that any other functions of unknown variables occurring in the analyses can be eliminated.

RESULTS OF ANALYSIS

Reproducibilities of the analytical results using acetanilide and ethyl *p*-aminobenzoate are listed in Table 1. Standardization of the sensitivities of the detectors was preliminarily carried out with a standard organic sample of antipyrine. The standard deviations of hydrogen and nitrogen

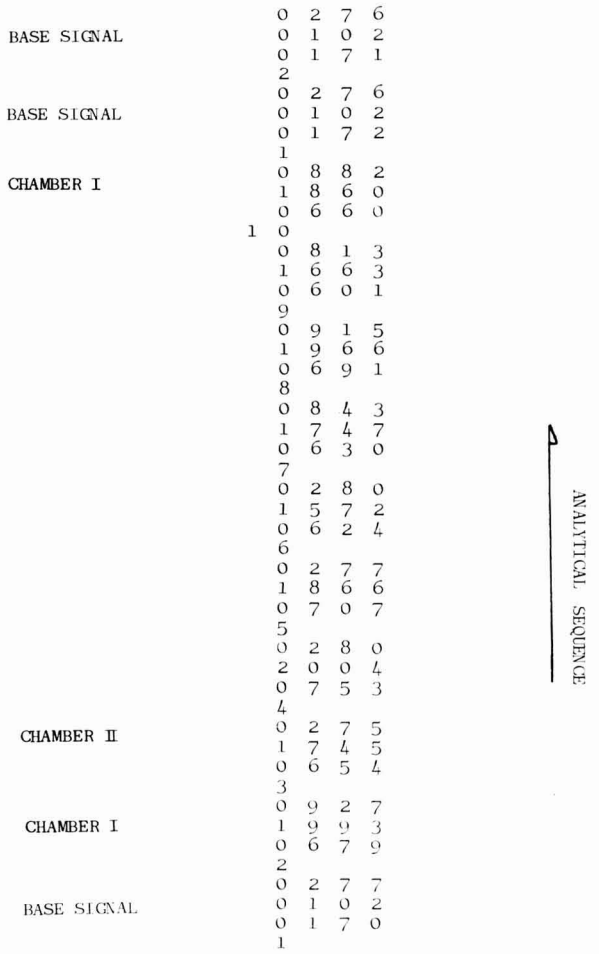


FIG. 10. Registered output signals.

values were calculated as 0.05–0.10% and that of carbon values was 0.10–0.12%.

Analytical data with more refractory substances are listed in Table 2. Polymers, coal, and pteridine compound resulted in carboneous residues, which requirered longer time for complete removal from the quartz capsules by oxygen containing helium. Cholesterol and benzene hexachloride form very stable fragments of methane and chlorinated carbon compounds, respectively, during the pyrolysis. It has been found that all the analytical data including some of the refractory substances were satisfactorily accepted in the traditional allowance of the microanalysis, although the theoretical compositions of coal were unknown.

TABLE 1

SEQUENTIAL ANALYTICAL DATA WITH STANDARD ORGANIC COMPOUNDS

Calc	C% = 71.09	H% = 6.71	N% = 10.36
Acetanilide 1	71.22	6.77	10.39
2	71.11	6.80	10.34
3	71.20	6.82	10.37
4	71.29	6.85	10.43
5	70.99	6.67	10.32
6	71.25	6.75	10.41
7	71.28	6.77	10.55
8	71.02	6.81	10.39
9	71.06	6.58	10.22
10	71.30	6.82	10.40
Mean	71.16	6.76	10.38
SD	0.12	0.08	0.09
	C% = 65.44		N% = 8.48
Ethyl *p*-aminobenzoate 1	65.60	6.80	8.47
2	65.46	6.78	8.45
3	65.43	6.70	8.43
4	65.50	6.74	8.45
5	65.48	6.78	8.57
6	65.36	6.61	8.47
7	65.52	6.72	8.55
8	65.22	6.68	8.57
9	65.58	6.78	8.53
10	65.51	6.80	8.41
Mean	65.47	6.74	8.49
SD	0.10	0.05	0.06

DISCUSSION

Combustion Time

An experiment was carried out to investigate the necessary time for complete combustion of sample using an arrangement as illustrated in Fig. 11. A thermal conductivity cell is inserted between the combustion system and the pump, the output signals being indicated by a strip chart recorder.

Several organic samples were burned in the combustion tube under operative conditions that the combustion furnace was kept at 800°C and the oxygen flow of 10 ml/min. The combustion patterns were obtained as illustrated in Fig. 12. It has been found that the ordinary compounds such as anthraquinone, sucrose, and cholesterol could be completely decomposed within 2.5–3 minutes, while some of refractory substances

TABLE 2

ANALYSES OF SEVERAL REFRACTORY ORGANIC COMPOUNDS

	Carbon (%)		Hydrogen (%)		Nitrogen (%)	
Sample	Found	Dev.	Found	Dev.	Found	Dev.
6-6 Nylon	63.55	−0.17	9.82	+0.09	12.32	−0.07
	63.49	−0.23	9.76	+0.03	12.34	−0.05
Poly-acrylonitril	67.64	−0.26	5.79	+0.09	26.22	−0.18
	67.88	−0.02	5.70	0.00	26.26	−0.14
Coal	77.38		5.72		0.98	
	77.37		5.89		1.03	
Cholesterol	84.06	+0.19	12.17	+0.18		
	83.83	−0.04	12.08	+0.09		
Benzene hexa-chloride	24.89	+0.11	2.13	+0.05		
	24.86	+0.06	2.09	+0.01		
2, 4-Dihydroxypteri-dine	44.06	+0.15	2.38	−0.08	34.51	+0.37
	43.98	+0.07	2.41	−0.05	34.32	+0.18

such as polyethylene and mineral oil pitch required more than 4 minutes. A heavy tailing probably due to water was encountered with the combustion of polyethylene. Higher oxygen flow rate of 20 ml/min against the helium flow rate of 180 ml/min gave little improvement of the

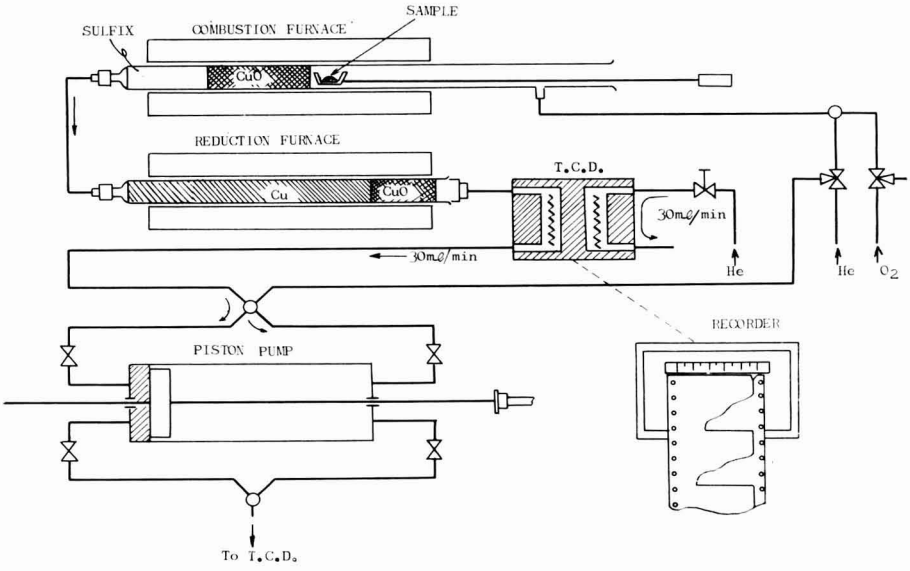


FIG. 11. Flow scheme for monitoring the combustion patterns.

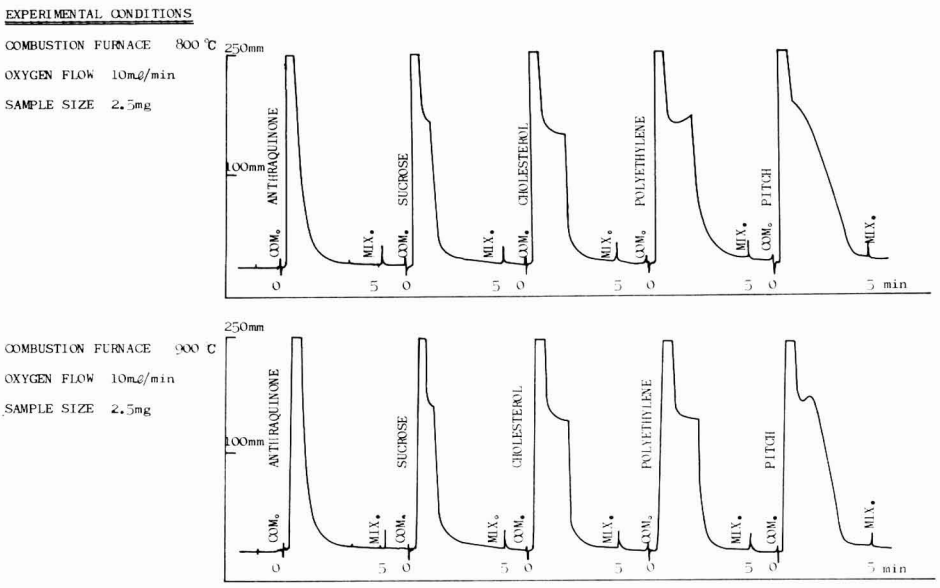


FIG. 12. Combustion patterns.

combustion speed. On the other hand, higher temperature of the combustion furnance of 900°C resulted in a significant improvement of the combustion speed so that the entire combustion products of polyethylene were introduced in the pump during the "Combustion" cycle.

Error Functions

Physical parameters functional to the analytical errors are listed in Table 3, where the individual relative error is evaluated under the given operative conditions. Since the reproducibility of the microbalance Mettler M5-SA presented a standard deviation of 1.5 μg, the weighing error of the samples should be evaluated as $\sqrt{2} \times 1.5$ nearly equal 2 (μg). The DC power supplier of 12 V with a guaranteed variation within 3 mV presented a relative error of 0.02%, but a threefold multiple of this value should be considered as a linear function of the analytical error because the sensitivity of thermal conductivity detector is proportional to three powers of the bridge current.

Variations of the temperature of the air oven and the final volume of the pump could not be precisely evaluated because a thermometer with graduations of 0.5°C and a precision millimeter scale measured no visual change of the oven temperature and the length of pistion stroke, respectively. Therefore an assumption was made that the maximum errors would have been equal or less than the detectable limits of 0.05°C and 0.05 mm, respectively.

TABLE 3

EVALUATIONS OF ERROR FUNCTIONS

Parameter	Range	Variation	Relative error (%)
Weighing	2.5 mg	2 μg	0.08
DC source	12 V	3 mV	0.025
Oven temperature (°K)	323	0.05	0.02
Piston stroke (mm)	100	0.05	0.05
Atmospheric pressure (mm Hg)	760	0.2	0.03
Analog readout	1 mV	2 μV	0.20
Digital readout	2.5 V	1 mV	0.04

Variation of the atmospheric pressure during 2–3 hours was practically negligible, at least in the normal weather. The main cause of variation was experienced by readjusting the level of mercury reservoir before reading the barometer scale. Repeated tests of the readjustments with ordinary care evaluated the variation of 0.2 mm.

Analog readout with the strip chart recorder is normally guaranteed as a precision of 0.5%, but actually most of the recorders for analytical purpose are qualified having a precision of approximately 0.2%. It is of interest that the helicalohm as a balancing potentiometer installed in the recorder is guaranteed to have a precision of 0.1% and probably will work with much higher precision. It follows therefore that most of the error sources exist in the transmission mechanism between the helicalohm and the recorder pen.

The use of the dual type helicalohm to amplify the bridge output signals and to operate the A–D converter as illustrated in Fig. 7 presented a considerable improvement of the reproducibility. The digital counter exhibited a precision of 1 mV for the full range of 2.5 V, resulting a relative error of 0.04%. Employing the digital readout system, the physical error functions listed in Table 3 are well balanced by each other. Other error functions coming from chemical problems have not been discussed here because of a lack of reasonable measuring mean.

SUMMARY

An automatic instrumentation for microdetermination of carbon, hydrogen, and nitrogen in organic compounds involving an automatic sample charger, a double action pump, and a data printing system has been described. A series of 12 quartz capsules containing weighed organic samples can be set in the automatic sample charger and are sequentially charged into a combustion tube. The combustion gas with a carrier gas of helium is withdrawn into one side of the double action pump during a period of 5 minutes and is kept standing for 2.5 minutes for homogenizing. The gas mixture is then pushed out of the pump towards a series of three differential thermal conductometers successively removing or retaining

water, carbon dioxide, and nitrogen components. While the double action pump is pushing out the combustion gas towards the thermal conductometers, the next sample can be burned and the combustion gas is withdrawn into the opposite side of the pump. Chargings of the samples are therefore carried out at every 7.5 minutes. An analog-digital converter is provided to print out the successive signals from the thermal condutometers eliminating a need of measuring the length of the bar-gram on strip chart recorder. Evaluations of some error functions of the apparatus have been discussed.

REFERENCES

1. Clerc, J. T., Dohner, R., Sauter, W., and Simon, W., Vollautomatische Anlage zur gleichzeitigen Bestimmung von Kohlenstoff, Wasserstoff und Stickstoff in organischen Verbindungen an Milligram- und Submilligram Mengen unter Verwendung einer selbst-integrierenden Wärmeleitfähigkeitmessmethodik. *Helv. Chim. Acta* **46,** 2369–2388 (1963).
2. Clerc, J. T., and Simon, W., Limitations of a self integrating method of the simultaneous C, H, and N determination by thermal conductivity measurement. *Microchem. J.* **7,** 422–436 (1963).
3. Condon, R. D., A new automatic organic elemental microanalyzer. *Microchem. J.* **10,** 408–426 (1966).
4. Duswalt, A. A., and Brandt, W. W., Carbon–hydrogen determination by gas chromatography. *Anal. Chem.* **32,** 272–274 (1960).
5. Francis, H. J., Jr., Automatic elemental microanalysis. *Anal. Chem.* **36,** 31A–47A (1964).
6. Haber, H. S., and Gardiner, K. W., A rapid instrumental method for the microdetermination of carbon and hydrogen in organic compounds. *Microchem. J.* **6,** 83–90 (1962).
7. Hozumi, K., An instrumental method for the simultaneous microdetermination of carbon, hydrogen, and nitrogen. *Microchem. J.* **10,** 46–60 (1966).
8. Hozumi, K., and Tamura, H., Theory on cell balance in differential thermal conductometer and a practical method for counterbalancing. *Jap. Anal.* **16,** 1193–1199 (1967).
9. Maresch, C., Sundberg, O. E., Hofstader, R. A., and Gerhard, G. E., Microdetermination of carbon, hydrogen and nitrogen by gas chromatography. *Microchem. J., Symp. Ser.* **2,** 387–396 (1962).
10. Miller, C. D., and Winefordner, J. D., An efficient instrumental procedure for the rapid determination of carbon and hydrogen in the submilligram range, and its extension to the determination of nitrogen. *Microchem J.* **8,** 334–348 (1964).
11. Mitsui, T., Yoshikawa, K., and Furuki, C., Microdetermination of carbon and hydrogen using nitrogen as a transport gas. *Mikrochim. Acta* **1962,** 385.
12. Nightingale, C. F., and Walker, J. M., Simultaneous carbon–hydrogen–nitrogen determination by gas chromatography. *Anal. Chem.* **34,** 1435–1437 (1962).
13. Shimizu, M., and Hozumi, K., Statistical elucidation on analytical error of an automatic carbon, hydrogen, and nitrogen analyzer involving differential thermal conductometers. *Jap. Anal.* **19,** 1041–1049 (1970).
14. Simon, W., Sommer, P. Z., and Lyssy, G. H., Complete automation of the microdetermination of carbon and hydrogen in organic compounds. *Microchem. J.* **6,** 239–258 (1962).

15. Sundberg, O. E., and Maresch, C., Application of gas chromatography to microdetermination of carbon and hydrogen. *Anal. Chem.* **32,** 274–277 (1960).

16. Tamura, H., and Hozumi, K., Effects of flow rate and temperature in differential thermal conductometry. *Jap. Anal.* **19,** 60–66 (1970).

17. Vogel, A. M., and Quattrone, J. J., Rapid gas chromatographic method for determination of carbon and hydrogen. *Anal. Chem.* **32,** 1754–1757 (1960).

18. Walisch, W., Eine Ultramikroschnellmethode zur gleichzeitigen Bestimmung des C-, H-, und N-gehaltes organischer Verbindungen. *Chem. Ber.* **94,** 2314–2317 (1961).

19. Walisch, W., A rapid ultramicro method for simultaneous determination of the carbon, hydrogen and nitrogen contents of organic compounds. *Trans. N. Y. Acad. Sci.* **25,** 693–704 (1963).

MICROCHEMICAL JOURNAL **15**, 498–507 (1970)

Book Reviews

Clinical Analysis by Thin-Layer Chromatography Techniques. By RONALD M. SCOTT, Ann Arbor *Sci. Pub.* Ann. Arbor, Mich. 1969 $18.75

In this monograph, the various precedures which involve thin-layer chromatography in clinical analysis are assembled and presented in a concise manner. First the general techniques of the chromatography itself are discussed briefly. This is followed by a series of chapters on carbohydrates, amino acids, steroid hormones, bile acids, lipids, organic acids and phenols, and heterocyclics. In each chapter, the chemistry of the compounds is summarized and the general chromatographic properties are discussed. Specific details are then given for the qualitative and quantitative assay of various substances within the class. The author appears to have had much experience in clinical analysis and with the procedures. Thus, the book is quite practical and should be most useful in clinical laboratories.

In the opinion of this reviewer, however, the overall quality of the book is not especially good. The section on techniques may be good enough for clinical analysis, but it is not a very complete presentation. No uniform attempt is made to tell the reader how to obtain the various gadgets described. For example, the useful fact that the Desaga equipment shown in many pictures is obtainable from Brinkmann Instruments is not obvious. The writing is spotty, varying in style almost from one paragraph to another. Although the book was published in 1969, there are no references after 1967. There is a lack of attention to detail. The author cites five books as general references on thin-layer chromatography and misspells the names of two of the authors. This does not bode well for the experimental procedures.

In summary, the book will be quite useful because it was written for one area of work, but it should be used in conjunction with some other book, perhaps Kirchner's "Thin-Layer Chromatography" [Wiley (Interscience)] or Stahl's book of the same name (Academic Press).

JAMES M. BOBBITT, *University of Connecticut, Storrs, Connecticut 06268*

Hackh's Chemical Dictionary, 4th ed. Revised and edited by JULIUS GRANT, McGraw-Hill, New York, 1969. xi + 738 pp. $29.50.

This dictionary will be a valuable addition to many libraries, whether general or specialized in their holdings, and, as the case for the 3rd edition of some 15 years ago, can be a friend to many chemically oriented marketing personnel, secretaries, writers, editors, and technologists. Although billed as a *chemical* dictionary, the coverage of other physical sciences is generous. Julius Grant in the Introduction states "the total . . . number of words now defined is nearly 55,000." The publisher's staff has done Dr. Grant an injustice by having the jacket copy read ". . . the total number of words now defined has risen to 80,000."

To establish the "score" for the inclusion of terms related to microanalytical techniques and recent advances in analytical chemistry, this reviewer prepared a list of about 500 terms, culled from the index to a recent textbook, a recent

instrument guide, and some back issues of this journal. The batting average of the new edition proved excellent.

To keep the work to reasonable length, Dr. Grant has been forced to extreme conciseness in most definitions. As a consequence, the dilemma of usefulness versus brevity, which is classically delineated by the description of a cow as a bovine animal, is encountered throughout this work.

A publisher has a critical decision in the pricing of any technical work. *Hackh's Chemical Dictionary* is now offered at almost 30 dollars. This reviewer wonders whether the return might not have been greater at a significantly lower price and whether an abridged version that could be placed on the desks of more chemical secretaries might be a feasible publishing strategy. In such an abridgement the coverage of organic compounds might be made more selective or more concise and the listing of the physical properties of many compounds might be omitted.

The format, typography, and binding are commendable.

A. J. BARNARD, JR., *J. T. Baker Chemical Company*
Phillipsburg, New Jersey 08865

Capillary Methods of Investigating Micro-organisms. By B. V. PERFIL'EV AND D. R. GABE; transl. by J. M. SHEWAN. Univ. of Toronto Press, Toronto, Canada, 1969. xi + 627 pp. $30.00.

This book, written by USSR scientists in the late 1950's, consists of descriptions of methods in four areas: (a) construction of capillaries and other microimplements from ribbon glass, (b) quantitative and qualitative examination of microflora of natural environments, (c) isolation of single microbial cells, and (d) continuous microbial cultures. Of most novelty and utility to Western readers is area (a); the other three areas have been well reviewed and updated in numerous Western publications during the 1960's. The translation into English and the approximately 300 diagrams are excellent; the index is somewhat lean. The bibliography contains a good representation of USSR papers published during the forties and fifties but references to United States publications are largely confined to the twenties and thirties. Thus, the authors are completely unaware of developments in this country in the forties in such areas as direct cell counting of bacteria (e.g., extensive use of the Petroff-Hausser and Helber chambers described by Simmons in Laboratory Methods of the U.S. Army, 1944). Despite its partial obsolescence, however, a copy of this book should be available in all libraries used by microbial ecologists.

Eugene D. Weinberg, Indiana University, Bloomington, Indiana 47401

Massenspektrometrie Organischer Verbindungen. By WOLFGANG BENZ. Akademische Verlagsgesellschaft, Frankfurt/Main, Germany, xii + 425 pp. 75 DM.

The catalog of books dealing with various aspects of organic mass spectrometry has been expanding steadily in recent years, a reflection, no doubt, of the rapidly increasing utilization of this technique in many phases of organic chemical research and of the rapidly accumulating information on electron impact-induced fragmentation processes of organic molecules. The work under review here, represents a comprehensive and up-to-date introduction into the organic chemical

applications of mass spectrometry ranging in scope from instructions on low resolution mass spectra counting to high resolution data reduction, from elementary aspects of spectra evaluation and presentation to some of the more recent results on fragmentation mechanisms and their interpretation. The organization of the book is of the more or less conventional sort in which chapters on basic instrumentation, sample introduction systems (including a very good discussion of gas chromatography–mass spectrometry coupling methods) and techniques of measurement, evaluation, and presentation of spectra are followed by a long (110 pp.) chapter covering both general fragmentation mechanisms and specific patterns and characteristic peaks observed for various individual compound classes. The latter section, although brief and limited to the most important aspects of the fragmentation pathway of a given class, includes most important organic structural types, with the exception of natural products. The remaining chapters concern the detailed interpretation of mass spectra with examples and 14 problems (for which solutions are given in the appendix), quantitative mixture and isotopic analysis, high resolution mass spectrometry and its applications, and finally a brief discussion of common methods of chemical modification of samples as an aid in mass spectral analysis.

The inclusion of an appendix in a book of this type appears a very happy idea, since it provides in readily accessible form some basic data useful to the practicing spectrometrist: ionization and appearance potentials of selected elements, molecules and ions, mass differences between some common doublets of the same nominal mass, a table for the determination of elemental composition from accurate masses, isotopic abundance tables of elements, and a catalog of about 350 mass spectra (in tabular form) listed according to compound class (and including all basic types) which can serve as a convenient small reference library.

The book, in short, contains all the important information an introductory volume on organic analytical applications of mass spectrometry should contain; basic concepts, important applications, as well as useful practical hints and procedures are presented—and presented well—but the distribution of emphasis in certain intances appears open to some criticism. A presentation, for example, which allocates barely more space to a discussion of the double focusing principle of high resolution mass spectrometers than is devoted to a description of a variable scale, must be judged somewhat unbalanced. Again, if a schematic drawing of such a variable scale is deemed useful, would an ilustration of the actual appearance of some metastable peaks not have been equally informative? Why also, in a book not notable for abundant mass spectral illustration, reproduce twice within 10 pages the element map of ethyl undecanoate, using it once as an illustration of element map format and once as an example of element map interpretation? A minor point, admittedly, but if such duplication is felt to be desirable, the discussion of additional element map examples would not be judged overly redundant, since the one chosen (although appropriate as a demonstration of basic approach) is so embarrassingly simple that it might best serve as an illustration of unnecesary mass spectral "overkill."

In over a hundred pages of discussion of fragmentation pathways—though a very good summary of current knowledge, competently presented, well documented and up-to-date—one finds only 12 mass spectra, a very stringent reduction to principles of the empirical information, ill-suited, one feels, for an introductory volume aimed at readers with limited previous exposure to mass spectrometry.

And if the 12 spectra shown (a rather uneven selection in itself: five alkynes three alkanes, and ethyl butyrate and three deuterated analogs), are considered illustrations appropriate to the discussion, the presentation of an occasional ether, ketone, or amine spectrum would not be judged excessive bulk. Natural products are discussed all too briefly (1 page) and while one readily agrees that no exhaustive treatment of so varied a topic could have been attempted within the scope of the book, one nevertheless wishes that the page had been expanded to a chapter, since much of the actual application of mass spectrometry concerns exactly this area. A few well-chosen examples would have sufficed to convey the utility of the method, and might have served also to amplify and illuminate basic concepts presented earlier, as well as to demonstrate the often considerable difficulties in properly applying principles derived from simple models to complex structures.

The above comments, while drawing attention to some of the omissions, should not be allowed to obscure the basic quality to this monograph: it is more current, more comprehensive and more useful than other introductroy texts of the same category.

H. K. SCHNOES, *Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706*

Ion Selective Electrodes. Edited by RICHARD A. DURST. Nat. Bur. Stand. Spec. Publ. 314, 1969. 474 pp. $3.50. Order from the U.S. Gov. Printing Office, Washington, D.C., Cat. No. C13.10:314.

This book is the report of the proceedings of the Symposium on Ion-Selective Electrodes that was held at the National Bureau of Standards in 1969, which the reviewer had the pleasure of attending. Included in the 12 chapters are discussions on the theory, characteristics and methodology of all types of ion-selective electrodes; their uses in thermodynamic, kinetic, and complex ion studies; pure and applied research in biomedical areas; industrial analysis and control systems; and applications to diverse analytical problems. The chapters, which are written by the 10 authors to whom an invitation was extended because of their extensive experience in the subject, show a continuity in subject and in style. Literature citations follow each chapter; the index, which is very complete, refers to all the chapters regardless of the author.

The book discusses membrane, liquid ion exchange, and solid-state electrodes. It presents applications of these electrodes, which are generally employed with an expanded scale pH meter, to the determination of both cations and anions (e.g., silver, calcium, cadmium, sulfide, nitrate, fluoride, chloride, perchlorate, etc.). The book is excellent. It provides a comprehensive treatment of the subject not only from a theoretical standpoint but also, and possibly more important, with details on the use of these electrodes, causes of difficulty, and means of overcoming problems, the accuracy and concentration limits that can be determined as well as interferences to be anticipated in practical analysis. It presents thoughts to the chemist on both past uses and future applications of ion-selective electrodes. At the low cost of $3.50, it is not only a fantastic bargain for a hard-cover book but a most authoritative work on the subject. Its purchase is highly recommended to any user of pH meter.

PETER F. LOTT, *Chemistry Department, University of Missouri–Kansas City, Kansas City, Missouri 64110*

Ancillary Techniques of Gas Chromatography. Edited by LESLIE S. ETTRE AND WILLIAM H. MCFADDEN. Wiley (Interscience), New-York, 1969. xi + 395 pp. $17.50

The book under review presents a treatment of the current techniques and analytical instruments used in conjunction with the gas chromatograph. It offers a clear and logical account of the present state of practical gas chromatography, with the inescapable conclusion that there exists a definite trend, a special characteristic that the coupling of the different instrumental or chemical methods with gas chromatography are usually carried out in one unified system. Thus all these manipulations or methods can be regarded in this respect as *ancillary techniques of gas chromatography*.

The book is divided into 10 chapters, each written by eminent researchers in the field of gas chromatography. Chapter 1 considers the principles and classifications of ancillary techniques. Chapter 2 deals with microreaction gas chromatographic techniques and introduces the reader to the field of microreaction techniques showing some examples of how microreactors coupled to gas chromatographs can be used for the investigation of chemical, mainly catalytic, reactions. Some examples are also cited for the application of gas chromatography for the determination of catalyst properties other than activity. Chapter 3 covers pyrolysis gas chromatography (PGC) which is growing rapidly in both technique and application. PGC applications at present are primarily qualitative, but as the authors point out, the use of computerized techniques for handling PGC data could greatly increase the speed and applicability for identification by comparison of pyrogram fingerprints with a library of standard fingerprints. Quantitative analytical data can also be obtained and the use of PGC for quantitative analysis should increase rapidly. Chapter 4 discusses precolumn reactions for structure determination; reactions may be carried out prior to or during gas chromatography. Analyses applicable at the microgram level are stressed. The techniques treated are carbon-skeleton chromatography in which functional groups are stripped from a molecule, hydrogenation—applicable to a wide variety of compounds and which is quantitative in many instances—can be be easily carried out within the gas chromatographic pathway, and subtractive reactions in which it is possible to remove or distinguish between compounds having diffierent functional groups. Chapters 5 through 7 deal with mass spectroscopy, infrared and Raman spectrometry, and nuclear magnetic resonance spectroscopy as adjuncts to gas chromatography. The topics covered include the principles involved, batch and continous sampling, recording techniques and scanning rates as well as sample requirements. Much of the difficulty encountered with the use of ancillary systems for identification involve trapping and maniipulation of milligram quantities. It is pointed out in these chapters that time-consuming systems are tolerated only if absolutely necessary, and sample techniques developed for frequent daily analyses are reviewed thoroughly. Chapters 8 and 9 deal with thin-layer chromatography and chemical identification of gas chromatographic fractions as ancillary systems. The last chapter is devoted to special identification detectors and covers detectors specific for determination of such elements as halides, phosphorus, sulfur, and nitrogen. Reviewed are the microcolorimetric titrating system, the thermionic detector, and the microwave emission detector. For the detection of specific organic functional groups, the electron capture detector, the acid–base titration cell, and the spectrofluorimetric detector come for specific treatment. The chapter concludes wih a brief review of the biological

detector as human sensor being the most sensitive detector of all capable of detecting materials in amounts of 10^{-15} g or less.

To sum up, the book under review represents a comprehensive treatise on the practical aspects of ancillary systems used in conjunction with gas chromatography. The principal aim of the book is to summarize the aspect of a particular system, discuss its pros and cons, describe the most important instrumental arrangements, and to discuss what kind of information can be obtained through its use. Although practitioners of the art may disagree on the extent of stress that should be placed on a particular technique, each described technique is adequately and concisely explained. Typographically the book is excellent and well produced. The book is well written and reads easily; the print is clear, and only a few minor errors of fact or transcription are apparent—obviously the result of good editorial work. Being moderately priced and containing a valuable review of the recent literature the book should be invaluable to a broad cross section of scientists engaged in the practical aspects of gas chromatography. It should appeal in particular to analytical and organic chemists to whom this book should be an absolute must and as such its acquisition is strongly recommended.

GEORGE WIENER, *Chas. Pfizer Co., Inc., Brooklyn, New York 11206*

Practical Optical Crystallography. 2nd ed. By N. H. HARTSHORNE AND A. STUART. Amer. Elsevier, New York, 1969. ix + 326 pp. $11.50.

The first edition of this book, published in 1964 [reviewed in *Microchem. J.* **11,** 146 (1966)], consisted of revisions of selected sections of the authors' *"Crystals and the Polarising Microscope."* This second edition retains the qualities of the first: it is a clearly written description of classical crystallography, of the wave properties of light, and of the use of the polarizing microscope for studying the interaction of crystals and light. The approach is practical and descriptive rather than theoretical or mathematical, and the book is an ideal guide to the use and care of the microscope.

Only minor changes have been made in the new edition. A few references have been added, a list of manufacturers has been appended and some small errors have been corrected. A 77% price increase seems to be the main reason for calling it a new edition instead of merely a new printing.

DONALD E. SANDS, *Department of Chemistry, University of Kentucky, Lexington, Kentucky 40506*

Chromatography. Edited by D. R. BROWNING. McGraw-Hill, New York/London, 1969. v + 151 pp. $7.50.

This book is designed to be used as a supplementary textbook for introductory courses in biochemistry, instrumental methods, and in the medical laboratory sciences. It appears to be intended for use at the undergraduate level, or for those with little or no previous knowledge of chromatography. The following areas are covered: Column, paper, thin-layer, ion-exchange, and gel permeation chromatography. In addition, zone electrophoresis is covered.

The book is well organized. Each chapter is subdivided along the same line. However, there are minimum visual aids in each one. The bulk of the subject material is devoted to discussions of column, paper, and thin-layer chromatography.

On the average, references were sparse with the exception of the chapter on thin-layer chromatography. They were fairly up-to-date, however. It would have been helpful in a book of this type to include a few select numbers of annotated references so that the novice could quickly gain a practical entrée into a specific area of chromatography. For example, in the chapter on paper chromatography there are no specific references to an exact procedure for amino acid separations.

The chapter on thin-layer chromatography was well written and contained good basic material and up-to-date general references. However, adsorbents and specific separation problems were not covered in depth. A potentially misleading statement is made that "a combination of more than two solvents should seldom be required in adsorption thin-layer chromatography." Many major classes of metabolites cannot be separated by the adsorption technique except by the use of multicomponent solvent systems (viz., neutral lipids). In addition, a statement is made that "temperature is far less important than with paper partition chromatography." This is quite relative since, in thin-layer chromatography, temperature and humidity also are important determining factors in the separation of many classes of compounds (viz., amino acids).

The chapter devoted to the separation of macromolecules by gel permeation chromatography was disappointingly brief.

More recent techniques such as ion-exclusion and dry-column chromatography are not included.

This book is recommended for use by nonspecialists because it is highly readable and because it will serve as a rapid introduction to the subject of chromatography.

IRWIN L. SHAPIRO, *J. T. Baker Chemical Co., Phillipsburg, New Jersey 08865*

Spectroscopy. Edited by D. R. BROWNING. McGraw-Hill, New York, 1969. vii + 183 pp. $8.50.

Within the space of 183 pages (21.5 × 13.5 cm), the editor has collected 10 chapters on spectroscopy written by six contributors. Tht titles are: Theory of Molecular Spectorscopy, Molecular Spectroscopy in the Visible and Ultraviolet, Infrared Spectroscopy, Raman Spectroscopy, Mass Spectrometry, Nuclear Magnetic Resonance Spectroscopy, Electron Spin Resonance Spectroscopy, Atomic Emission Spectroscopy, Atomic Absorption Spectroscopy, and Atomic Fluorescence Spectrophotometry. A list of selected references is given at the end of each chapter—except, inexplicably, the NMR chapter. A brief list of suggstions for further reading is given at the end of the book.

Too much is attempted in too little space; some of the shorter chapters are compressed recitals of topics, formulas, and definitions. However, the book may serve the practicing chemist who must decide on a spectroscopic application to his problem.

R. M. SILVERSTEIN, *State University College of Forestry at Syracuse University, Syracuse, New York 13210*

Electrometric Methods. Edited by D. R. BROWNING, McGraw-Hill, London/New York, 1969. viii + 131 pp. $7.50.

This book is one of a three-volume series on instrumental methods in chemistry. The intention of the series is to provide the chemist with an authoritative and

accurate account of the subject and to serve as a textbook in college-level courses. The first half of this volume contains an introduction to conductance and potentiometry. With a few exceptions, some of which are noted below, these first three chapters are well written, contain few errors, and should be well suited to the avowed purpose of the book. However, the quality deteriorates in the second half, which contains many serious factual errors, much careless presentation of material, and does not contain adequate discussions of numerous necessary topics. For these reasons the volume cannot be recommended by this reviewer.

In the first chapter, which deals with low-frequency conductance, no mention is made of the correction which can be made for dilution effects in conductance titrations. The second chapter, on high-frequency titrimetry, fails to discuss adequately the morphology of the titration curves and the use of "anode current" for plate current in a vacuum tube might be confused with cell current. Also the use of c/s instead of Hz seems unfortunate. All in all, however, these two chapters are quite good.

In chapter three, on potentiometry, the commonly used convention of writing cells, so that the right-hand electrode is positive, is employed. In the experience of the reviewer, this is an unfortunate practice because the beginning student would like to write down the symbol of the cell *before* he deduces which electrode is positive. On page 44 this is extended to include oxidation at the left-hand electrode and reduction at the right-hand electrode, all of which is correct only so long as the cell operates spontaneously but not if it is operated as an electrolysis cell. Of more serious consequence is the labeling of electrode potentials as "Standard Oxidation Potentials." The potentials listed are in agreement with the IUPAC convention, and in the United States would be referred to as reduction emf values. The use of symbols "E" and "π" for cell voltage and electrode potential, respectively, seems good, but unfortunately is not followed in the next two chapters. On page 55 the reader is likely to conclude that hydrogen ions actually pass through the glass blub of a glass electrode. On page 57 it is stated that the ratio of activities of iron(III) to iron(II) remains constant after the equivalence point in the permanganate titration of iron!

The remainder of the book suffers primarily from the absence of a chapter introducing the reader to the concepts involved in electrolysis. This chapter should cover, at a very introductory level, such diverse ideas as exchange current, heterogeneous rate constant, reversibility, back emf, concentration polarization, diffusion processes, and electrode double layer. Without such a background, much of the material in the second half of the volume is unintelligible to the beginner.

Chapter four, on voltammetry, contains an overemphasis on chronopotentiometry and essentially nothing of the important techniques of AC, square wave, and pulse polarography. No mention is made of modern electronic circuits and the importance of three electrode cells for IR compensation. In Fig. 4.8 the X amplifier of the oscilloscope is not properly connected.

Chapter five, on classical polarography, has several serious errors. The sweep rate of 1 v/min, suggested on page 92, does not seem to be a typographical error since it is repeated on page 97. The authors seem to confuse the RC damping of drop oscillations with compensation for the double-layer charging current (pp. 96–97). The claim of $\pm 5\%$ accuracy at a contentration of 10^{-7} M is especially amazing! The reviewer is happy with that accuracy at 10^{-5} M and most authors set the detection limits between 10^{-5} and 10^{-6} M! The example, on page 100, of the effect of complexation could hardly have been more atypical. The last para-

graph of page 103 makes sense only if one is restricted to the two electrode cells used in this chapter. No mention is made of the problem of analyzing for a low concentration of a metal ion in the presence of a higher concentration of a more readily reducible ion. Finally, the discussion on instrumentation is hopelessly out of date.

Chapter six, on coulometry is adequate, except that the discussion on coulometers again ignores modern electronic instrumentation. The term "standard potential of the amalgam electrode" (p. 110) seems rather unusual. The discussion of the mercury cathode is not in this chapter, but is in the following chapter on electrogravimetry. The reviewer would like to have seen a more complete discussion of biamperometric techniques (pp. 115–116) included with amperometric titrations in chapter five.

Chapter seven contains an especially poor discussion of electrogravimetry. The first example (p. 121) has several errors. The decrease in copper ion concentration leads to an *increase* in back emf (as is indicated on the next page) and the oxygen pressure will not be in equilibrium with the atmosphere, but will be much closer to 1. The voltmeter referred to is missing from Fig. 7.1a, and the necessity for and use of two controls in Fig. 7.1 is unexplained. Changing the setting of R (Fig. 7.1b) does not adjust the potentiometer as stated, but instead changes the electrode potential which the potentiometer circuit measures. The description of internal electrolysis is simply incredible! The discussion of the separation of copper and lead is nonsense, since lead will go to the anode and be deposited as PbO_2 under these conditions. This discussion is also obscured by lack of distinction between cell voltage (either back emf or applied) and electrode potential. The discussion of the separation of copper and bismuth is also seriously in error.

PETER E. STURROCK, *School of Chemistry, Georgia Institute of Technology, Atlanta, Georgia 30332*

Progress in Thin Layer Chromatography and Related Methods. Vol. 1. Edited by A. NIEDERWIESER AND G. PATAKI. Ann Arbor-Humphrey Sci. Publ. Ann Arbor, Mich., 1970. xv + 224 pp. $15.00.

The book under review is the first volume in a series of monographs written by a group of international specialists devoted entirely to recent advances in thin layer chromatography (TLC). Because different chromatographic techniques are used simultaneously as well as in connection with many other physiochemical methods, the editors also decided to include the combination of TLC with other analytical techniques such as radioactive techniques, gas chromatography, and fluorometric analysis, a most welcome development, indeed. The rapidly growing output of papers dealing with TLC has caused biologists, pharmacologists, and chemists seeking information in particular fields to resort increasingly to authoritative reviews such as these where the main trends of development are summarized by experts. The present volume contains seven reviews covering the literature thoroughly through 1967 with an incomplete literature survey of 1968, and but a handful of 1969 listed references. Pertinent material is correlated under select headings, an arrangement useful for those seeking methods to solve special problems.

The scope of the book is considerable, although it is not, and does not claim to be comprehensive. The first two chapters deal with the general problems of the

R_M-function and its use in structural analysis, and of thin layer radiochromatography. The other five chapters describe specialized subjects such as the separation of lipids by argentation-TLC, TLC of DANS-amines, TLC of iodoamino acids, the use of TLC in structural elucidation of glycoproteins, and the separation of oligonucleotides. Each review begins with a well written introduction to that particular TLC field and ends with a summary of the state of the art today along with a complete list of references. It provides the latest information on principles, methods, and applications of TLC for the researcher with discussions that relate physical concepts to practical applications. Recent advances in the field through 1968 are reviewed, assessed, and critically evaluated.

The book is stoutly bound and well written. The print is clear and mistakes are few. In view of its moderate price and a valuable review of the recent literature, this first excellent volume is recommended to scientific libraries and to analysts engaged in specific separation problems. It should also be helpful as an aid to chemists, biochemists, biologists, clinical chemists, and medical scientists in keeping step with the techniques of TLC, and it is hoped that it will help in the practical performance of their work.

GEORGE WIENER, *Pfizer Inc., Brooklyn, New York 11206*

Announcement

EASTERN ANALYTICAL SYMPOSIUM
Statler Hilton Hotel, New York City
November 18–20, 1970

Program

Wednesday, November 18; 9:00–12:00 a.m.

ATOMIC ABSORPTION AND EMISSION SPECTROMETRY—NEW HORIZONS

T. C. RAINS, Chairman

1. "Nonflame Methods for Atomic Absorption Spectrometry." R. Woodriff, Montana State University, Bozeman, Montana.
2. "Flames for Special Applications." A. Hell and J. Ramírez-Muñoz, Beckman Instruments, Inc., Fullerton, California.
3. "Modulation Techniques in Flame Spectrometry." V. G. Mossotti, University of Illinois, Urbana, Illinois.
4. "Critical Evaluation of Resonance Lines for Atomic Absorption Spectrometry." T. C. Rains, T. A. Rush, and O. Menis, National Bureau of Standards, Washington, D.C.

APPLICATIONS OF ANALYTICAL TECHNIQUES IN WATER POLLUTION RESEARCH

L. L. CIACCIO, Chairman

1. "Sampling and Analysis of Hudson River Water." T. J. Kneip, N.Y.U. Institute of Environmental Medicine, Sterling Forest, New York.
2. "The Monitoring of Pesticides and Lead in Lake Michigan." H. Mancy, University of Michigan, Ann Arbor, Michigan.
3. "Use of Automated Analytical Techniques in the Evaluation of a Model Stream." R. Cardenas and A. Molof, New York University, The Bronx, New York.
4. "The Analysis of Secondary Waste Effluents." L. L. Ciaccio, General Telephone and Electronics Labs, Bayside, New York.

THE ROLE OF PRECISION AND ACCURACY IN MODERN ANALYTICAL RESEARCH

J. E. OBERHOLTZER, Chairman
Arthur D. Little, Inc., Cambridge, Massachusetts

1. "Instrumentation for Improved Resolution, Accuracy, and Precision in Chemical Analysis." J. W. Amy, Purdue University, Lafayette, Indiana.
2. "The Significance of Precision—an Industrial Viewpoint." E. P. Przybylowicz, Eastman-Kodak Company, Rochester, New York.
3. "Precision and Accuracy in the Clinical Laboratory." D. Seligson, School of Medicine, Yale University, New Haven, Connecticut.
4. "Analyzing the Air—How Specific, Accurate, and Precise Should We Be?" R. G. Smith, University of Michigan, Ann Arbor, Michigan.

QUANTITATIVE X-RAY SPECTROGRAPHY—COMPARISON OF EMPIRICAL REGESSION AND FUNDAMENTAL PARAMETER METHODS

W. C. CAMPBELL, Chairman
U.S. Bureau of Mines, College Park, Maryland

1. "Empirical Methods." H. J. Rose and N. F. Cuttitta, U.S. Geological Survey, Washington, D.C.
2. "Regression Methods." S. D. Rasberry, National Bureau of Standards, Washington, D.C.
3. "Fundamental Parameter Methods." L. S. Birks and J. W. Criss, U.S. Naval Research Laboratory, Washington, D.C.
4. Panel Discussion, Chairman and Speakers.

DESK-TOP COMPUTER WORKSHOP

R. MICHAEL CROCCO, Chairman
Ortho Pharmaceutical Corp., Raritan, New Jersey

Wednesday, November 18; 2:00–5:00 p.m.

ADVANCES IN PERMEATION CHROMATOGRAPHY

J. N. LITTLE, Chairman
Waters Associates Inc., Framingham, Massachusetts

1. "The Mechanism of the Separation in Permeation Chromatography." E. F. Casassa, Mellon Institute, Pittsburgh, Pennsylvania.
2. "Data Handling in Permeation Chromatography." J. Cazes, Mobil Oil Research Center, Paulsboro, New Jersey.
3. "The Influence of Permeation Chromatography on Polymer Science." D. Bly, DuPont Co., Wilmington, Delaware.
4. "Rigid Glass Substrates for Permeation Chromatography." W. Haller, National Bureau of Standards, Washington, D.C.

DIFFRACTION GRATINGS

B. SHERMAN, Chairman

1. "Historical Background." B. Sherman, General Telephone & Electronics Labs Inc., Bayside, New York.
2. "The Largest Diffraction Gratings." G. W. Harrison, Massachusetts Inst. of Technology, Cambridge, Massachusetts.
3. "Gratings for Raman Spectroscopy." D. O. Landon, Spex Industries, Inc., Metuchen, New Jersey.
4. "Characteristics of Gratings in the Ultraviolet." J. Ferris, Jarrell-Ash Div., Fisher Scientific Co., Cambridge, Massachusetts.
5. "Holographic Gratings." P. Foote, Angenieux Corp. of America, Oceanside, New York.

COULOMETRY

A. J. BARD, Chairman

University of Texas, Austin, Texas

1. "Coulomb—The Absolute Chemical Standard." G. Marinenko, National Bureau of Standards, Washington, D.C.
2. "Galvanic Coulometry." P. A. Hersch, Gould National Batteries, Minneapolis, Minnesota.
3. "Coulometry in Organometallic Chemistry." M. D. Morris, University of Michigan, Ann Arbor, Michigan.
4. "Determination of S, N, and Cl in Petroleum Fraction and GLC Effluents by Microcoulometry." H. V. Drushel, Esso Research Labs, Baton Rouge, Louisiana.

GENERAL PAPERS

D. L. NASH, Chairman

Bell Telephone Labs, Murray Hill, New Jersey

1. "Measurement of Smoke Density by TGA/Photometric Analysis." A. A. Loehr and P. F. Levy, DuPont Co., Wilmington, Delaware.
2. "High-Resolution Mass Spectrometric Investigation of Treated Solid Waste and Airborne Particulates." J. L. Shultz, R. A. Friedel, and A. G. Sharkey, Jr., Bureau of Mines, Pittsburgh, Pennsylvania.
3. "Transmission and ATR Infrared Spectroscopy in the Analysis of Normal and Diseased Tissues, and Identification of Certain Biochemical Compounds." F. S. Parker, New York Medical College, New York.
4. "Optical Activity of Cu(II) Complexes of Dipeptides with Aliphatic Side Chains and a Procedure for the Analysis of Mixtures of Di-

peptides Differing in Location of Asymmetric Center." B. Verma and Y. P. Myer, SUNY at Albany, Albany, New York.
5. "Identification of Compounds Including Polymers Using Molecular Weight Chromatography." D. G. Paul and C. E. Bennet, Chemalytics Corporation, Unionville, Pennsylvania.
6. "Pulse Polarographic Determination of Trace Elements in Alkali Salt Solutions." R. G. Greene and R. H. Lansing, Eastman Kodak Co., Rochester, New York.

DESK-TOP COMPUTER WORKSHOP

R. MICHAEL CROCCO, Chairman
Ortho Pharmaceutical Corp., Raritan, New Jersey

Thursday, November 19; 9:00–12:00 a.m.

PLENARY SESSION

B. L. KARGER, Chairman
Northeastern University, Boston, Massachusetts

1. "Some Topics of Recent Interest in Analytical Atomic Spectroscopy." P. W. J. M. Boumans, Philips Research Labs., Eindhoven, Netherlands.
2. "Two-Phase Reactions in Analytical Chemistry." R. Belcher, The University of Birmingham, Birmingham, England.
3. "New Ion-Selective Electrodes." W. Simon, Eidgenossenschaft Technische Hochschule, Zurich, Switzerland.

RADIOACTIVE ISOTOPES IN ANALYTICAL CHEMISTRY

H. H. Ross, Chairman

1. "Nuclear and Radiochemical Methods in Environmental Analysis." W. S. Lyon, Oak Ridge National Laboratory, Oak Ridge, Tennessee.
2. "Radioactive Kryptonates: A Versatile Analytical Tracer." Philip Goodman, Panametrics Inc., Waltham, Massachusetts.
3. "New Tracer Techniques in Clinical Analysis." William G. Myers, College of Medicine, Ohio State Univ., Columbus, Ohio.
4. "Analytical Applications of the Secondary Effects of Radiation." H. H. Ross, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

LIQUID–LIQUID CHROMATOGRAPHY WORKSHOP

P. W. ALMQUIST, Chairman
Waters Associates, Inc., Framingham, Masschusetts

Thursday, November 19; 2:00–5:00 p.m.

SPECTROSCOPY IN FORENSIC SCIENCE

J. M. ENGLISH, Chairman
Georgetown University Law and Medical Centers, Washington, D.C.

1. "X-Ray Milliprobe Applications in Forensic Science." K. Rayburn, University of Maryland, College Park, Maryland.
2. "Applications of Spectroscopic Techniques to the Identification of Inks." C. F. Hammer, Georgetown University, Washington, D.C.
3. "Problems in Analysis of Oil Paintings." C. H. Olin, Smithsonian Institution, Washington, D.C.
4. "Remote Optical Detection of Trace Materials." H. Tannenbaum, Edgewood Arsenal, Maryland.

REVIEW OF APOLLO 11 AND 12 LUNAR SAMPLES

I. ADLER, Chairman

1. "Selected Trace Element Distributions in Lunar Samples." C. C. Schnetzler, NASA Goddard Space Flight Center, Greenbelt, Maryland.
2. "Mineralogy of Lunar Samples." L. S. Walter, NASA Goddard Space Flight Center, Greenbelt, Maryland.
3. "Lunar Sample Chemical Studies." I. Adler, NASA Goddard Space Flight Center, Greenbelt, Maryland.
4. "Geochemical Evidence for the Origin of the Moon." J. A. O'Keefe, NASA Goddard Space Flight Center, Greenbelt, Maryland.

COMPUTER APPLICATIONS IN KINETIC ANALYSIS

H. L. PARDUE, Chairman

1. "Computer Processing of Kinetic Data from Parallel Chemistry Rotors." N. G. Anderson, Molecular Anatomy Program, Oak Ridge National Laboratory, Oak Ridge, Tennessee.
2. "Instrumentation for Computer-Controlled Kinetic Studies." S. N. Deming, Emory University, Atlanta, Georgia.
3. "Application of the LINC Computer to Enzyme Kinetic Studies." G. P. Hicks, University of Wisconsin Medical Center, Madison, Wisconsin.
4. "On-Line Computer-Processing of Stopped-Flow Kinetic Data." H. L. Pardue, Purdue University, Lafayette, Indiana.

GENERAL PAPERS

P. CUKOR, Chairman

1. "Structural Analysis of Organic and Inorganic Materials by Electron Spectroscopy (ESCA)." W. A. Wolstenholme, B. N. Green, M. Barber, and P. Swift, AEI Scientific Apparatus Ltd., Manchester, England.
2. "Mercury Pollution Monitoring by Atomic Absorption Utilizing a Gas Cell Technique." A. L. Malenfant, S. B. Smith, and J. Y. Hwang, Instrumentation Laboratory Inc., Lexington, Massachusetts.
3. "Nonflame Atomization in Atomic Absorption and Fluorescence Spectroscopy." P. W. Y. Lung and J. P. Matousek, Varian Pty., Ltd., Victoria, Australia, and D. P. Sandoz, Varian Techtron, Walnut Creek, California.
4. "The Determination of Boron, Phosphorus, and Sulfur by Molecular Flame Emission Spectroscopy." J. D. Kerber and F. J. Fernandez, The Perkin-Elmer Corporation, Norwalk, Connecticut.
5. "State of the Art in the Microcoulometric Determination of Total Bound Nitrogen, Sulfur, and Halogen: 0.02 ppm to 1%." R. J. Joyce and R. T. Moore, Dohrmann Instruments Company, Mountain View, California.
6. "Thin-Film Standards Prepared by Pyrolysis of Metallo Organic Compounds." D. Oblas, P. Lublin, P. Cukor, H. Hoda, and W. Shelby, General Telephone and Electronics Labs., Bayside, New York.

LIQUID–LIQUID CHROMATOGRAPHY WORKSHOP

P. W. ALMQUIST, Chairman

Friday, November 20; 9:00–12:00 a.m.

ANALYTICAL USES AND FUNDAMENTALS OF INORGANIC FLUORESCENCE AND PHOSPHORESCENCE

G. SCHENK, Chairman

1. "Metal Chelates and the Determination of Inorganic Iions." C. E. White, University of Maryland, College Park, Maryland.
2. "Charge Transfer Luminescence of Some Ruthenium(II) and Iridium(III) Chelates." E. Ohnesorge, Lehigh University, Bethlehem, Pennsylvania.
3. "Luminescence Studies of Group VIII Polypyridine Chelates." F. E. Lytle, Purdue University, Lafayette, Indiana.

4. "Fluorescence Quenching and Phosphorescence in Trace Analysis." G. Schenk, Wayne State University, Detroit, Michigan.

ARTIFICIAL INTELLIGENCE APPLIED TO CHEMISTRY

T. L. ISENHOUR, Chairman

1. "On the Design and Evaluation of (Semi-)Empirical Processors for Computerized Interpretation of Spectral-Type Data." J. W. Ashley, Northern Illinois University, DeKalb, Illinois.
2. "Techniques for Improving the Predictive Ability and Reliability of Computerized Learning Machines." P. C. Jurs, Pennsylvania State University, University Park, Pennsylvania.
3. "On a Self-Evolving, Multicategory, Pattern Classifier." N. M. Frew, L. E. Wangen, and T. L. Isenhour, University of North Carolina, Chapel Hill, North Carolina.
4. Paper to be announced.

RECENT INSTRUMENTAL APPLICATIONS IN CLINICAL CHEMISTRY

J. S. ANNINO, Chairman

1. "A Spectrophotometric Critique in Automated Analysis." B. Zak, Wayne State University School of Medicine, Detroit, Michigan.
2. "Enzyme and Substrate Electrochemical Probes." G. G. Guilbalt, Louisiana State University, New Orleans, Louisiana.
3. "The Fast Analyzer: A New Approach to Automated Chemical Testing." N. G. Anderson, Molecular Anatomy Program, Oak Ridge National Laboratory, Oak Ridge, Tennessee.
4. "The Future of Automated Analysis in Clinical Chemistry." Panel Discussion, J. S. Annino, N. G. Anderson, G. G. Guilbalt, B. Zak.

Friday, November 20; 2:00–5:00 p.m.

ORGANIC ELECTROCHEMISTRY

A. J. DIEFENDERFER, Chairman

1. "Electrochemical Oxidations of Aromatic Hydrocarbons." D. A. Aikens, Rensselaer Polytechnic Institute, Troy, New York.
2. "Electrochemistry of Carbonyl Compounds." P. Zuman, Clarkson College of Technology, Potsdam, New York.
3. "Prospects for Organic Synthesis through Electrochemistry." N. Weinberg, American Cyanamid Co., Stamford, Connecticut.

4. "Electrochemistry of Organic Halogen Compounds." A. J. Diefenderfer, Lehigh University, Bethlehem, Pennsylvania.

ADVANCES IN AIR POLLUTANT ANALYSIS

R. K. STEVENS, Chairman

1. "Carbonate and Noncarbonate Carbon in Atmospheric Particles." P. K. Mueller, R. W. Mosley and L. B. Pierce, Air Pollution Industrial Hygiene Laboratory, Department of Public Health, Berkeley, California.
2. "Use of Chemiluminescence for Detection of Ozone and Excited Oxygen." J. A. Hodgeson and K. J. Krost, National Air Pollution Control Administration, Raleigh, North Carolina.
3. "Rotational Microwave Spectroscopy and Air Pollution Measurements." H. W. Harrington, Hewlett-Packard, Palo Alto, California.
4. "Measurement of Hydrogen Sulfide, Sulfur Dioxide, and Methyl Mercaptan in Ambient Air by Gas Chromatography." A. E. O'Keefe, R. K. Stevens, and J. D. Mulik, National Air Pollution Control Administration, Cincinnati, Ohio.

CHEMICAL STRUCTURE BY MOLECULAR SPECTROSCOPY

J. P. LUONGO, Chairman

1. "Raman Spectra of Graphite Fibers." F. Tuinstra and J. L. Koenig, Case Western Reserve Univ., Cleveland, Ohio.
2. "Orientation in Nylon-6 Films as Determined by the Three-Dimensional Polarized Light Technique." J. P. Sibilia, Allied Chemical Corporation, Morristown, New Jersey.
3. "Studies of the Low Temperature Spectra of Ethylene–Propylene Co-polymers." J. J. Elliott, Esso Research and Engineering Company, Linden, New Jersey.
4. "Metal Isotope and High Pressure Effects on Low Frequency Vibrations of Coordination Compounds." J. R. Ferraro, Argonne National Laboratory, Argonne, Illinois.

GENERAL PAPERS

A. Z. CONNER, Chairman
Hercules Inc., Wilmington, Delaware

1. "Solution Thermodynamics of Geometrically Isomeric Olefins by Gas–Liquid Chromatography." R. L. Stern and T. R. Faulkner, Oakland University, Rochester, Michigan.

2. "The Role of the Solvent in Ion Exchange." G. E. Janauer, State Univ. of New York at Binghamton, Binghamton, New York.
3. "Quantitative Aspects of Spectrodensitometry of Thin-Layer Chromatograms." J. C. Touchstone, S. S. Levin, and T. Murawec, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.
4. Analysis of Mixtures of Functional Group-Containing Organic Compounds." S. F. Sarner and E. J. Levy, Chemical Data Systems, Oxford, Pennsylvania.
5. "Determination of α-Hydroxy Fatty Acids and α-Glyceryl Ethers as their Cyclic Boronate Derivatives." S. Ramachandran, R. F. Kruppa, and R. S. Henley, Applied Science Laboratories, Inc., State College, Pennsylvania.
6. "Vapor Programming Techniques for Thin-Layer Chromatography." H. S. Hirsch, Brinkmann Instruments Inc., Westbury, New York.

Erratum

Vol. 15, No. 2 (1970), in the article "Solvent Effects in Photometric Analysis," by E. Sawicki, T. W. Winfield, and C. R. Sawicki, pp. 294–363:

Page 347, the second column under structure III consists of λ max obtained in water; the third column λ max obtained in pyridine.

Pages 348 and 349, structures V and VI should be interchanged; i.e., the structure now labeled V is structure VI and vice versa.

The Elucidation of Organic Electrode Processes

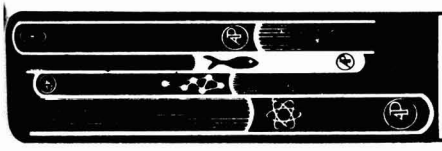
A Current Chemical Concepts Monograph of the Polytechnic Institute of Brooklyn

By **P. Zuman**
Department of Chemistry
University of Birmingham
England

This book deals primarily with techniques used in the elucidation of polarographic current-voltage curves of organic compounds. The approach is unique in that the elucidation of electrode processes is discussed from the point of view of experimental results. Empirical considerations determine the selection of the most plausible mechanism for an understanding of the nature and implications of the processes and possibilities involved in polarographic analysis with separate discussion of systems manifested by one, two, and three or more waves. The use of controlled potential electrolysis and the nature of structural changes on polarographic curves under conditions of prolonged electrolysis are discussed in some detail.

1969, 184 pp., $9.00

AP 2597



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

Cell Separation:
METHODS IN HEMATOLOGY

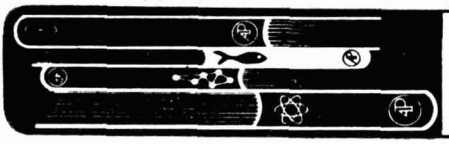
By **J. Harry Cutts**
Department of Anatomy
School of Medicine
University of Missouri, Columbia, Missouri

This book describes the various methods of cell separation that have been applied to the isolation of cell types from blood and hemopoietic organs, and discusses their principle and relative usefulness. Following an introductory chapter in which are outlined some of the initial considerations of cell separation including the choice of anticoagulant, the effects of temperature, treatment of glassware and equipment, each succeeding chapter is devoted to a specific method of cell separation. The principles on which each method is based are discussed, following which the various modifications used by different workers are presented. Pertinent data taken from the literature are used to illustrate the relative efficiencies of the methods presented, and details of special equipment are illustrated.

This volume will be of interest to microbiologists, cell biologists, pathologists, and comparative pathologists as well as a valuable addition to the libraries of research institutions and transfusion and pathological services in hospitals.

1970, 228 pp., $12.50

AP 2554



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