Volume 16, Number 1, March 1971

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

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

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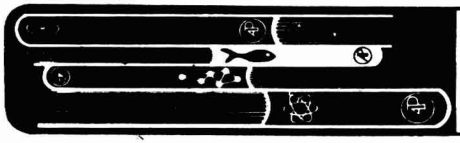

Academic Press
NEW YORK AND LONDON
111 FIFTH AVENUE, NEW YORK, N.Y. 10003
BERKELEY SQUARE HOUSE, LONDON W.1

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Published quarterly at Exchange Place, Hanover, Pa. 17331
by Academic Press, Inc.,
111 Fifth Avenue, New York, New York 10003
In 1971, Volume 16 (4 issues) 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. 16, No. 1

Briefs

Spot Test of Carboxylic Acids. L. LÉGRÁDI, *Nitrochemical Industrial Plants, Füzfögyártelep, Hungary.*

A new spot test is proposed for the detection of carboxylic acids, on the basis of the reaction with thionyl chloride and then with *o*-nitrophenylhydrazine and alkali. Dicarboxylic acids, α-hydroxy acids, amino acids, di- and trichloroacetic acids are not detectable with this method.

Microchem. J. **16,** 1 (1971).

Thermal Properties of Ethambutol (Myambutol). The Detection of the *meso* Isomer in the Presence of Ethambutol by Differential Thermal Analysis. HAL FERRARI, *American Cyanamid Company, Lederle Laboratories Division, Pearl River, New York 10965;* AND D. G. GRABAR, *American Cyanamid Company, Central Research Laboratories, Stamford, Connecticut 06904.*

Thermal analysis was used to detect small concentrations of the *meso* isomer in the presence of ethambutol by the possible mechanism of solid solution formation. Ethambutol exists in at least three polymorphic modifications and the *meso* isomer exists in at least two polymorphic modifications.

Microchem. J. **16,** 5 (1971).

Determination of Copper, Iron, and Zinc from a Single Small Sample. RUTH WATKINS, LAWRENCE M. WEINER, AND BENNIE ZAK, *Departments of Biochemistry Pathology and Microbiology, Wayne State University School of Medicine and Detroit General Hospital, Detroit, Michigan 48207.*

Procedures are described for the sequential determination of copper and iron in one aliquot of a trichloroacetic acid–ascorbic acid filtrate followed by the determination of zinc in a second aliquot. Copper, iron, and zinc can also be determined individually on three aliquots of the same solution. All reactions are selective for the three metals under the described conditions.

Microchem. J. **16,** 14 (1971).

Detection of Amines by Using the Depolymerization of Poly(chloroaldehydes). THADDEUS J. NOVAK AND EDWARD J. POZIOMEK, *Physical Research Laboratory, Edgewood Arsenal, Maryland 21010.*

A detector tube is described in which amine vapors cause decomposition of a chloral–dichloroacetaldehyde copolymer. Detection signal is based on the dissolution of the dye pinacyanole by the liberated chloroacetaldehyde monomers. The limit of detection of diethylamine is approximately 2 μg.

Microchem. J. **16,** 24 (1971).

The Analysis of Tensides by Automatic Radio-Frequency Titrations. W. J. Scott and G. Svehla, *Department of Chemistry, Queen's University, Belfast, Northern Ireland.*

The advantages of radio-frequency end-point detection in titrimetric analysis are discussed and the design of an automatic titrimeter, equipped with variable frequency and employing the heterodyne-beat principle, is outlined. Titration of cationic tensides with silver nitrate, of sulfated anionic tensides with barium acetate or benzidine hydrochloride, and of the alkali metal ion in tensides with lithium chloride in nonaqueous solution are accurate, precise, and simple analytical procedures.

Microchem. J. **16,** 37 (1971).

A Modified "Sandwich" Microapparatus for Horizontal Thin-Layer Chromatography. Anthony V. DeThomas, Carolyn R. DeThomas, Robert Lazar, and Donald Verrastro, *Plainville High School, Department of Science, between School, and Plainville, Plainville, Connecticut 06062.*

A modification of the so-called continuous horizontal development technique using a "sandwich" microapparatus is described.

Microchem. J. **16,** 52 (1971).

The Introduction of Microtechniques in Elementary Science Courses. I. Blood Glucose Determination. Alice Laughlin and Catherine O'Neill, *Division of Science, Jersey City State College, Jersey City, New Jersey 07305.*

The introduction of microtechniques in the teaching of elementary science courses is described. Beginning students have been found to learn small scale operations equally as well as those normally taught students at this level. The determination of blood glucose is described in this first of the series on the subject.

Microchem. J. **16,** 57 (1971).

Microprocedure for Serum Triglyceride Estimation. Peter Haux and Samuel Natelson, *Department of Biochemistry, Michael Reese Hospital and Medical Center, Chicago, Illinois 60616.*

A microprocedure is described for estimating the triglycerides in blood serum. One hundred microliters are used per determination. Protein and phospholipids are removed before hydrolysis with potassium and barium hydroxides. Barium hydroxide is used so that on eventual acidification, the fatty acids coprecipitate with barium sulfate. The glycerol formed is determined by oxidation to formaldehyde and reaction with chromotropic acid.

Microchem. J. **16,** 68 (1971).

Sources of Error in the Mercury(I) Titration of Low Concentrations of Hexacyanoferrate(III) in Alkaline Iodide Medium. John T. Stock and Robert J. Merrer, *Department of Chemistry, University of Connecticut, Storrs, Connecticut 06268.*

Although submillimolar concentrations of hexacyanoferrate (III) in acid thiocyanate medium can be accurately titrated amperometrically with mercury (I) perchlorate, the titration is unsatisfactory when carried out in alkaline iodide medium. The decay of hexacyanoferrate(III) current in alkaline iodide medium and the effect of cyanide ion on the titration have been examined. The results provide an explanation for the titration errors.

Microchem. J. **16,** 77 (1971).

Determination of Silver with the Thiosemicarbazone of picoline-2-aldehyde. D. J. LEGGETT AND B. W. BUDESINSKY, *Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada.*

Picoline-2-aldehyde thiosemicabazone forms a stable complex with silver in the pH range 5.0 to 6.0. The complex has the metal:ligand ratio of 1:1. Complexation with silver at pH 5.5 in the presence of EDTA allows a selective determination to be performed.

Microchem. J. **16,** 87 (1971).

Identification of Several Ions by Nonaqueous Solutions of Tin (II) Chloride. FRANCISCO BERMEJO-MARTINEZ AND MARIA DEL PILAR SOUZA-CASTELO, *Department of Analytical Chemistry, Faculty of Sciences and Analytical Chemistry Section of High Council Scientific Research, University of Santiago de Compostela, Santiago de Compostela, Spain.*

Nonaqueous solutions of tin(II) chloride are used for spot tests of mercury(II), molybdenum(VI), bismuth(III), tellurium(IV), tungsten(VI), and arsenic(III).

Microchem. J. **16,** 94 (1971).

A Cooperative Study of the Determination of Lead in Milk. MANUEL BRANDT AND JEROME M. BENTZ, *Ethyl Corporation, Ferndale, Michigan 48220.*

Tests made by eight laboratories suggest that previously reported data on the lead content of market whole milk are too high by an order of magnitude or more.

Microchem. J. **16,** 113 (1971).

The Preparation and Properties of the Acetate Complex of Trivalent Cobalt. J. BUDĚŠÍNSKÝ, J. DOLEŽAL, B. ŠRÁMKOVÁ, AND J. ZÝKA, *Department of Analytical Chemistry, Charles University, Prague, Czechoslovakia.*

A reasonably stable solution of the cobalt(III) acetate complex in glacial acetic acid has been prepared by the anodic oxidation of the corresponding cobalt(II) acetate solutions at a platinum electrode in a closed system. The current efficiency was in the range of 60 to 70% and the best reproducibility was obtained using a porous glass membrane.

Microchem. J. **16,** 121 (1971).

Research in the Olfactory Detection of Chemical Agents. E. J. POZIOMEK, G. L. BOSHART, E. V. CRABTREE, R. DEHN, J. M. GREEN, D. J. HOY, R. A. MACKAY, G. T. PRYOR, H. STONE, AND M. TANABE, *Physical Research Laboratory, Edgewood Arsenal, Maryland 21010; and Stanford Research Institute, Menlo Park, California 94025.*

Research on the olfactory detection of various electrophiles including toxic organophosphorus compounds is described. An odoriferous alkyl isocyanide is formed from the reaction of the electrophile with *N*-alkylformamides.

Microchem. J. **16,** 136 (1971).

Physicochemical Measuring Methods for Endpoint Determination in Organic Elemental Microanalysis. F. SALZER, *Untersuchungsabteilung, Chemische Werke Hüls A.G., 437 Marl, Kreis Recklinghausen, West Germany.*

In the last 10 years a series of physicochemical methods of measuring have been developed which could replace the classical weighing of combustion products in microelemental analysis. These methods have been used to automate the various determinations; and these principles, and the commercial units using them for microanalysis, are discussed.

Microchem. J. **16,** 145 (1971).

MICROCHEMICAL JOURNAL 16, 1–4 (1971)

Spot Test of Carboxylic Acids

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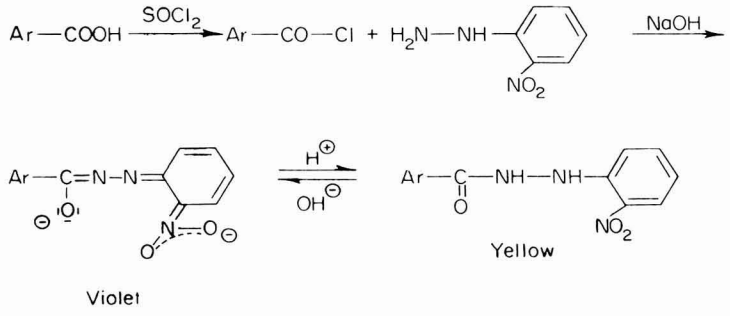
Received February 28, 1970

Carboxylic acids and its derivatives which are convertible into hydroxamic acids can be detected by the ferric hydroxamate test (*1*). In this paper a new method is described for the detection of carboxylic acids. An acid chloride must be formed first by the action of thionyl chloride, as in the case of the hydroxamate test, then it gives an acid–base indicator on treatment with *o*-nitrophenylhydrazine and alkali. The acid color of this indicator is yellow, the alkaline color, violet or blue. The violet or blue color in alkaline medium is due to the nitronic acid structure and the keto–enol tautomerism:



o-Nitrophenylhydrazine is not an acid–base indicator, so it does not change color in the presence of alkali. *p*-Nitrophenylhydrazine and 2,4-dinitrophenylhydrazine are not usable in place of the *o*-derivative for detection of carboxylic acids, as these compounds are indicator acids changing color in alkaline medium also without the reaction of acid chloride.

If the carboxylic acid is not convertible into acid chloride by the action of thionyl chloride, it cannot be detected by this test. But this restriction is valid just so for the ferric hydroxamate test, too. Aliphatic dicarboxylic acids, HOOC-$(CH_2)_n$–COOH, especially if $n>1$, give acid anhydride instead of acid chloride by the action of thionyl chloride.

Whereas *o*-nitrophenylhydrazine reacts also with acid anhydride yielding the same color as with acid chloride. α-Hydroxy acids give formic acid by the action of thionyl chloride, but formic acid cannot be detected by this test. In addition the procedure given here for the detection of carboxylic acids cannot be applied in the case of amino acids, dicarboxylic acids, e.g., oxalic acid, di-, and trichloroacetic acid. These compounds do not react with thionyl chloride. On the basis of this test monochloroacetic acid can be detected in the presence of dichloroacetic acid or glycine. A negative reaction was obtained with the alkali salts of carboxylic acids, e.g., sodium acetate. On the basis of this behavior, acetic acid can be detected in the presence of sodium acetate. Some sulfonic acids give also a positive reaction.

The advantage of the new method is its greater sensitivity in some cases e.g., citric acid over the hydroxamate test, and its selectivity. The reaction of this test occurs not only with acid chlorides but also with acid anhydrides in the presence of acetic acid, naturally without addition of thionyl chloride. On the other hand the amides, imides, and esters of carboxylic and sulfonic acids as well as peroxides do not show this reaction. In addition a positive reaction was obtained without thionyl chloride in the presence of acetic acid with some carbonyl compounds, e.g., benzoylacetone, acetylacetone, nitrobenzaldehydes, *p*-nitroacetophenone, ω-chloroacetophenone. No reaction was given by: acetone, acetaldehyde, acetophenone, *p*-dimethylaminobenzaldehyde, diacetone alcohol, benzophenone, benzaldehyde.

EXPERIMENTAL METHODS

Preparation of o-nitrophenylhydrazine

Dissolve 13.8 g (0.1 mole) of *o*-nitroaniline in a mixture of 30 ml of water and 30 ml of 37% hydrochloric acid and add 6.9 g of sodium nitrite dissolved in 40 ml of water at below 5°. Neutralize the solution with fine powdered sodium carbonate and finally with sodium hydrocarbonate at this temperature with stirring. Filter the diazonium salt solution and add it to 13.3 g (0.06 mole) of potassium pyrosulfite and 4 g of sodium hydroxide dissolved in 25 ml of water at below 10° with stirring. Then add 40 g of potassium chloride and 20 ml of 37% hydrochloric acid. After standing a night filter off the separated product and add it to 56 ml of 37% hydrochloric acid and heat on water bath for 1 hour. Cool the solution, filter off the crystalline product, dissolve it in 120 ml of hot water, filter the solution and neutralize with ammonium hydroxide, then with 22–23% of sodium acetate. Filter off the separated product and dry it. Yield about 12 g, mp, 90°.

Spot Test of Carboxylic Acids

Evaporate to dryness a drop of the aqueous or alcoholic test solution and treat it or the solid test with 1 drop of thionyl chloride, warm it in a water bath and spot on the residue 3 drops of 0.1% of alcoholic solution of *o*-nitrophenylhydrazine and 1 drop of 2*M* sodium hydroxide. The appearance of a violet color signals the presence of carboxylic acids. Limits of identification:

15 μg	benzoic acid	1 μg	citric acid
8 μg	*p*-nitrobenzoic acid	100 μg	salicylic acid
10 μg	monchloroacetic acid	8 μg	stearic acid
10 μg	acetic acid	15 μg	tartaric acid
8 μg	ethylenediaminetetraacetic acid disodium salt (EDTA)	10 μg	3,5-dinitrobenzoic acid
		10 μg	metanilic acid
15 μg	anthranilic acid	15 μg	sulfanilic acid
15 μg	nicotinic acid		

Negative responses are given by sodium acetate, glycine, *p*-hydroxyphenyl glycine, trichloroacetic acid, dichloroacetic acid, formic acid, oxalic acid, ortanilic acid and sodium benzenesulfonate.

Detection of Acetic Acid in the Presence of Sodium Acetate

Evaporate to dryness 1 drop of 10% of aqueous test solution of sodium acetate, which contains about 0.1–0.4% of acetic acid, treat it with 1 drop of thionyl chloride. Evaporate the mixture almost to dryness, add 3 drops of 0.1% of alcoholic solution of *o*-nitrophenylhydrazine, 2 drops of 2 *M* sodium hydroxide and 5 drops of ethanol. An orange color appears, which changes to red in the presence of acetic acid. A blank test is advisable.

Detection of Monochloroacetic Acid in the Presence of Glycine or Di- and Trichloroacetic Acid

(a) Evaporate to dryness 1 drop of about 7.5% of aqueous test solution of glycine, which contains monochloroacetic acid in trace amount, treat it with 1 drop of thionyl chloride and evaporate again, then add 3 drops of 0.1% alcoholic solution of *o*-nitrophenylhydrazine and 2 drops of 2 *M* sodium hydroxide. The appearance of a violet color indicates the presence of monochloroacetic acid. Near the limits of identification the orange color darkens in the presence of monochloroacetic acid. With this method 0.3% of monochloroacetic acid is detectable in the presence of glycine and 0.03% of that in 7.5% of aqueous solution of glycine applying a blank test.

(b) To 1 drop of the test dichloroacetic acid, which contains also monochloro derivative, add 1 drop of thionyl chloride, evaporate to

dryness, then treat with 3 drops of 0.1% alcoholic solution of *o*-nitrophenylhydrazine and 2 drops of 2 *M* sodium hydroxide. A positive response is indicated by a violet shade in the orange color. A blank test is necessary. Limit of identification: 0.2% of monochloro- in the presence of dichloro- or trichloroacetic acid.

SUMMARY

A new spot test is proposed for the detection of carboxylic acids, on the basis of the reaction with thionyl chloride and then with *o*-nitrophenylhydrazine and alkali. Dicarboxylic acids, α-hydroxy acids, amino acids, di- and trichloroacetic acids are not detectable with this method. A negative response is given also by the sodium salts of carboxylic acids. This test may be employed for selective detection, e.g., acetic acid can be detected in the presence of sodium acetate, or monochloroacetic acid in the presence of glycine or di- and tricloroacetic acid. Acid anhydrides and some carbonyl compounds give also a positive reaction with o-nitrophenylhydrazine in the presence of acetic acid and then with alkali, but without thionyl chloride. Amides, imides, and esters of carboxylic acids as well as peroxides do not show this reaction.

REFERENCE

1. Feigl, F., "Spot Tests in Organic Analysis," 7th ed., p. 212. Elsevier, Amsterdam/New York, 1966.

MICROCHEMICAL JOURNAL **16**, 5–13 (1971)

Thermal Properties of Ethambutol (Myambutol)

The Detection of the *meso* Isomer in the Presence of Ethambutol by Differential Thermal Analysis

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Received June 12, 1970

INTRODUCTION

This work represents a more detailed study of the thermal properties of Myambutol (*4*) than previously reported (*1*). The preliminary work included only the heating cycle of the individual isomers (*d, l, meso*). The nature of the endotherms other than the final melting endotherm had not been investigated at that time.

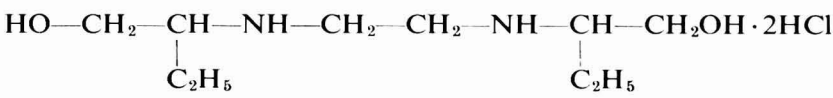
Visser and Wallace (*3*) used differential thermal analysis (DTA) to determine *ortho*-toluenesulfonamide in *para*-toluenesulfonamide by taking advantage of an eutectic system resulting from a mixture of the two isomers. Brancone and Ferrari (*1*) employed DTA to detect the presence of small amounts (1–2%) of a *cis* isomer in a *trans* isomer by using the differences in their sublimation temperatures. Ferrari [in Ref. (*2*)] employed the differences in the decomposition temperatures in air vs reduced pressure to detect one isomer in another.

This work demonstrates the interaction of two isomers by the possible mechanism of a solid–solution effect to yield an additional endotherm which is related to the *meso* isomer concentration. The values obtained on the two known synthetic mixtures are obviously not precise values but are good approximations in spite of the small peak areas involved and lack of satisfactory base lines.

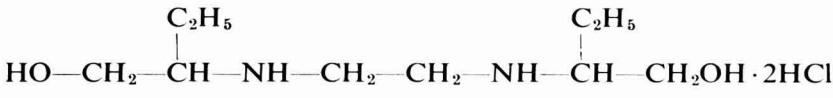
EXPERIMENTAL METHODS

Equipment. Du Pont No. 900 Differential Thermal Analyzer.

Reagents. Ethambutol—*d*-2,2′(ethylenediimino)di-1-butanol dihydrochloride:



meso-2,2′(ethylenediimino)di-1-butanol dihydrochloride:



Thermograms were run on each of the pure isomers. The cyrstals of each isomer were pulverized first, then transferred to the capillary tube and heated at a programmed rate of 10°C/minute at a ΔT of 0.5°C/inch. All the temperatures shown are adjusted for the chromel-alumel thermocouple correction.

Synthetic mixtures of the *d* and *meso* isomers were prepared to contain a concentration range of 1–4% of the *meso* form. After the isomers were weighed, they were placed in a mortar and pulverized. A 0.2-ml volume of water was added to solubilize each mixture, followed by 0.2 ml of isopropanol. The solution was transferred to a 5-ml beaker and then placed in a vacuum desiccator containing conc. H_2SO_4. The pressure was reduced by a water aspirator. The resulting crystals were dried in a vacuum desiccator over P_2O_5 for 7 hours. From these dried crystals, accurate weighings were made directly in the appropriate DTA capillary tubes. Thermograms were recorded on each mixture. A calibration curve was plotted for the concentration of *meso* isomer in ethambutol. The peak heights were used to measure the *meso* concentrations.

Two synthetic mixtures were prepared as sample knowns, as described, to fall within the limits of the calibration curve for the *meso* concentration. Thermograms were obtained on the mixtures and the *meso* concentration was obtained from the calibration curve.

Table 1 shows a comparison of the *meso* concentrations found compared with the calculated amount of *meso* added. Again, this was not meant to be a highly precise analytical method. Therefore, a large number of determinations, duplicate values, etc., were not done. It demonstrates that DTA can show the presence of the *meso* isomer and can approximate its concentration.

TABLE 1

Sample no.	*meso* (%)	
	Calc.	Found
9377B-3-1	4.05	3.8
9377B-3-2	1.75	2.0

DISCUSSION

The use of differential thermal analysis was considered because of the relative ease with which the results can be obtained and the satisfactory results previously obtained with *cis* and *trans* isomers (*2*, *4*). However, an inspection of the thermograms run on both the *d* (Fig. 1) and the *meso* isomers (Fig. 2) by thermal analysis did not seem very promising for determining small amounts of the *meso* isomer in the presence of the *d* isomer. The only suitable point for differentiation appears to be the area of 40°C endotherm of the *meso* isomer. With

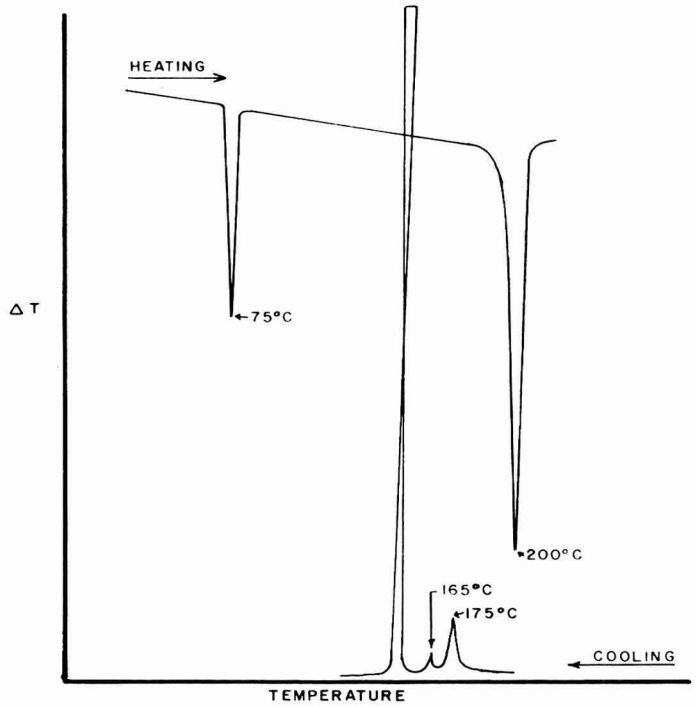


FIG. 1. Thermogram of ethambutol, heating and cooling cycle.

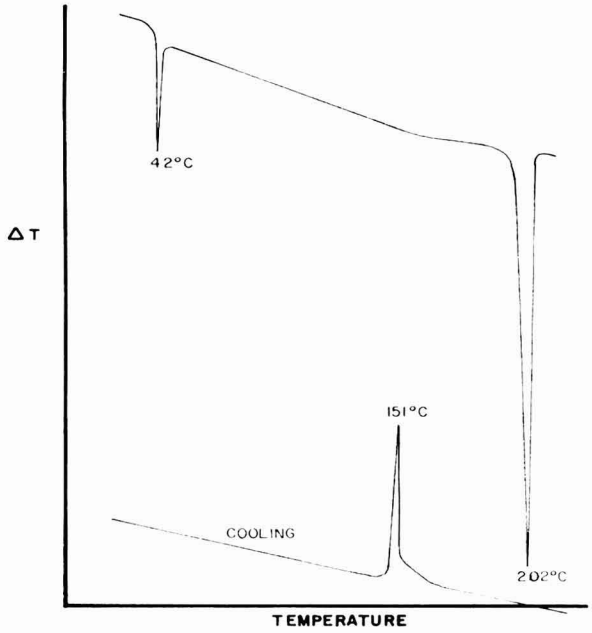


FIG. 2. Thermogram of the *meso* isomer, heating and cooling cycle.

small concentrations of the *meso* isomer, the 40°C endotherm would be barely visible at the 4% level and probably not visible at the 1% level.

The more significant finding is the presence of an additional small endotherm in the 175°C region for a prepared mixture (Fig. 3) of the isomers which was not observed in the thermograms for either the *d* or *meso* isomers. This exploratory run was made from a mixture of the isomers in the dry form—no weighed amount of samples was used—but a substantial amount of *meso* was used to magnify any possible effect from the presence of the *meso* isomer.

DTA of ethambutol. The heating of ethambutol results in two endotherms, 75 and 200°C (Fig. 1). The cooling cycle has three exotherms (Fig. 1), two very small exotherms, one at 175°C and the other at 165°C, plus a large major exotherm at 150°C. A reheat of the recrystallized material now has an additional endotherm at 44°C, the absence of the 75°C endotherm and the presence of the melting endotherm at 198°C (Fig. 4). The same two endotherms, 75 and 200°C, are observed whether the sample is run in an atmosphere of air or reduced pressure. This indicates that these endotherms are not the result of a loss of solvent. The disappearance of the 75°C endotherm in the reheat thermogram of ethambutol suggests the possibility of a polymorph.

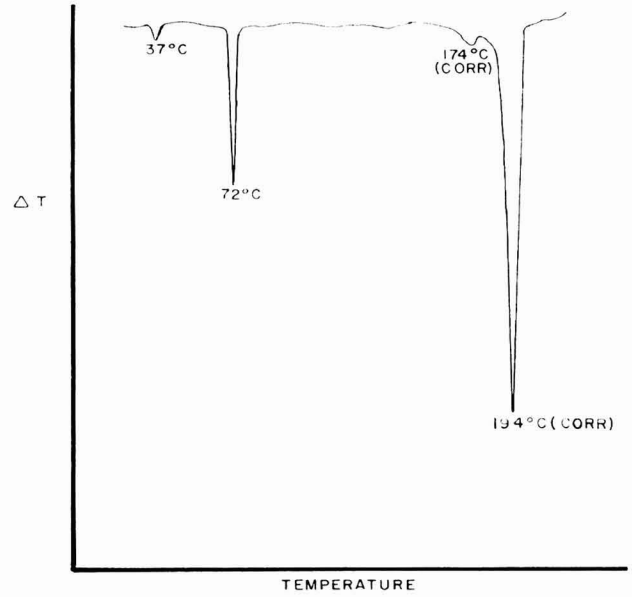


FIG. 3. Thermogram of a mixture of the dry crystals of *d* and *meso* isomers.

DTA of meso *isomer.* The heating of the *meso* isomer results in two endotherms, 42 and 200°C (Fig. 2). The thermogram of the cooling cycle has one exotherm at 151°C (Fig. 2). A reheat of the recrystallized material has the same endotherms as the original heating cycle (42°C and 200°C) (Fig. 5), except that the 42°C endotherm has broadened. The thermograms for the *meso* isomer are the same whether they are run in an atmosphere of air or reduced pressure. Again, as in the case of ethambutol, the 42°C endotherm appears to represent a polymorph.

DTA of d/meso *isomer mixtures.* The heating of a mixture of the *d* and *meso* isomers results in four endotherms; 37, 72, 174, 194°C (Fig. 3). Thermograms run under reduced pressure do not show any change in the position of the endotherms. This indicates that the endotherms are not the result of solvation. The 37°C endotherm can be associated with the *meso* isomer, the 72°C endotherm with the *d* isomer and the 194°C endotherm with the melting of the *d* or *meso* isomer and/or the *d/meso* mixture. The 174°C endotherm is unexplainable by DTA at this point. Repeated runs of the mixtures always showed the presence of the 174°C endotherm. The greater the *meso* content the greater the 174°C endotherm (Fig. 6 and 7). It seems obvious that the 174°C endotherm is some function of the *meso* isomer concentration. A plot

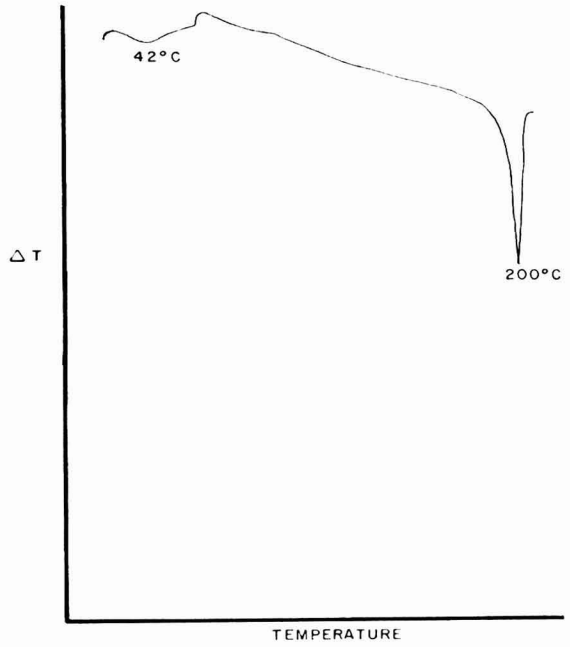


FIG. 4. Thermogram of the reheating of ethambutol.

of the *meso* isomer concentration vs mv/inch results in a linear response (Fig. 8).

Hot-stage microscopy of ethambutol. Ethambutol exists in at least three polymorphic modifications. When crystals of ethambutol are heated, a transformation occurs at approximately 75°C which results in the formation of form I (form II + 75°C → form I). Form I remains unchanged until it melts at 200°C. A third modification (form III) is obtained by a solid–solid transformation following recrystallization from the melt. When form III is heated, it undergoes a transformation at about 40°C, which is reversible to form I, and remains unchanged until it melts at 200°C.

Hot-stage microscopy of the meso *isomer.* The *meso* isomer exists in at least two polymorphic modifications. The original crystals exist in a modication designated as form II. When form II is heated, it undergoes a transformation at approximately 42°C, which is reversible to form I, which remains unchanged until it melts at 200°C.

Hot-stage microscopy of the d/meso *isomer mixtures* (synthetically prepared co-crystallized mixtures). The microscopical examination of the mixed fusions indicates that solid solutions with a minimum melting at 175°C are formed between the *d* I and the *meso* I polymorphs. The

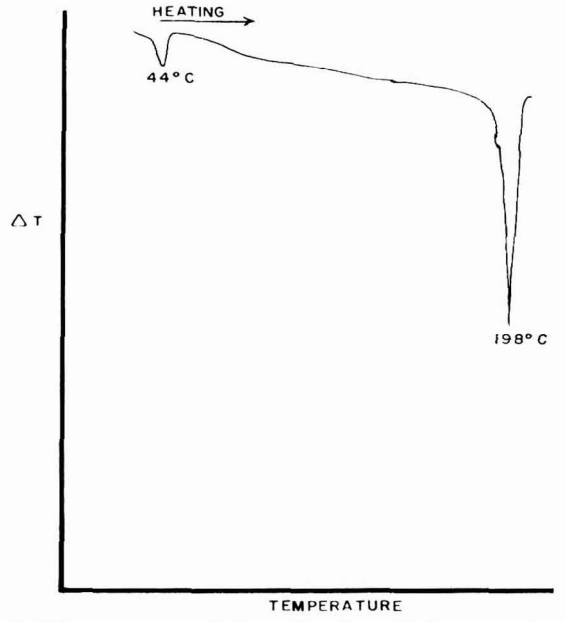


FIG. 5. Thermogram of the reheating of the *meso* isomer.

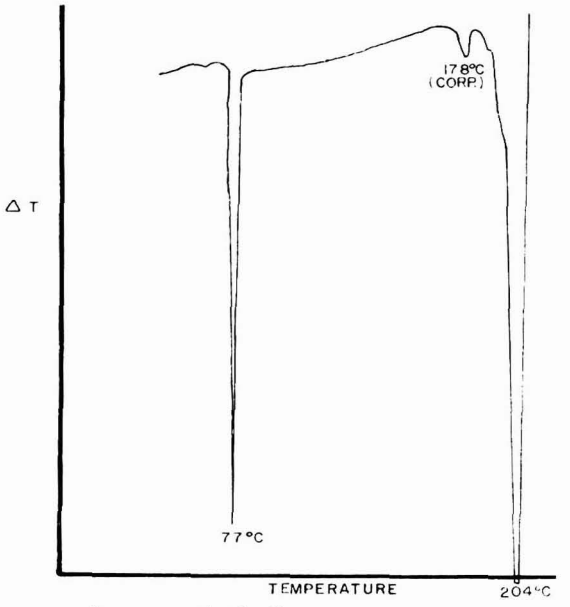


FIG. 6. Thermogram for a synthetically prepared co-crystallized mixture of the *d* and *meso* isomer containing 2% *meso* and 98% *d*.

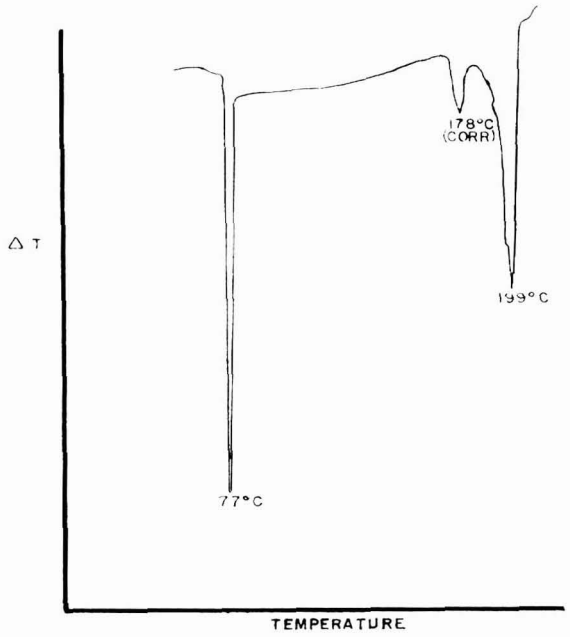


FIG. 7. Thermogram for a synthetically prepared co-crystallized mixture of the *d* and *meso* isomers containing 4% *meso* and 96% *d*. The 178°C endotherm is larger than the 178°C in Fig. 6.

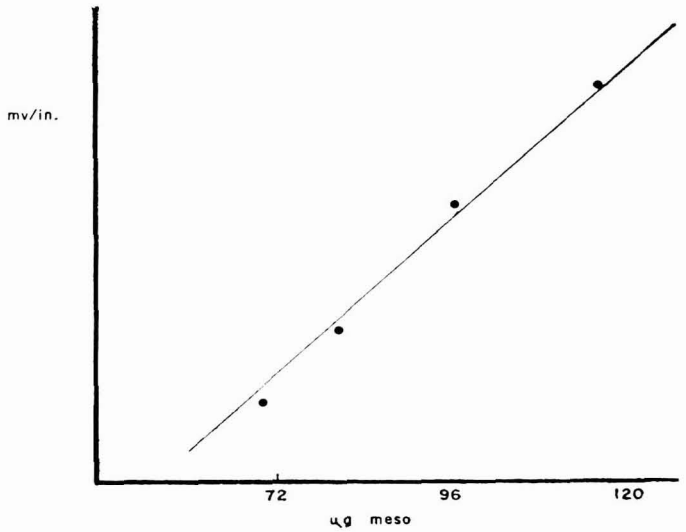


FIG. 8. Calibration curve for concentrations of the *meso* isomer in synthetically prepared co-crystallized mixtures of *d* and *meso* isomers.

X-ray pattern of the recrystallized mixtures also suggests a crystallographic form different from *d* II, *d* III, and *meso* II.

CONCLUSION

Although the exact nature of the *d*/*meso* system has not been established, the increase in the size of the 175°C endotherm with a corresponding increase in the *meso* concentration has been used to determine the approximate concentration of the meso isomer in ethambutol by thermal analysis.

SUMMARY

Thermal analysis can detect small concentrations of the *meso* isomer in the presence of ethambutol by the possible mechanism of solid solution formation. Ethambutol exists in at least three polymorphic modifications and the *meso* isomer exists in at least two polymorphic modifications.

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MICROCHEMICAL JOURNAL 16, 14–23 (1971)

Determination of Copper, Iron, and Zinc from a Single Small Sample

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Received June 17, 1970

INTRODUCTION

The three most common trace metals present in serum are copper, iron, and zinc and all three metals are of current interest (*1–5*). Their concentrations are relatively similar in the normal range but the values for each metal may vary somewhat differently in pathological circumstances. There is evidence that a measure of interrelationship exists (*6–8*) and therefore it would be useful if a single, relatively small sample could be treated in such a way that any or all of the three metals could be determined during the same procedure. However, two difficulties, both relating to sensitivity, are encountered in dealing with trace constituents of serum. One is the limiting factor of the available amount of sample, and the other the necessity to end the procedure by dilution to a small fixed volume. The primary purpose of this report then is to demonstrate with simulated filtrates of serum how one can determine the three metals from the same small volume of original sample by a relatively simple spectrophotometric procedure. An acid filtrate will be used as is common practice since all three metals are quantitatively released from their protein bonds into the supernatant fluid for subsequent quantification (*9–12*). There were several possible alternatives for the spectrophotometric determinations but only two methods were selected as being most practical. In one procedure, the determinations of copper and iron were made on one aliquot of filtrate by two sequential reactions followed by the determination of zinc on a second aliquot by differential demasking. In the alternative procedure,

[1] Supported in part by grants-in-aid from the Detroit General Hospital Research Corporation and the Michigan Heart Association. In partial fulfillment of Master of Science Degree in Biochemistry.

the determinations of the three metals were carried out on three small individual aliquots of the filtrate using similar methodologies to the first procedure.

EXPERIMENTAL METHODS

In order to avoid contamination, all test tubes used were chemically clean throw-away tubes which were not reused. Wherever possible, aliquots were taken automatically. All reagents were delivered from automatic diluters to ensure precision and minimize contamination.

Reagents

Borate buffer. Add 4.5 g of sodium borate decahydrate to a 50-ml flask containing approximately 10 ml of H_2O. Add 75 mg of NaCN and 20 ml of 4 *N* NaOH. Dissolve the borate and dilute the solution to 50 ml with metal-free H_2O. When 1.0 ml of buffer is mixed with 4.0 ml of working standard or blank, the pH should fall between 8.5–9.5. Usually, if it does not, it is too low. The preparation must then be repeated, 2.0 ml of extra alkali are added and the pH is checked again. Once the final conditions have been established for this solution, it is easy to prepare new buffer when it is needed.

Chloral hydrate solution. Dissolve 6 g to a final volume of 10 ml with metal-free water.

Trichloroacetic acid stock solution. Dissolve 100 g of metal-free trichloroacetic acid (TCA) to a final volume of 100 ml with metal-free water.

Trichloroacetic acid–ascorbic acid precipitating agent (TCA-AA). Pipet 20 ml of 100% TCA into a 100-ml volumetric flask containing about 80 ml of metal-free H_2O. Add 1 g of ascorbic acid and dilute to the mark of the flask with metal-free water.

Stock copper, iron, and zinc solutions. Prepare each solution to contain 50 mg/liter of each metal.

Standard iron solutions. Prepare standard solutions corresponding to 0–300 μg/100 ml of filtrate by adding 0.0, 1.0, 2.0, and 3.0 ml of iron to 100-ml volumetric flasks. Add 50 ml of TCA-AA solution and dilute to the mark of the flask with metal-free distilled water. These solutions can also be prepared by using one tenth volume relationships.

Standard copper solutions. Prepare standard solutions corresponding to 0–300 μg/100 ml as described for iron.

Standard zinc solutions. Prepare standard solutions corresponding to 0–300 μg/100 ml as described for iron.

Bathocuproine sulfonate (BCS). Dissolve 30 mg of BCS/100 ml of 50% ammonium acetate. Warm to dissolve the BCS.

Bathophenanthroline sulfonate (BPS). Dissolve 50 mg of BPS in 5.0 ml of metal-free water. Also prepare a 30 mg/100 ml solution in 50% ammonium acetate to be used if iron is the only desired constituent.

Zincon solution. Dissolve 35 mg of Zincon into 2 ml of Brij 35 solution (Technicon Reagent) in a 10-ml volumetric flask. Dilute the solution to the mark with metal-free distilled water.

In the previous work on the determination of serum zinc (*12, 13*) the detergent, acationox, was used as a solubilizer for Zincon. However, its makeup was and is unknown to us although it is probably one of the sterox family. Therefore, a study was made to find a suitable substitute of known composition. Brij-35 (polyoxyethylene lauryl ether) a surface active agent in common use in on-stream automation was selected over several other detergents tested because of its availability, low trace metal concentration, known composition and excellent solubilizing characteristics.

Sequential Procedure for Copper and Iron

Pipet 2.0 ml of sample into 2.0 ml of TCA-AA precipitating agent. Add 0.5 ml of BCS reagent to 2 ml of filtrate and read the absorbances of the solution at both 484 nm and 534 nm against the reagent blank to get readings A_1 for copper and A_2 for the iron blank. Add 20 μl of the 1% BPS solution to both blank and sample and read the sample again at 534 nm against the blank to get A_3. The reading A_1 is proportioned to the copper concentration where (A_3 - A_2) is proportional to the iron concentrations. The small change in volume from the addition of the BPS solution does not significantly affect the total absorbance since it only increases the volume from 2.50 to 2.52 ml. However, identical treatment of the iron standards does correct for the less then 1% decrease in absorbance due to volume change.

Procedure for Zinc (and copper and iron)

Pipet 3.0 ml of sample into 3.0 ml of TCA-AA solution. A 2.0-ml aliquot is used for the sequential determination of copper and iron as previously described. Take a second 2.0-ml aliquot containing the ferrous, cuprous, and zinc ions and add, in order, 0.5 ml of borate buffer and 50 μl of Zincon reagent. Set the instrument at 0 absorbance at 630 nm with this solution and then add 50 μl of chloral hydrate solution. Read the quickly formed absorbance at 630 nm. The color is stable for more than 20 minutes and is linearly proportional to the zinc concentration as determined by treating the standards in the same manner. If only zinc is to be determined, a 2-ml sample can be used and a 2.0-ml aliquot of filtrate is treated as described.

Procedure for Copper

Pipet 3 ml of solution to be analyzed into 3 ml of TCA-AA solution. Add 0.25 ml of BCS solution to a 1-ml aliquot and read the instantly developed color at 484 nm against a reagent blank made with metal-free water replacing the sample. If copper is the only desired constitutent use 1 ml of sample and 1 ml of TCA-AA solution.

Procedure for Iron

Treat a second 1-ml aliquot from above as described for copper but use the BPS solution in 50% ammonium acetate to replace the BCS solution and read the instantly developed color at 534 nm against a reagent blank. If iron is the only metal to be determined use a 1-ml aliquot as described for copper.

Procedure for Zinc

Treat a 2-ml aliquot from above as described under zinc procedure.

DISCUSSION

In order to determine copper and iron sequentially in the same aliquot of serum filtrate by similar reacting ligands it is essential to demonstrate lack of interference. If one ligand reacts with both metals and forms no color with one of them, there might be inadequate remaining ligand to combine with the metal of interest and this would result in low absorbance values for the second metal. In another circumstance, a metal might form an interfering color. To prove the lack of interaction in the described procedure the reactions were carried out both individually in separate aliquots as well as by the sequential technique of the described procedure to obtain a mixture of colors. Figure 1 (left) shows the spectrum of the mixture, curve C, along with the individual spectra for BCS·Cu(I), curve A; and BPS·Fe(II), curve B. To show that no interaction had occurred after the formation of both colors in the mixture because one metal might have a strong enough binding constant to displace the other metal, curve B was spectrally subtracted from curve C in a double beam spectrophotometer to result in curve D which is the same as curve A, the copper spectrum. In similar manner, curve A. was spectrally subtracted from curve C to yield curve E which is the same as curve B, the iron spectrum. Obviously this is a safe procedure for stoichiometric formation of the two colors in the presence of each other with no interaction apparent in the procedure.

The determination of zinc as the zinconate in a serum filtrate containing interfering copper and iron was made possible by the use of a system in which all three metals were first bound as their cyanide com-

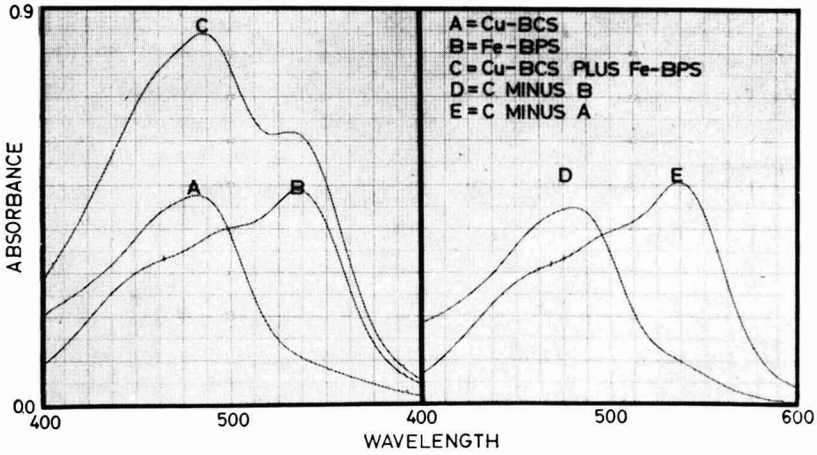

FIG. 1. (left graph) The spectra of Cu • BCS (curve A); Fe • BPS (curve B); and a mixture of A and B. (right graph) The spectrophotometric subtraction of curve A from curve C resulting in curve E and curve B from curve C resulting in curve D.

plexes (*11–15*). Then zinc, the easiest of the three complexes to destroy was differentially demasked by reaction with chloral hydrate to instantly form the zinconate which was measured quickly before the other metals were also released from their complexes to show interference. If other interfering colors or reactants were present but unreactive in the cyanide complexation step they presumably became a part of the blank of the sample, they were read before the addition of chloral hydrate and were unaffected by the demasking step which released the zinc ions. However, in the previously described procedure there was a need to measure the zinconate quickly (*12, 13*) because of instability of the cupric and ferric cyanide complexes in the presence of chloral hydrate and subsequent interference from the ions when they were released from their cyanide complexes. The necessity to measure within 1 minute (*11, 13*) decreased the effectiveness of the determination and increased the tedium of measurement. The stabilization of the cyanides of copper and iron by reduction to cuprocyanide and ferrocyanide complexes was decided upon as the simplest way to simultaneously stabilize the zinc–zinconate complex. An analogous stabilization of a complex cyanide had been shown previously where cuprous cyanide resisted reaction with formaldehyde whereas cadmium cyanide was quickly demasked and the released cadmium then determined (*16*).

In order to be certain that the characteristics of the zinc determination were now properly established, three studies seemed essential. In the first, the various parameters of reagent addition must be at plateaus. In the second, copper and iron, the most likely suspects for interference

with zinc in the procedure must not interfere during the period of time of color formation and color measurement. In the third, stability of the color with time must be increased and a study made to determine the safe period of measurement. In Fig. 2, the curves show the plateaus of formation of color of the zinc–zinconate in testing Zincon, chloral hydrate, and cyanide concentrations. For the latter, a stepwise curve implies that different species of cyanide complexes were formed as the cyanide concentration was increased. The other plateau of reaction to be determined for zinc was the optimum pH at which the determination was to be carried out. Figure 3 shows a pH study for the zinc–zinconate spectrum covering the range from 8.0 to 10.0. If the pH rises above 10.0, a heavy precipitate forms which interferes markedly in the reaction. It is rather obvious from these spectra that peak formation is quite consistent over a rather wide range of pH. Once the buffer solution was prepared as described under procedure, pH formation should be constant and the amount of color obtained would be dependent from thereon only on pipetting accuracy. Some latitude in pH is obvious and in the range shown, no hypo- or hyperchromic effect or hypsochromic or bathochromic shift need be expected with any small pH changes one might encounter preparing buffered serum filtrates.

The measure of the effectiveness of maintaining the lower valence

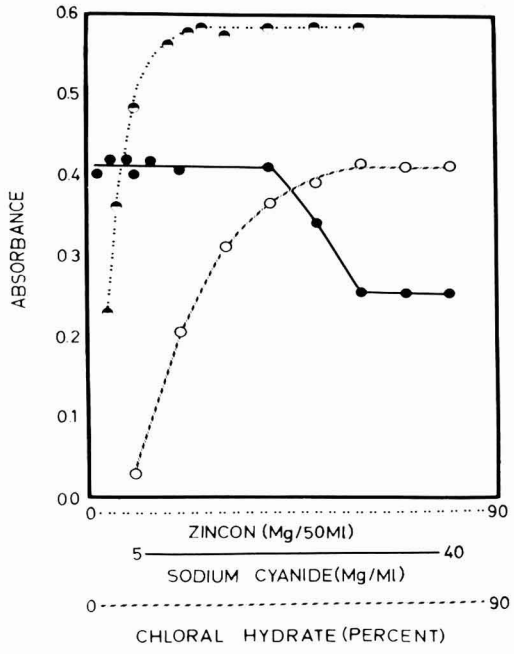

FIG. 2. Changes in absorbances to plateaus for continuous variation of reagent concentrations for the Zincon reaction for zinc.

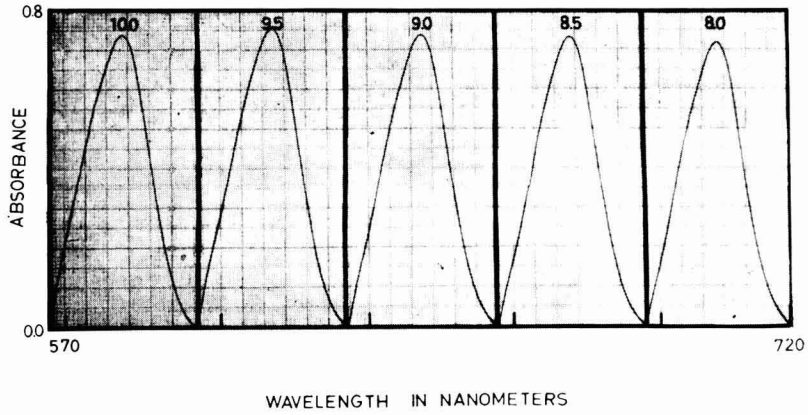

FIG. 3. Effect of variation of pH from 8.0–10.0 on peak position and absorbance intensity of the zinc–zinconate reaction.

states of iron and copper in the cyanide complexes is graphed in Fig. 4. In all three circumstances shown, the reaction is not stable for long in the absence of ascorbic acid but is quite stable in its presence. When all

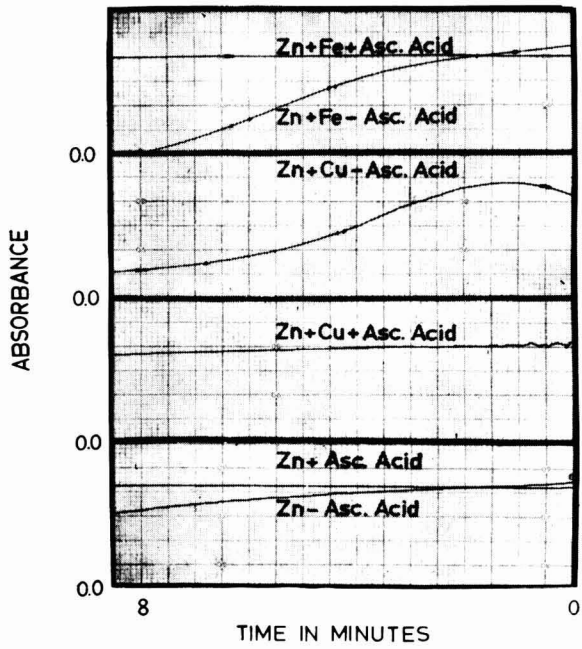

FIG. 4. Effect of presence of ascorbic acid in stabilizing the zinc–zinconate color by maintaining lower valences and stronger complexes of cuprocyanide and ferrocyanide.

three metals are together in the same matrix and a reducing atmosphere is maintained by the addition of ascorbic acid, the reaction is stable for a relatively long period of time as shown in Fig. 5. Here the same solution is scanned at the time of release of zinc from cyanide and then continuously over a period of 30 minutes. The spectral curves of Fig. 5 show freedom from the previously described fading effect (*12, 13*).

A measure of the precision and accuracy of the procedures for the three metals is demonstrated by the data shown in Table 1. Various mixtures of absolute quantities of the three metals were carried through the described processes. The results indicate that the values found coincide with the amounts known to be present within reasonable analytical limits and that the presence of the other two metals is not detrimental during the measurement of the third metal.

SUMMARY

Procedures have been described for the sequential determination of copper and iron in one aliquot of a trichloroacetic acid–ascorbic acid filtrate followed by the determination of zinc in a second aliquot. Alternatively, copper, iron, and zinc can be determined individually on three aliquots of the same solution or either can be determined separately on a smaller sample. All reactions are selective for the three metals under the described conditions. The various parameters of the procedure are shown to be optimum for color formation and without interference or interaction for any metal by either of the other two metals.

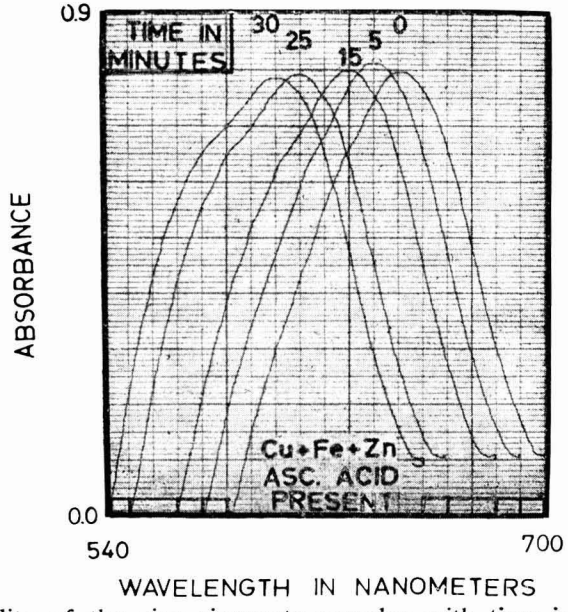

FIG. 5. Stability of the zinc–zinconate complex with time in the presence of both cuprous and ferrous complexes of cyanide.

TABLE 1

RECOVERY OF ABSOLUTE QUANTITIES OF COPPER, IRON, AND ZINC FROM MIXTURES

Present (μg/100 ml)			Found (μg/100 ml)		
Zinc	Copper	Iron	Zinc	Copper	Iron
100	50	100	100	50	100
150	100	50	151	100	47
50	200	100	50	200	103
100	150	100	103	149	101
100	50	150	101	50	151
100	100	50	105	97	50
100	100	100	105	100	100
50	50	50	46	48	50
150	150	150	154	148	140
150	150	150	147	146	50
50	150	150	55	148	143
150	50	150	154	50	151
100	100	150	100	100	148

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Detection of Amines by Using the Depolymerization of Poly(chloroaldehydes)

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Received June 23, 1970

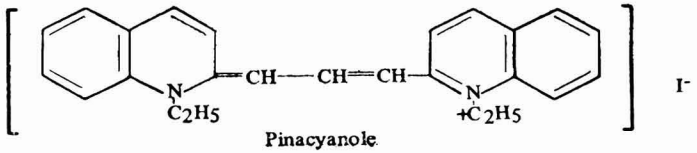
INTRODUCTION

In a recent communication (*10*), we introduced a technique for the detection of amines in aqueous solution which involved the amine catalyzed depolymerization of trifluoroacetate capped chloral-dichloroacetaldehyde copolymer. Detection signal was based on the dissolution of a fluorescent derivative of 2-diphenylacetyl-1,3-indandione. The present paper describes in more detail a detector tube in which amine vapors cause decomposition of a chloral–dichloroacetaldehyde copolymer.

MATERIALS

A. Detector Tube Description

The detector tube consists of a band of approximately 5 mg of dry pinacyanole (Eastman Organic Chemicals No 622) adjacent to a band



Pinacyanole

of approximately 10 mg of acetate capped chloral dichloroacetaldehyde copolymer. The two segments are packed tightly and held together in a glass tube (2.5 mm i.d.) with organdy cloth plugs (Fig. 2). A positive test is the formation of a blue color.

B. Polymer Capping Procedures

The preparation of chloral–dichloroacetaldehyde copolymer was described previously *(10)*.

1. Acetate Cap. Approximately 113 g of acetyl chloride was placed in a 250 ml round bottom flask equipped with a thermometer, drying

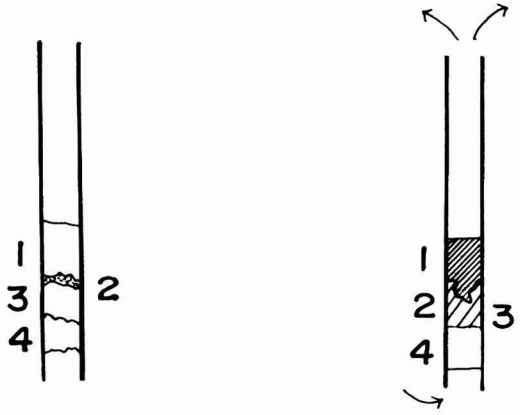


FIG 2. (left) Detector tube before testing or negative test: (1) and (4) organdy cloth plugs (white); (2) dry pinacyanole dye (green); and (3) polymer (white). (right) Detector tube strong positive test (approx 5 seconds after drawing diethylamine vapor): (1) organdy cloth plug (dark blue); (2) decomposing polymer (blue); (3) chloroacetaldehyde liquid (transparent blue); and (4) organdy cloth plug (white).

tube, and containing a magnetic stirring bar. The flask was cooled to 4° C with an ice bath. Chloral–dichloroacetaldehyde copolymer (10.0 g) was added and the mixture was stirred at 4–5°C for 1 hour. The ice bath was removed and the flask was allowed to reach room temperature. The mixture was then stirred for 2 hours. The flask was again cooled to 4°C and 50 ml of methanol was added during a 2–hour interval. An additional 100 ml of methanol was added quickly and the mixture was stirred for 15 minutes. The mixture was filtered and the remaining solid was washed with 50 ml of methanol. The solid was dried by allowing air to pass through the funnel. Yield: 9.0 g of colorless solid; the infrared absorption spectrum is given in Fig. 3.

2. 2-Tetrahydropyranyl Cap. Anhydrous ether (25 ml) was placed in a round bottom flask equipped with a thermometer, drying tube, and containing a magnetic stirring bar. 3,4-Dihydropyran (10 ml) and 1 drop of concentrated sulfuric acid were added. The flask was cooled to 10°C with an ice bath. Chloral–dichloroacetaldehyde copolymer (1.11 g) was added. The mixture was stirred at 10–15°C for 30 minutes. The ice bath was removed and the flask was allowed to reach room temperature while the mixture was being stirred an additional 30 minutes. The mixture was filtered under vacuum overnight. The funnel was covered with a piece of rubber film during the filtration. Yield: 0.48 g of tan solid.

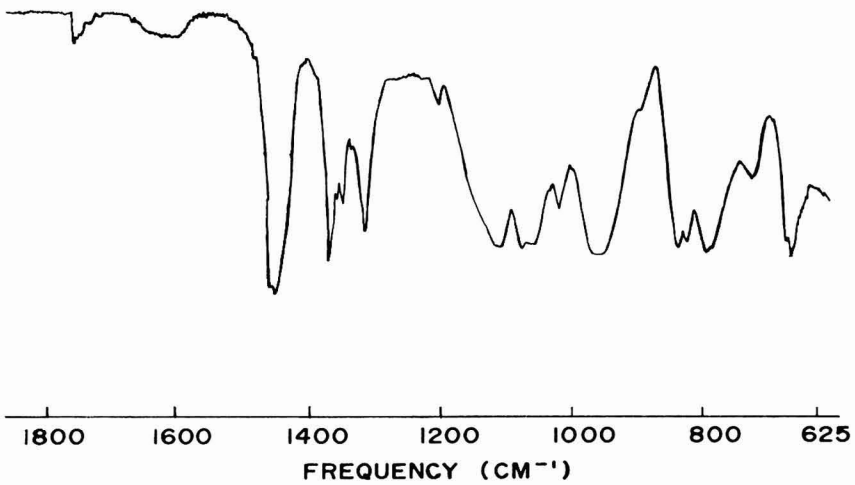


FIG. 3. Infrared absorption spectrum of acetate-capped chloral–dichloroacetaldehyde copolymer in Nujol mull.

RESULTS

A. Selectivity

The detector tube was placed just above the liquid level of each test compound contained in a 15–20 ml bottle. Most test compounds were part of a "Lab Assist" kit, Chem Service Inc., Media, Pa. The compound vapor and air were drawn through the tube using a rubber bulb (35-ml capacity). If a blue color did not appear in the polymer after 5 minutes, approximately 10 μl of petroleum ether (bp, 30–60°C) was added to the polymer end of the tube. The tube was again checked for a blue coloration.

Positive tests were grouped according to strength in the following classifications:

a. Strong with vapor: immediate blue color produced in polymer upon drawing vapor; little or no polymer remained after 1 minute.

b. Moderate with vapor: definite blue color produced in polymer within 5 minutes after drawing vapor; most of the polymer remained colorless for at least 5 minutes.

c. Moderate with vapor and solvent: strong blue color produced in polymer within 5 minutes after adding petroleum ether to the polymer; most of the polymer remained colorless.

d. Weak with vapor and solvent: blue color could just be detected at the interface of polymer and dye within 5 minutes after adding petroleum ether.

Whenever a positive vapor test was obtained a control experiment was performed. Approximately 5 mg of pinacyanole was placed between two organdy cloth plugs in a glass tube (2.5 mm i.d.). The test procedure was then repeated with this tube. A blue discoloration in the tube was considered an interference due to direct dissolution of dye by the compound tested. The selectivity profile of the detector tube is outlined in Table 1. Compounds which were found to interfere are listed in Table 2.

TABLE 1

SELECTIVITY

Compound	bp (°C)	mp (°C)
Tests with vapor		
"Strong" test with approx 25 ml of vapor		
Ammonium hydroxide		
Allylamine	52.5–53.5	
n-Butylamine	76.6–77.9	
Isobutylamine	65–67	
sec-Butylamine	62–64	
Cyclohexylamine	133–135	
Diethylamine	55.5	
Dibutylamine	158–161	
Dipropylamine	108–110	
Piperidine	105.5–107.5	
"Moderate" test with approx 25 ml of vapor		
Diallylamine	108–110	
2-Ethylhexylamine	168–169	
Hexylamine	129–130	
Triallylamine	146–150	
Tributylamine	88.5–90	
Triethylamine	88–90	
Tests with vapor and solvent		
"Moderate" test with approx 25 ml of vapor only after polymer was wetted with petroleum ether		
N,*N*-Dimethylformamide	152–154	
N,*N*-Dimethyldodecylamine		−21–−11
1-Amino-2-propanol		−2–0
3-Amino-1-propanol		10–12
1-(Dimethylamino)-2-propanol	117–126	
Ethanolamine		9–10
2-(Ethylamino)ethanol	160–167	

TABLE 1. continued

3-Methoxypropylamine	113–119	
N-Methylmorpholine	113–115	
Benzylamine	70–71 @ 10 mm	
Dibenzylamine	175–176 @ 12 mm	
o-Aminobenzenethiol	114–116 @ 11 mm	
"Weak" test with approx 25 ml of vapor only after polymer was wetted with petroleum ether		
2-(Dibutylamino)ethanol	110–114 @ 18 mm	
Diethanolamine		26–28
2-(Diethylamino)ethanol	161–163	
2-(Dimethylamino)ethanol	133–135	
2-(Methylamino)ethanol		−6– −4
N-Ethylmorpholine	136–139	
Morpholine	126–130	
3,3′-Diaminodipropylamine	113–116 @ 9 mm	
Diethylenetriamine	102–105 @ 22 mm	
N,N-Diethylethylenediamine	145–147	
N,N-Diethyl-1,3-propanediamine	169–172	
N,N-Dimethyl-1,3-propanediamine	129–134	
Ethylenediamine	118	
1,2-Propanediamine (90%)	170	
N,N-Dimethylaniline		1.5–2.5
2,4-Dimethylaniline	93–94 @ 9 mm	
2,4-Lutidine	157–159	
2,6-Lutidine	142–144	
2-Picoline	126–130	
3-Picoline	143.5	
4-Picoline	143–145	
Pyridine	115–115.6	
Quinoline	235–238	
2,4,6-Trimethylpyridine	169–170	

Compound	bp (°C)	mp (°C)
Nitrogen compounds that gave negative tests		
Approx 25 ml of vapor drawn and polymer was wetted with petroleum ether		
Dicyclohexylamine		−6– −4
2-Amino-2-methyl-1-propanol	61.5 to 63.5 @ 8 mm	
Triethanolamine		20–22
2-Pyrrolidinone		22–25
Triethylenetetramine	155–161 @ 18 mm	
2-[(2-Aminoethyl)amino)]ethanol	129–130 @ 12 mm	
N,N-Diethylaniline		
o-Toluidine	83–85 @ 15 mm	
m-Toluidine	92–93 @ 15 mm	
o-Anisidine		5–6
o-Chloroaniline		−2– −1

TABLE 1. continued

Nitrogen compounds that gave negative tests—continued

m-Chloroaniline		−11–−9
5-Chloro-2-methylaniline		20–22
2-Anilinoethanol	150–152 @ 10 mm	
N-Methyl-*N*-nitrosoaniline		14–15
Isoquinoline		14–17
N,*N*-Diethyldodecanamide	156–158 @ 1.5 mm	
N,*N*-Dimethylacetamide	58–60 @ 9 mm	
N-Methylacetamide		29–31
Benzoxazole		26–28
Benzothiazole		0–2
2-Methylbenzothiazole		12–14
Aniline		−6
Acetonitrile		
Benzonitrile		
Nitromethane		

Other compounds that gave negative tests

Approx 25 ml of vapor drawn and polymer wetted with petroleum ether

Compound	Compound
Acetals	Pyruvic acid
1,1-Dimethoxyethane	3-Mercaptopropionic acid
Acids, monobasic	Thioglycolic acid
Acetic acid	Acid anhydrides
Caproic acid	Acetic anhydride
Cyclohexanecarboxylic acid	Butyric anhydride
2-Ethylbutyric acid	Acyl halide
2-Ethylhexanoic acid	Benzoyl chloride
Heptanoic acid	Alcohol, monohydric
Linoleic acid	Benzyl alcohol
Nonanoic acid	Methyl alcohol
Octanoic acid	Alcohol, ether
Oleic acid	Tetrahydrofurfuryl alcohol
Propionic acid	Alcohols, other
10-Undecenoic acid	2-Mercaptoethanol
Valeric acid	Aldehydes
Isovaleric acid	*n*-Butyraldehyde
Acids, halogen	Formaldehyde
2-Bromo-*n*-butyric acid	Furfural
Dichloroacetic acid	Carbonate
Acids, hydroxy	Dimethyl carbonate
Gluconic acid	Esters
Lactic acid	Ethyl acetate
Acids, other substituted	Ethyl bromoacetate
Methoxyacetic acid	Ethyl cyanoacetate
Levulinic acid	Triethyl orthoformate

TABLE 1. continued

Other compounds that gave negative tests—continued

Compound	Compound
Ethers	Lactone
Butyl ether	γ-Butyrolactone
Tetrahydrofuran	Phenol, monohydroxy
Halogen compounds	Phenol
Methyl iodide	Phosphorus compounds
1,1,1-Trichloroethane	Dibutyl phosphite
Heterocyclic compounds	Diethyl ethyl phosphonate
2-Methylbenzothiazole	Tributyl phosphite
2,5-Dibromothiophene	Dichlorophenyl phosphine oxide[a]
Tetrahydrothiophene	Dichlorophenyl phosphine sulfide
Thiophene	Sulfate
Hydrocarbons	Dimethyl sulfate
Octane	Sulfide
Cyclopentane	Carbon disulfide
2-Heptene	Sulfonate
Benzene	Methyl benzenesulfonate
Ketones	Sulfoxide
Acetone	Methyl sulfoxide
Acetylacetone	Mercaptan
Acetophenone	1-Octanethiol

[a] Causes dye to become black.

TABLE 2

DETECTOR TUBE INTERFERENCES

Compound	Test strength[a]	Remarks
Acetyl bromide[b]	A	
Formic acid	B	88–90% acid
Butyric acid	C	
Isobutyric acid	C	
Glycolic acid	C	

[a] The test strength results are noted as follows: (A) moderate test with vapor alone (5 minutes); (B) moderate test after drawing vapor and wetting polymer (5 minutes); and (C) weak test after drawing vapor and wetting polymer (5 minutes).

[b] Vapors cause the dye itself to turn blue.

B. Vapor Sensitivity

1. Procedure A. The detector tube was inserted into a one-holed stopper which was placed in the center outlet of a three-necked 250-ml round bottom flask. A thermometer was placed into the second outlet and a glass capillary tube was fitted into the remaining outlet. A solu-

tion was made up by placing 10 μl to 1 ml of amine into 100 ml of distilled water. The solution was stirred for 15 minutes with the system closed to the atmosphere. Vapor from the flask was drawn through the detector tube using a rubber aspirator bulb. The detector tube was placed in an oven at 110°C, and after 5 minutes, the polymer in the tube was observed for a blue discoloration.

The amount of amine actually drawn through the tube was roughly estimated on the assumption that the system was ideal and obeyed Rauolt's law, $P = P^0N$, where P^0 = vapor pressure of the pure amine, N = mole fraction of amine in solution, and P = partial pressure of the amine. The partial pressure P thus obtained was substituted in the ideal gas equation $PV = nRT$, where V = volume of vapor drawn through the tube, n = number of moles, R = gas constant and T = temperature (°K).

In a control experiment it was found that water vapor alone did not cause a positive test. Also the detector tube itself appeared stable at 110° for at least 15 minutes. After that a yellow discoloration became evident.

Results with diethylamine and triethylamine appear in Tables 3 and 4. Vapor pressure data for these amines can be found in Ref. (*2*).

2. *Procedure B.* The apparatus and technique previously described by Crabtree *et al. (3)* were used. A 2 × 9-cm test tube with a capacity of about 12 ml was fitted, by means of a 19/38 standard taper joint, with an adapter supporting a sidearm air inlet and a length of glass tubing that extended to within 5 to 10 mm of the bottom of the test tube. Detector tubes were connected by a short length of rubber tubing to the upper end of the glass. Vapors from the test tube were drawn through the detector tube by compressing a rubber bulb.

Samples of varying amounts of triethylamine dissolved in petroleum ether (10 μl, bp 30–60°) were measured with disposable pipettes and placed in the test apparatus described above. The amine vapor is collected on the tube, the detector tube is removed from the apparatus, and heated in a 110°C oven for 5 minutes. The appearance of a blue color is a positive test for amine. Results appear in Table 3.

C. *Solution Sensitivity Studies*

Solutions of triethylamine were made up in petroleum ether (bp 30–60°) at concentrations equivalent to 0.5, 1.0, 5.0, and 10.0 μg/10 μl solvent. A 10 μl sample of the solution was injected (using a hypodermic syringe) into the detector tube at the interface of dye and polymer. Results appear in Table 4.

TABLE 3

SENSITIVITY OF THE DETECTOR TUBE WITH AMINE VAPOR [a]

Procedure A		Procedure B	
Quantity of amine vapor drawn (μg)	Test result	Quantity of amine vapor drawn (μg)	Test result
	Diethylamine		
11	Strong	7	Moderate
8	Moderate	4	Weak
7	Negative	2	Negative
		5[b]	Moderate
		2[b]	Weak
		1[b]	Negative
	Triethylamine		
17	Strong	7	Moderate
8	Moderate	4	Weak
5	Negative	2	Negative
		4[b]	Moderate
		2[b]	Weak
		1[b]	Negative

[a] Detector tube was heated at 110°C for 5 minutes after the amine vapor was drawn.
[b] Detector tube was pretreated by drawing through it 175 ml of air saturated with water vapor.

TABLE 4

SENSITIVITY OF THE DETECTOR TUBE WITH TRIETHYLAMINE SOLUTION

Quantity of triethylamine (μg)	Test result	Time required for test[a]
10	Strong	Immediate
5	Moderate	30–45 seconds
1	Weak	60 seconds
0.5	Negative	—

[a] The test was performed at room temperature.

DISCUSSION

A. Selectivity

The selectivity profile (Table 1) for the detector tube appears to depend on several properties of the amine including volatility and basicity. As readily seen from Table 5, compounds which are the most basic and the most volatile generally give the strongest tests. However, triethylamine, which is very volatile and a strong base, is bulky sterically;

TABLE 5

A COMPARISON OF AMINE PROPERTIES WITH THE STRENGTH OF THE DETECTION TEST

Compound	Vapor pressure [mm Hg (°C)]	Strength of test[a]	Approximate amount tested (μg)	$K_{(b)}$aq (*6*)	Vapor pressure (ref.)
n-Butylamine	105(32.2)	A	10,200	4.1×10^{-4}	(*1*)
Isobutylamine	199(32.2)	A	14,500	3.1×10^{-4}	(*1*)
sec-Butylamine	287.5(32.2)	A	27,900	3.6×10^{-4}	(*1*)
Diethylamine	316(32.2)	A	30,800	9.6×10^{-4}	(*1*)
Piperidine	40(29.2)	A	4500	1.6×10^{-3}	(*5*)
Triethylamine	468(29.0)	B	62,000	5.7×10^{-4}	(*2*)
Benzylamine	1(29.0)	C	140	2.4×10^{-5}	(*5*)
N,*N*-Dimethylaniline	1(29.5)	D	160	1.2×10^{-9}	(*5*)
2-Picoline	10(24.4)	D	>1240	3×10^{-8}	(*5*)
Pyridine	40(38.0)	D	<4200	1.7×10^{-9}	(*5*)
Quinoline	1(59.7)	D	<<170	6.3×10^{-10}	(*5*)
Aniline	1(34.8)	E	<124	3.8×10^{-10}	(*5*)
Isoquinoline	1(63.5)	E	<<170	—	(*5*)
2-Anilinoethanol	1(104.0)	E	<<180	—	(*5*)

[a] (A)—strong test with vapor alone (1 minute); (B) moderate test with vapor alone (5 minutes); (C) moderate test after drawing vapor and wetting polymer (5 minutes); (D) weak test after drawing vapor and wetting polymer (5 minutes); (E) negative test.

it gave weaker tests than primary amines which are less volatile and weaker bases.

B. Sensitivity

1. Vapor. Sensitivity differences obtained using procedures A and B (Table 3) are most likely due to deviations from Raoult's Law and/or the Ideal Gas Law in procedure A. Reproducible results were not obtained with less than 150 μg of amine using either test procedure unless the tubes were heated after the amine vapors were sampled.

2. Solution. Sensitivity was found to vary considerably depending on the technique of injecting the solution into the detector tube. As low as 1 μg of triethylamine gave a positive test at room temperature if the solution was injected at the dye–polymer interface. Injecting 30 μg of triethylamine (in 10 μl of petroleum ether) just below the dye–polymer interface did not result in a positive test. Heating the detector tubes at 110°C for 5 minutes did not increase the sensitivity.

When diethylamine was tested, a positive result was obtained with 2 μg without heating. However, unlike the tests with triethylamine the sensitivity could be increased to 0.4 μg by using the heating technique. Instead of obtaining a low intensity discoloration over a large surface of polymer near the dye–polymer interface, an intense blue color formed only at the point of sample injection (0.4 or 0.7 μg of diethylamine/10 μl of solution). This suggests that the amine attack was concentrated at the point at which the test solution was injected. The positive blue color signal occurred because dye was present in the immediate area where the polymer had unzipped.

3. Comparison to other methods. A variety of methods for the detection and analysis of amines are known (*4, 11*). Lower limits for some of these tests are given in Table 6. The detector tube describe in this paper appears to compare favorably. However, an adequate comparison cannot be made without more data and a consideration of the specific detection problem involved.

C. Mechanism.

The synthesis and properties of poly(chloroaldehydes) are well described [see, e.g. (*7–9*)]. The polymers have an acetal structure (*A*). The polymer used in the present work is acetate capped (*B*). A small amount of 2-tetrahydropyranyl capped polymer (*C*) was found to

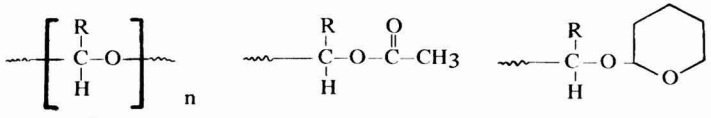


TABLE 6

DETECTION LIMITS OF SOME REPORTED TESTS FOR AMINES (*4*)

Amine	Limit of detection (μg); Test method[a]									
	a	b	c	d	e	f	g	h	i	j
Diethylamine		4		4						
Dipropylamine	125									
Isobutylamine	8									
Benzylamine	8.5			10	1.6	1	.25			
Piperidine	4.5	5	0.2	4	3.3					
Aniline					1.6	0.2	50		0.1	0.5
Isoquinoline					5					
α-Naphthylamine									0.05	2
Pyridine								2		

[a] Key to test methods: (a) conversion to dithiocarbamates; (b) reaction with sodium nitroprusside and acetaldehyde; (c) formation of copper dithiocarbamates; (d) melting with fluorescein chloride; (e) reaction with 1-chloro-2,4-dinitrobenzene; (f) reaction with *p*-(dimethylamino)benzaldehyde; (g) fusion with potassium thiocyanate; (h) citric acid and acetic anhydride; (i) reaction with glutaconic aldehyde; and (j) reaction with sodium pentacyanoaquoferrate.

unzip as rapidly with diethylamine as did the acetate-capped polymer. From a practical point of view the nature of the cap does not appear to be important. From a mechanistic point of view more evidence is needed to unequivocally decide between end group versus backbone attack.

Knowledge of the susceptibility of poly(chloroacetaldehydes) to depolymerization with amines is based on unpublished work by G. H. McCain and J. M. Sanders, Diamond Shamrock Corporation, T. R. Evans Research Center, Painesville, Ohio 44077.

SUMMARY

A detector tube is described in which amine vapors cause decomposition of a chloral–dichloroacetaldehyde copolymer. Detection signal is based on the dissolution of the dye pinacyanole by the liberated chloroacetaldehyde monomers. Of the 77 amines tested, ammonia, allylamine, *n*-butylamine, isobutylamine, *sec*-butylamine, cyclohexylamine, diethylamine, dibutylamine, dipropylamine, and piperidine gave strongest tests. The limit of detection of diethylamine is approximately 2 μg. The sensitivity is increased about one order of magnitude if solution techniques are employed. Interferences appear to be few since 71 compounds representing 21 classes of compounds did not give a test.

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MICROCHEMICAL JOURNAL **16**, 37–51 (1971)

The Analysis of Tensides by Automatic Radio-Frequency Titrations [1]

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Received July 8, 1970

Electrometric titration methods may be employed for the determination of tensides (surface active agents), which are used as constituents in detergents. Unfortunately, if electrodes have to be immersed into the solution for the purpose of electrical contact, tensides tend to absorb onto their surfaces and this may cause errors in the results (*39*). Radio-frequency titrations offer great advantages in this respect, but only a few attempts have so far been made to apply them to studies in this field. Thus, methods have been described for the titration of sodium dodecyl sulfate with dodecylamine hydrochloride (*33*), for the determination of phosphate tensides by titration with iron(III) solutions (*18*), and for the estimation of magnesium and calcium using sodium oleate as titrant (*16*) in all cases applying radio-frequency monitoring. In the present study the determination of various tensides based on precipitation reactions in both aqueous and nonaqueous solutions is attempted.

CHOICE OF A RADIO-FREQUENCY TITRIMETER

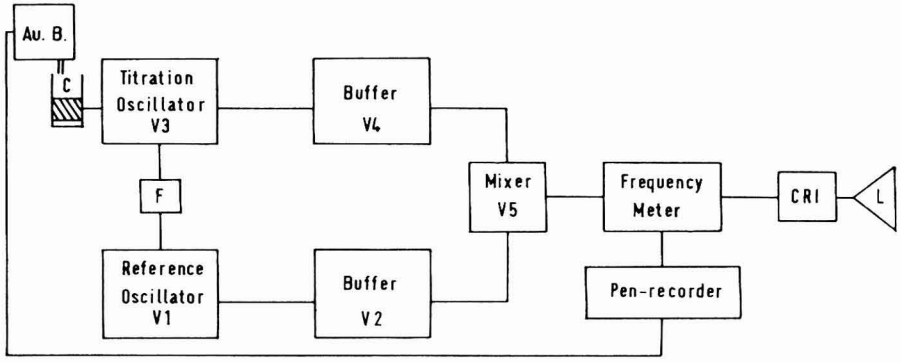
The accuracy and precision of radio-frequency titrations depend largely upon the sensitivity of response of the instrument towards changes in the chemical composition of the sample solution. This sensitivity depends mainly on three variables, namely the operational frequency of the instrument, the cell geometry and the dielectric constant of the solution (*3, 5, 32*). From a purely chemical standpoint, the dielectric constant of the solution, and this of course implies composition of the solvent, should not be interfered with unless absolutely necessary, since the solvent itself is normally chosen so that the particular chemical reaction in question proceeds to best advantage. Cell geometry may be varied relatively easily within certain limits (*2, 4, 7, 8, 29, 30*) but

[1] Presented at the 3rd International Symposium on Oscillometry, Horny Smokovec, (Czechoslovakia), September, 1969.

if a wide range of solvents of different dielectric constants are to be employed then this is no longer a simple proposition and it becomes necessary to vary the operational frequency if adequate sensitivity is to be maintained over a range of concentrations. Most solvents in common usage have dielectric constants less than that of water and it is known that the sensitive region at a given frequency moves to higher concentrations as the dielectric constant of the solvent is decreased (*22, 31*). From all these arguments it follows that the most suitable instrument for both aqueous and nonaqueous titrations should have operational frequencies variable within a suitable range.

Instruments employing a variable operational frequency have been very seldom mentioned in the literature (*10, 12, 23–25*) and none of these has been used to monitor titrations in nonaqueous solutions; neither have any been based on the heterodyne-beat principle. Both these reasons, and personal experience gained with commercial titrators operating at fixed frequencies, encouraged us to build an automatic radio-frequency titrimeter with variable frequency, based on the heterodyne-beat principle, with a view to employing it in a study of nonaqueous titrimetry.

The block-diagram of the instrument, which is continuously operable over the range 0.5 to 30 MHz, is shown in Fig. 1. The capacitive titration cell is connected to the tuned circuit of one of two virtually identical radio-frequency oscillators, the other oscillator acting as reference. Unit F is used to vary the operational frequency. The outputs of



F frequency-control; C capacitive cell; CRI cathode ray indicator; L loudspeaker; Au. B. auto-burette.

FIG. 1. Block-diagram of the titration assembly: (F) frequency-control; (C) capacitive cell; (CRI) cathode ray indicator; (L) loudspeaker; (Au. B.) auto-burette.

the titration and cell oscillators are fed separately via buffer stages to a mixer, and thence through a filter to the frequency meter, loudspeaker, and cathode ray indicator. The latter two devices facilitate the operator in the adjustment of beat-frequency which may be measured on nine scales, the lowest reading 100 Hz, the highest 1 MHz at full-scale deflection. The titrant is delivered by a Radiometer piston burette, the titration curve being recorded by a Sunvic pen recorder connected to the output stage of the frequency meter. The titration cell is a glass cylinder to the outside of which the capacitor plates are attached with adhesive tape. The cell is sealed with a glass stopper which incorporates facilities for introducing gases into the cell. A mechanical stirrer is employed. The photograph of the titrimeter is shown in Fig. 2. Electrical circuitry has been described in detail elsewhere (*35*).

EVALUATION OF TITRIGRAMS

Since the accuracy of the results depends on both the accuracy with which the end point is located on the chart and on the accuracy of the measurement of volume, preparation and evaluation of the titrigrams must be carried out with the greatest care. We adopted the following method. Before commencing the titration, the sensitivity is turned up momentarily with the pen in contact with the paper, thereby obtaining a reference line marking the start of the titration. Then the titration is commenced by operating the start switch. When a suitable trace is

FIG. 2. View of the automatic titrimeter (the rubber-tubing arrangement leads inert gas into the cell when required).

obtained on the chart, from which the end point can be established, the switch is turned off and a second reference line is obtained on the titrigram, marking the end of the titration curve. The volume corresponding to this position (a) is read from the burette and the total length between the two reference lines (l_a) is measured. Now the end point is located by extrapolating the relevant branches of the titration curve and determining the position of the point of intersection. The length between this point and the initial reference line (l_v) is then measured. The volume (V) of the titrant consumed at the end point may then be calculated by the formula.

$$V = a \frac{l_v}{l_a}$$

TITRATION OF TENSIDES WITH ONE ANOTHER

When the aqueous solution of, for example, a cationic tenside is added to that of an anionic one, a complex is formed which precipitates when the molar ratio of the two tensides present is (approximately) 1:1. These end points are, however, difficult to estimate precisely and results are not too satisfactory. Better results are obtained when a water-immiscible organic phase is also present and a two-phase titration is performed *(11)*. For radio-frequency end point detection with automatic titrant delivery and recording, this is, however, an impractical proposition, as there is no time to allow the separation of phases after the addition of each portion of titrant. Accordingly we carried out one-phase titrations employing a 1 + 1 (v/v) mixture of water + methanol as solvent. Precipitation was seemingly more complete in this mixture than in water alone, and foaming was eliminated by the alcohol. Salton and Alexander (*34*) applied the same principle with success for visual titrations.

The following substances were used both as reagents and titrants: sodium dioctyl sulfosuccinate (Manoxol OT, or MOT); cetylpyridinium chloride (CPC); cetyltrimethylammonium bromide (CTAB); tetra-n-butylammonium iodide (TBAI); and sodium lauryl sulfate (NaLS). With the exception of titrations involving tetra-*n*-butylammonium ion, the high solubility of whose salts is widely recognized, precipitation occurred and elbow-type titration curves were obtained yielding sharp end points (see Fig. 3). Unfortunately the results were not reproducible (the coefficient of variation of results being about 5% in each case). Further evidence from the literature (*14, 15, 19–21, 28, 36*) suggests that nonstoichiometric precipitates are formed during these titrations. Although we tried our best to reduce the scatter of results by

applying strictly the same experimental conditions in each case, we could not obtain a better reproducibility than that quoted above.

As such a procedure would have no practical importance, we abandoned the idea of applying reactions between cationic and anionic tensides as a basis for titrimetric determination. Instead, we attempted to precipitate the tensides with reagents of simpler composition, thereby expecting better reproducibility in the results.

TITRATION OF CATIONIC TENSIDES WITH SILVER NITRATE

The halide content of a cationic tensides can be precipitated by titration with silver ion. The titration can be carried out in 1 + 1 methanol–water solvent mixtures, though other solvents with lower dielectric constants may also be employed.

Reagents

0.01 M solutions of tensides. These were prepared from the purest available samples in 1 + 1 solvent. MOT was dried at 60°C over phosphorus pentoxide, while the other tensides were dried over phosphorus pentoxide in a vacuum desiccator.

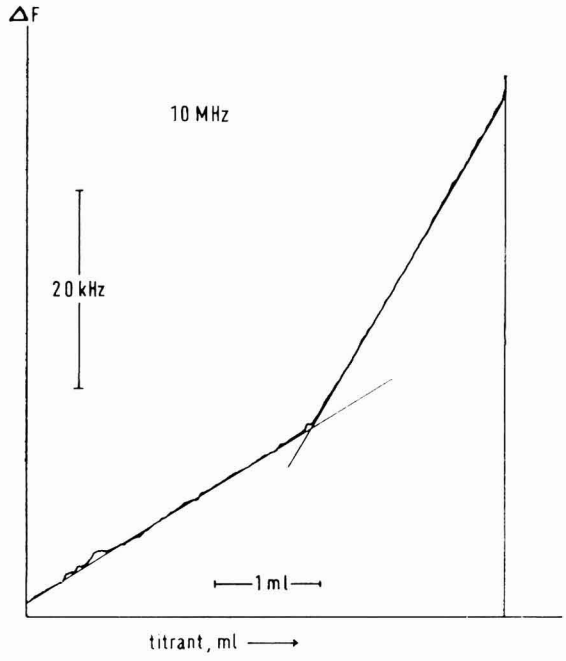


FIG. 3. Titration of 50 ml of 5 × 10^{-3} *M* CPC versus 0.1 *M* NaLS: operational frequency, 10 MHz; sensitivity, 100 kHz; ΔF is the beat-frequency.

Titrant

0.1 *M* silver nitrate was prepared from analytical grade reagent using distilled water as solvent. The solution was standardized against 50 ml of 5×10^{-3} *M* sodium chloride by radio-frequency titration at 30 MHz using 100 KHz sensitivity on the beat-frequency meter.

Solvents

Methanol and 1,4-dioxan were used in the purest available form.

Procedure

To 25 ml of 0.01 *M* solution of the tenside, 25 ml of methanol or 1,4-dioxan were added to lower the dielectric constant of the solution. Titrations were carried out at an operational frequency of 3 MHz, using 100 KHz sensitivity. The titrant was added at (approx) 2 ml/min^{-1}, while a 5 cm/min^{-1} chart speed was employed.

Results

Results (together with equivalent weights of the substances) are shown in Table 1. These results indicate that the attainable accuracy and precision are in all cases satisfactory.

Notes

Dioxan may not be used as a solvent if iodide is present. The solvent invariable contains traces of hydrogen peroxide, which oxidizes iodide partly to iodine (this is indicated also by the slight yellow color of the

TABLE 1

TITRATION OF CATIONIC TENSIDES: DETERMINATION OF HALIDE[a]

Reagent (25 ml of 0.01 *M* in 1+1 solvent)	Equiv. wt	Diluting solvent (25 ml added to titration cell)	Found (ml)	No. of results	SD	Coefficient of variation (%)
CPC	340.00	1,4-Dioxan	2.48	7	0.01	0.3
CTAB	364.46	1,4-Dioxan	2.76	10	0.01	0.4
TBAI	369.38	1,4-Dioxan	2.17	9	0.01	0.5
	369.38	Methanol	2.49	8	0.01	0.4

[a] Titrant, 0.0992 *M* aqueous silver nitrate; operational frequency, 3 MHz; sensitivity, 100 kHz.

mixture), causing negative errors. Methanol however is suitable in this case.

The tritration can be carried out with satisfactory accuracy and precision even in the presence of 25-fold amounts of anionic tensides. In such a case the composition of the solvent should approximate to 3 parts methanol and 1 part water. Owing to the higher electrolyte concentration, a higher operational frequency is necessary. Results are shown in Fig. 4 and Table 2, respectively.

TITRATION OF THE QUATERNARY AMMONIUM ION

Precipitation of the quaternary ammonium ion has previously been achieved using such ions as hexacyanoferrate(III), dichromate, and phosphotungstate, the gravimetric technique being usually employed. Such a procedure is laborious and time consuming and it would be more convenient to use a titrimetric procedure. Tetraphenyl borate is a widely accepted precipitant for cations like the quaternary ammonium

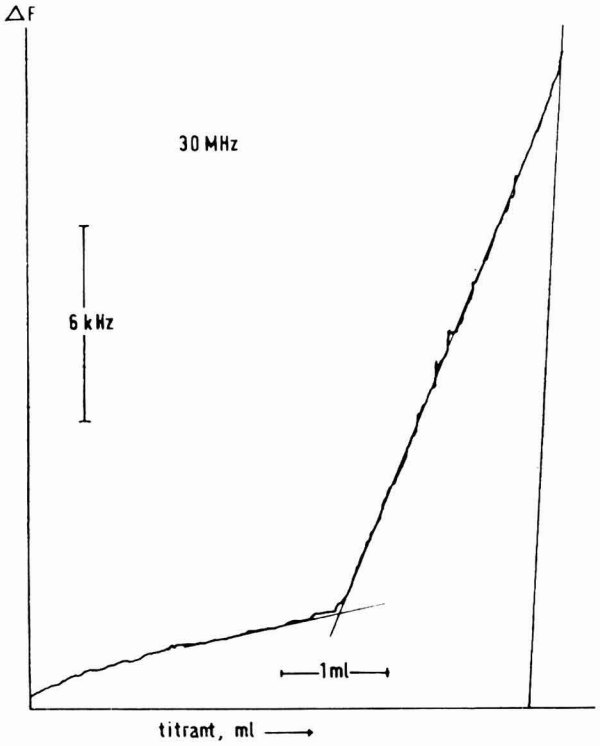


FIG. 4. Titration of 100 ml of 2.5 × 10^{-3} *M* TBAI and 6.25 *M* MOT versus 0.0992 *M* $AgNO_3$: operational frequency, 30 MHz; sensitivity kHz; ΔF is the beat-frequency.

TABLE 2

TITRATION OF CATIONIC TENSIDE IN THE PRESENCE OF EXCESS ANIONIC TENSIDE[a]

Reagent (25 ml of 0.01 *M* in 1+1 solvent)	Diluting solvent (25 ml added to titration cell)	Anionic tenside added (50 ml of 0.125 *M* in 3+1 methanol + water)	Sensitivity (kHz)	Found (ml)	No. of results	SD	Coefficient of variation (%)
CPC	1,4-Dioxan	NaLS	100	2.51	8	0.01	0.4
	1,4-Dioxan	MOT	300	2.57	7	0.01	0.3
CTAB	1,4-Dioxan	NaLS	100	2.83	8	0.01	0.5
	1,4-Dioxan	MOT	100	2.83	7	0.01	0.3
TBAI	Methanol	NaLS	30	2.61	7	0.02	0.8
	Methanol	MOT	30	2.60	7	0.02	0.9

[a] Titrant, 0.0992 *M* aqueous silver nitrate; operational frequency, 30 MHz.

ion, and recent work has shown that it may be employed to determine cationic tensides in aqueous or aqueous–alcoholic media (*9, 38*). However, on applying radio-frequency titrations, we obtained very obtuse angles on the titration curve and this prevented the determination of the end point with suitable exactness. Although the position of the equivalence point may be located the results cannot be expected to be as reliable as when a more acute angle is measured, and the estimation of cationic tensides by this particular method cannot be recommended.

TITRATION OF SULFATED ANIONIC TENSIDES

Sulfated anionic tensides can be hydrolyzed with dilute acid, and the sulfate ion is determined in the solution. We attempted to titrate sulfated tensides in aqueous solution using various barium salts as titrant. Of the nitrate, acetate, and hydroxide salts, barium acetate gave the best results. The nitrate yielded reproducible, but somewhat low results, while with the hydroxide the titration curves were almost completely linear, and the equivalences could not then be evaluated.

Benzidine hydrochloride can, in some cases, also be applied as a titrant. During the reaction, benzidine sulfate is precipitated, resulting in a sharp end point. This titrant gave very accurate results for the titration of Manoxol OT.

Reagents

Non aqueous solutions cause solubilization of the precipitates, so to reduce foaming to an acceptable level and yet not affect precipitation efficiency, 0.01 *M* solutions of the tensides were prepared in 9 + 1 (v/v) water + methanol and used for these experiments (cf. notes under "Titration of cationic tensides with silver nitrate").

Titrants

0.05 M barium acetate. The analytical grade reagent (mol. wt. 255.43) can be weighed and dissolved in boiled-out distilled water. It can be standardized against analytical grade zinc or magnesium sulfate, using radio-frequency end point detection.

0.025 M benzidine hydrochloride. Analytical grade benzidine base (mol. wt. 184.10) is weighed and agitated with 100 ml of *N* hydrochloric acid until complete dissolution, then diluted with water to 1 liter. It can be standardized against analytical grade zinc or magnesium sulfate using radio-frequency end point detection.

Procedure

Place 10–25 ml of aqueous solution, containing 0.1–0.5 mmole of the sulfated tenside, into the titration cell and dilute with water to 50 ml. Adjust operational frequency to 30 MHz, meter sensitivity to 100 kHz, and achieve zero beat by readjusting the frequency of the reference oscillator. Titrate automatically with 0.05 *M* barium acetate or 0.025 *M* benzidine hydrochloride. A sharp break on the titration curve indicates the end point.

Results

Some results are shown in table 3.

Note

Monoxol OT cannot be titrated with barium salts with a good accuracy.

TITRATION OF THE ALKALI METAL IN ANIONIC TENSIDES

A very simple and accurate way of analyzing anionic tensides is to titrate the alkali metal content with chloride ions in acetone solution. Using lithium chloride as titrant dissolved in absolute ethanol the titration can be carried out with normal speed as precipitation of the alkali chloride is instantaneous (*13*). The method is especially recommended for the titration of Manoxol OT, where the barium acetate method is not satisfactory.

TABLE 3

TITRATION OF ANIONIC TENSIDES: DETERMINATION OF THE ANION[a]

Reagent 50 ml	Titrant	Found (ml)	No. of results	SD	Coefficient of variation (%)
0.005 *M* NaLS	0.05 *M* $Ba(OAc)_2$	2.51	7	0.00	0.2
	0.05 *M* $Ba(NO_3)_2$	2.20	7	0.01	0.6
	0.05 *M* $Ba(OH)_2$	Curves linear; no equivalences detected			
0.005 *M* MOT	Ba titrants	Curve shapes badly defined in equivalence region			
0.0025 *M* NaLS	0.0249 *M* benzidine hydrochloride	2.60	8	0.01	0.3
0.0025 *M* MOT	0.0249 *M* benzidine hydrochloride	2.55	8	0.01	0.2

[a] Operational frequency, 30 MHz; sensitivity, 100 kHz; equiv wt of NaLS is 288.38 and of MOT is 444.57.

Titrant

0.1 M lithium chloride in ethanol. Dry "anhydrous" lithium chloride (mol. wt. 42.40) at 140°C for 24 hours, then allow it to cool in a vacuum desiccator over phosphorus pentoxide. Dissolve 4.24 g of the solid in anhydrous ethanol and dilute to 1 liter. The method of Lund and Bjerrum (*27*) is recommended for drying the solvent.

Procedure

Dissolve a sample of Manoxol OT, containing 0.125–0.25 mmole active ingredient, in 50 ml of anhydrous acetone. Transfer the solution into the dry titration cell, adjust operational frequency to 3–5 MHz, meter sensitivity to 30 KHz, and achieve zero beat by readjusting the frequency of the reference oscillator. Titrate with 0.1 *M* lithium chloride, dissolved in anhydrous ethanol. Using an automatic titrimeter, apply a burette speed of 2 ml min^{-1} and a chart speed of 5 cm min^{-1}. Obtain the titration curve; a sharp break indicates the end point.

Results

Some results are shown in Table 4.

THE APPLICATION OF MANOXOL OT AS A TITRANT FOR THE DETERMINATION OF ALKALOIDS

The idea of applying anionic tensides as titrants originates from Carkhuff and Boyd (*6*) and has been extended by others (*17*). An extrac-

TABLE 4

TITRATION OF ANIONIC TENSIDES: DETERMINATION OF THE CATION[a]

Reagent (50 ml in anhydrous acetone)	Titrant (in abs. ethanol)	Found (ml)	No. of results	SD	Coefficient of variation (%)	Operational frequency (MHz)
0.005 *M* MOT	0.1016 *M* LiCl	2.45	7	0.02	0.6	5
0.0025 *M* MOT	0.1016 *M* LiCl	1.25	10	0.01	0.7	3
NaLS	Insoluble in ketonic solvent					

[a] Sensitivity, 30 kHz.

tive technique is employed in conjunction with visual indication. The radio-frequency method, combined with the fact that Manoxol OT is available in pure form from which a standard solution can be readily made up (*1, 17, 26, 37*) offers advantages in this respect. Alkaloids form precipitates when titrated with Manoxol OT, and though the precipitation reactions are not adequately explained in the literature (*1*), it is known that the nitrogen atoms of the alkaloids play a special role in the process. Not all the nitrogen atoms in the molecules are necessarily active. For example the aminacrine hydrochloride molecule contains two nitrogen atoms, but only one of these is active. Alkaloid solutions are prepared in completely aqueous media at an acid pH since the presence of nonaqueous solvent causes solubilisation of the precipitates. However, the titrant is prepared in 3+1 (v/v) methanol + water to obtain a sufficiently concentrated solution. Aminacrine, papaverine, brucine, quinine, cinchonine, and aneurine can be determined with good accuracy.

Titrant

0.1 M Manoxol OT. Dissolve 44.457 g of anhydrous sodium dioctyl sulfosuccinate in 750 ml of methanol, add 200 ml of water, allow the mixture to cool to room temperature, then make up the volume to 1 liter with water.

Sodium Acetate–Acetic Acid Buffer (pH = 2.8)

Dissolve 4 g of anhydrous sodium acetate in water, dilute to 800 ml, add glacial acetic acid (about 150 ml) until the pH of the solution reads 2.8. Dilute with water to 1 liter.

Procedure

Dissolve a sample containing about $2.5 \times 10^{-4}/n$ moles of active ingredient (n is the number of active nitrogen atoms per molecule, cf.

Table 5) in water, add 10 ml of sodium acetate–acetic acid buffer and dilute with water to 50 ml. Transfer the mixture into the titration cell, adjust operational frequency to 30 MHz and the meter sensitivity to 100 KHz. Achieve zero beat by readjusting the frequency of the reference oscillator, then titrate with 0.1 *M* Manoxol OT. Using an automatic titrimeter apply a burette speed of 2 ml min^{-1} and a chart speed of 5 cm min^{-1}. Obtain the titration curve; a sharp break indicates the end point.

Results

A typical titration curve is shown on Fig. 5 and results are presented in Table 5.

SUMMARY

The advantages of radio-frequency end-point detection in titrimetric analysis are discussed, and the design of an automatic titrimeter, equipped with variable frequency and employing the heterodyne-beat principle, is outlined. While determinations of cationic tensides by titrating with anionic ones and vice versa cannot be carried out with sufficient precision, the titration of cationic tensides with silver nitrate, titration of sulphated anionic tensides wth barium acetate or benzidine hydrochloride, and the titration of the alkali metal ion in tensides with

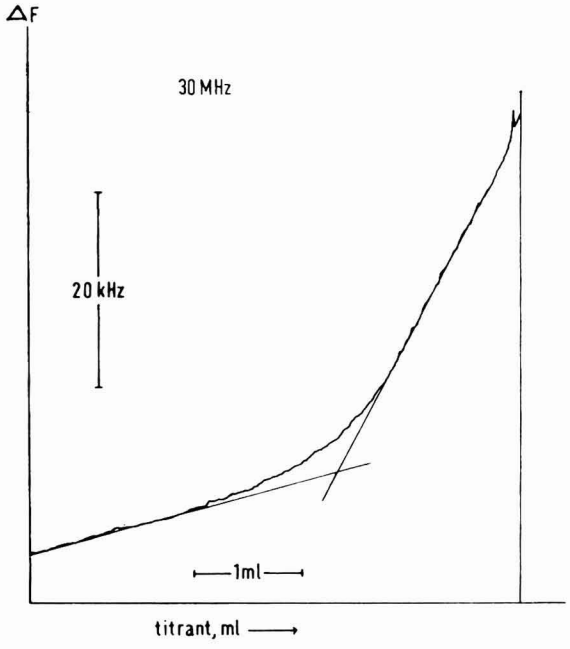


FIG. 5. Titration of 50 ml of 2.5×10^{-3} *M* cinchonine versus 0.1 *M* MOT: operational frequency, 30 MHz; sensitivity, 100 kHz; ΔF is the beat-frequency.

TABLE 5

TITRATION OF ALKALOIDS VERSUS ANIONIC TENSIDE

Reagent (50 ml/titration)	Molarity	Found (ml)	SD	Coefficient of variation (%)	No. of nitrogen atoms/molecule	No. of active nitrogen atoms, *n*
Aminacrine hydrochloride	0.0034	1.73	0.01	0.8	2	1
Papaverine	0.005	2.53	0.01	0.4	1	1
Brucine	0.005	2.62	0.02	0.7	2	1
Quinine	0.0025	2.61	0.01	0.4	2	2
Quinine sulfate	0.00125	2.52	0.01	0.4	4	4
Cinchonine	0.0025	2.56	0.01	0.4	2	2
Anuerine hydrochloride	0.00125	2.47	0.02	0.8	4	4

[a] Seven replicate titrations were carried out for each sample; titrant 0.0992 *M* MOT in 3 + 1 (v/v) methanol + water; operational frequency, 30 MHz; sensitivity, 100 kHz.

lithium chloride in nonaqueous solution are accurate, precise and simple analytical procedures. Manoxol OT can be employed as a titrant in the determination of alkaloids.

ACKNOWLEDGMENT

One of the authors (W.J.S.) thanks the Government of Northern Ireland for the provision of a research grant.

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MICROCHEMICAL JOURNAL 16, 52–56 (1971)

A Modified "Sandwich" Microapparatus for Horizontal Thin-Layer Chromatography

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Received August 15, 1970

The so-called "sandwich" developing chambers have been used for demonstrating ascending chromatography,[2] radial chromatography (*1*), descending chromatography (*2*), and electrophoresis on papers (*3*). The main advantages in using these chambers are (a) the low solvent requirement, (b) the excellent chamber saturation, and (c) the increased speed of separation of the components.

Brenner *et al.* (*4, 5*) used a horizontal development technique in which solvent was fed to one end of the thin layer by a filter paper wick. By allowing the solvent to evaporate from the other end of the thin layer, these workers made use of the so-called "continuous" development technique which resulted in the separation of substances that would otherwise have near-zero R_f values.

Modification of the Brenner and Niederwieser technique (*4*) resulted in the apparatus which is described presently and which was used to study the separation of the yellow, red, and blue components in the Camag[3] Dye Mixture No. 1.

The apparatus diagrammed in Fig. 1 is a modified "sandwich" because, instead of having a cardboard gasket around the three peripheries of a plate, a channel has been machined through the length of a plate. In the absence of a suitable milling machine, the channel in lower plate (A) may be formed by cementing one narrow strip of Lucite along each of the lengths of the plate.

Lower plate (A) and cover plate (B) are cut from colorless and trans-

[1] Please address communications for reprints, etc., to senior coauthor whose present address is 36 Oakridge Road, Bristol, Conn. 06010.

[2] Item No. 3093–B15 in Catalog No. 68, known as the Camag sandwich apparatus and obtainable from the Arthur H. Thomas Co., Vine St. at Third, P.O. Box 779, Philadelphia, Pa.

[3] Obtained from Arthur H. Thomas Co., address given in footnote No. 2.

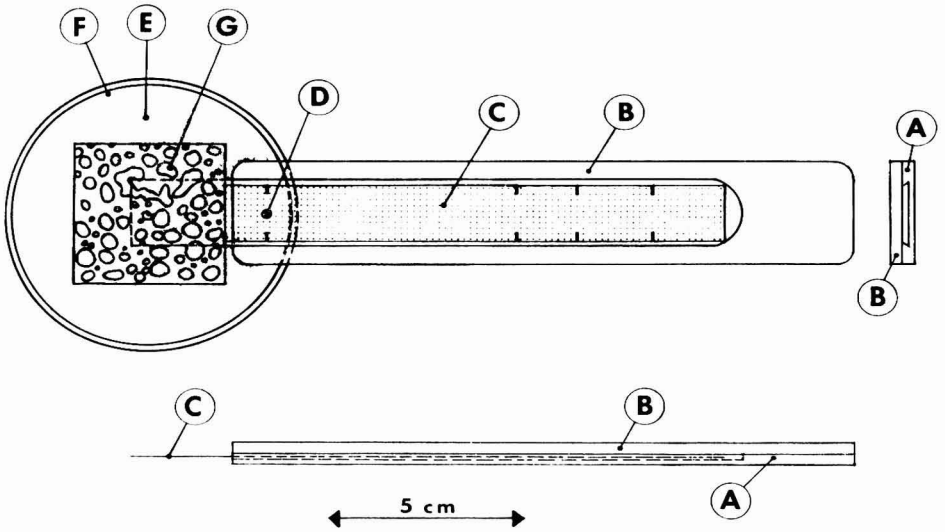

FIG. 1. Apparatus for horizontal thin-layer chromatography: (upper left) top view; (upper right) end view; and (bottom) side view.

parent ⅛-inch thick Lucite to a size of about 3 by 16 cm. A channel about 1.5 mm deep and about 20 mm wide is machined through the center line of lower plate (A) so that it may accommodate a strip of Eastman[4] Chromagram sheet (C) which has been cut to a size of about 2 by 15 cm.

To use the apparatus, the appropriate volume of the liquid test sample is applied by means of either a 1- or 2-μl spotting pipet[5] at a point (D) on the center line about 3 cm from one end of the sheet. After allowing the spot to dry in the air, about 1.5 to 2 mm of adsorbent is scraped off along each of the lengths of the sheet by means of a razor blade. Two short scratches are made near the long edges of the sheet to mark both the origin and the finish lines. The sheet is inserted beneath the two small grooves filed along the lower lengths of the channel in lower plate (A). (These grooves prevent the Chromagram sheet from buckling in the channel.) Cover plate (B) is aligned as shown in Fig. 1, and the resulting assembly is held intact by means of two bulldog clips. (Alternatively, the plates may be sealed permanently along three edges by means of an adhesive such as epoxy glue.) Next, 10 to 15 ml of de-

[4] Silica gel adsorbent thickness, 100 μ, Type K301R. Obtained from Distillation Products Industries, Division of Eastman Kodak Co., Rochester, New York.

[5] Obtained from Labindustries, Inc., 1802 Second St. Berkeley, California 94710.

TABLE 1

SUMMARY OF EXPERIMENTAL RESULTS

No.	Sample size (μl)	Solvent dist. (mm)	Av R_f values [a]			Approx develop. time (min)	Comments
			Blue	Red	Yellow		
1	1.0	65	0.28 ± 0.01	0.34 ± 0.01	0.72 ± 0.01	19–27	— [b]
2	1.0	80	0.32 ± 0.05	0.40 ± 0.05	0.75 ± 0.04	31–40	— [c]
3	1.0	100	0.38 ± 0.05	0.46 ± 0.08	0.78 ± 0.04	50–70	— [c, d]
4	2.0	65	0.36 ± 0.06	0.42 ± 0.08	0.77 ± 0.09	25–40	— [e]
5	2.0	80	0.33 ± 0.06	0.40 ± 0.05	0.77 ± 0.05	32–50	— [e]
6	2.0	100	0.34 ± 0.03	0.42 ± 0.05	0.78 ± 0.04	50–81	— [e]

[a] Averages and standard deviations calculated for 6 runs at each level.

[b] Best precision for all measurements.

[c] R_f values of yellow and red components relatively imprecise.

[d] Best resolution of all zones.

[e] R_f values of all components generally imprecise; poor resolution of red and blue zones.

veloping solvent (E) is added to the 3-inch watch glass (F), which also holds a cube (G) made from sponge [6] having dimensions of about 40 mm on an edge. A slit about 2 cm long and 2.5 cm deep is made along the center line of the face of the cube, and about 2.5 cm of the exposed end of the Chromagram sheet is inserted into the slit. The developing chamber is maintained in a horizontal position by means of a clamp. The timing is commenced when the solvent front reaches the origin and is terminated when it reaches the finish line. At the end of a run, the Chromagram sheet is removed from the apparatus and allowed to air-dry in a horizontal position. R_f values are calculated in the usual way.

The results in Table 1 indicate that the R_f values increase in the order: blue, red, and yellow components (*6, 7*) when benzene is used as the developing solvent.

Entry No. 1 in Table 1 indicates the conditions leading to the best precision for R_f measurements. By allowing the solvent migration distance to increase progressively from 65 to 100 mm (entries 1 to 3), better resolution of the red and blue zones is achieved but at the expense of the precision of the measurements.

Entries 1 and 4 reveal that doubling the sample size (a) greatly reduces the precision of the measurements, and (b) further impairs the resolution of the red and blue zones.

Entries 4 to 6 indicate that by allowing the migration distance to increase progressively as before, (a) a somewhat better resolution of the red and blue zones is observed, and (b) the precision generally improves.

Generally speaking, it was found that (a) the red zones give nearly circular spots, (b) the blue zones produce tailing, and (c) the yellow zones show sidewise migration.

ACKNOWLEDGMENTS

The authors are indebted to the personnel of the Department of Industrial Arts at Plainville High School for technical assistance received and also to the Division of Chemical Education-DuPont Small Grants Program for partial financial support received during this investigation.

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MICROCHEMICAL JOURNAL **16**, 57–67 (1971)

The Introduction of Microtechniques in Elementary Science Courses

I. Blood Glucose Determination

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Received August 21, 1970

The professional chemist looks upon microanalytical techniques as a highly valuable tool by which much information can be gained from very little material. In most high school and first year college curricula there is little or no emphasis on this form of analysis while laboratory time is given over to macrotechniques of analysis. A feeling seems to prevail that a certain maturity is needed before students can master the techniques of microanalysis. For this or similar reasons, these procedures are usually introduced no earlier than the second or third year of college chemistry in connection with a course in Analytical Chemistry. It must also be realized that today many graduates of high schools will not enter colleges but will be trained for technological work in special programs that are organized to give the greatest amount of training in a given period of time. These training programs perform a valuable service to their students and to the future employers of these students. However, with today's use of automated apparatus it is especially important for students entering such training programs to understand and have some experience with the theory and techniques that are basic to the procedures that they will perform. The authors are of the opinion that microtechniques can be profitably introduced much earlier in the curricula of both chemical and biological studies, even in high school science courses.

To prove the worth of these early introductions to microanalytical procedures a quantitative microanalytical technique utilizing three different methods was taught in a combined investigative venture involving first year college students enrolled in a biology and a chemistry class. The blood glucose levels of albino mice were determined simultaneously in the laboratory experiments of each class using the Folin micromethod for blood glucose analysis (*1*). The procedure was repeated

during a combined laboratory session to determine the glucose tolerance curve of one of the students.

When the colored solutions had been prepared by the students, the milligrams of glucose per 100 ml of blood (mg %) were determined by using each of three instruments, the spectrophotometer, the photoelectric colorimeter, and the color-comparison block. The values obtained by the three means were then compared.

The reagent solutions were prepared by the instructors ahead of time, as was the standard stock glucose solution. A set of standard dilute glucose solutions was also prepared against which the students' dilute standards were checked.

Since small blood samples (0.1 ml) were used in this procedure, any errors in the techniques were magnified, thus giving false blood glucose readings. Accuracy in measurement was therefore essential to the success of this technique. Micro volumetric pipettes were required for such accuracy. The technique for the proper use of the pipettes, such as the positioning of the tip under the solution to avoid sucking the solution into the mouth, the measuring of the meniscus on the etched line, and the wiping of the pipette tip with a piece of paper tissue before transferring the solution were demonstrated to the students. The students were also shown the proper balancing and operation of the clinical centrifuge. Procedure for taking the blood samples was demonstrated as well.

MATERIAL AND METHODS

Blood Drawing Procedures

About 1.5 inches of the mouse tail was cut off with a sharp scissors, and the 0.1 ml of blood was drawn from the bleeding stump with a calibrated micropipette. The stump was then cauterized with a hot flat blade of a scalpel to prevent infection. The students worked in pairs and performed the test on a normal mouse and on five mice who had received 2 units of insulin 1 hour prior to the test. An alternate method of obtaining the blood sample is to draw up a few drops of blood from the caudal artery using a sterile No. 22-gauge needle and syringe.

A student volunteered to come to class in a fasting condition. One of his fingers was placed in moderately hot water for a few minutes to stimulate circulation in the finger. The finger was wiped dry and was then wiped with a gauze sponge saturated with a 70% ethyl alcohol solution. A sterile lancet was used to prick the finger to a depth that permitted free flow of blood. The first drop of blood was wiped away with a sterile gauze pad (the first drop is usually diluted with tissue fluid). Blood was then taken from the student's finger in a manner similar to

that used to collect blood from the mice. The finger was wiped with the alcohol sponge and pressure was applied with a sterile dry gauze pad to halt the bleeding. This blood drawing process was repeated at time intervals following the ingestion of a 100-g glucose meal (in water flavored with lemon juice) by the student.

Test Procedure

Reagents. Sulfate-tungstate solution, containing 20 g of sodium sulfate and 30 ml of 10% sodium tungstate solution/liter.

Potassium ferricyanide solution, containing 4 g/liter (store in dark brown bottle).

Sodium cyanide-carbonate solution, containing 16 g of sodium carbonate and 300 ml of 1% sodium cyanide/liter.

Ferric gum ghatti or ferric polyvinyl alcohol solution, containing a saturated solution of the ferric compound, 5 g of ferric sulfate, 15 ml of 1% potassium permanganate solution, and 75 ml of 85% phosphoric acid.

Stock standard glucose solution, containing 1 g of benzoic acid and 980 mg of anhydrous dextrose/liter.

Standard dilute glucose solutions were prepared containing the following amounts of stock standard glucose solutions diluted to 100 ml with aqueous 0.1% benzoic acid solution (ml): 0.70, 1.0, 1.2, 1.3, 1.5, 1.7, 2.0, and 2.2. These corresponded to 70, 100, 120, 130, 150, 170, 200, and 220 mg % blood glucose. (The difference between the actual mg % glucose and the assigned mg % glucose in these standards allows for the dilution of 0.01 ml of blood to a volume of 5.1 ml.

Method. 1. Transfer 4 ml of the sulfate-tungstate solution to a clean, dry centrifuge tube.

2. Collect exactly 0.1 ml of blood from the mouse tail. Transfer this at once to the solution in the centrifuge tube. Rinse the pipette with the solution several times to draw out all the blood. Let stand for 15 minutes.

3. Add 1 ml of the acid sulfate solution and stir with a thin glass stirring rod.

4. Centrifuge for 5 minutes.

5. Transfer 2 ml of the clear supernatant solution in the centrifuge tube to a test tube graduated to 25 ml; and add 2 ml of distilled water.

6. To similar test tubes add 4 ml of the dilute standard dextrose solutions.

7. To a similar test tube, add 4 ml of 0.1% benzoic acid to act as a reagent blank.

8. Add 1.5 ml of the potassium ferricyanide solution and 1 ml of the

cyanide-carbonate solution to each of the tubes containing standards, test solution, and blank.

9. Heat the test tubes in a beaker of boiling water for 8 minutes, cool, and add to each tube 5 ml of the ferric gum ghatti solution.

10. Dilute the contents of all tubes to 25 ml and mix thoroughly. Colors are relatively stable after 10 minutes for about 1 hour.

Colorimetric Procedure

The photoelectric colorimeter used in these tests was of the simple single-beam variety (see Fig. 1), consisting of a tungsten filament bulb, a lens to give a parallel beam of light, a variable filter for selection of specific wave lengths, a cuvette holder, and a photovoltaic cell (Bausch and Lomb, Spectronic 20). The amount of light passing through the cuvette activates the photovoltaic cell and the current produced in this *cell* is measured with a microammeter. The reading of the scale of the microammeter gives direct readings of percentage transmittance (%*T*) on the upper scale and the negative logarithm of the percentage transmittance, optical density (OD), on the lower scale. A cuvette containing only the reagent blank was placed in the light path and the power produced by the light passing through this solution was adjusted until the microammeter needle indicated 100% T (zero OD). This procedure automatically subtracts any color in the reagent blank from the reading of the sample leaving only the color produced by the reaction of the reagents with the glucose as significant. Samples of the standards and unknown solutions were then placed in the cuvette and the OD was read directly from the scale. A standard curve was drawn by plotting the OD of each of the eight standards against its corresponding concentration

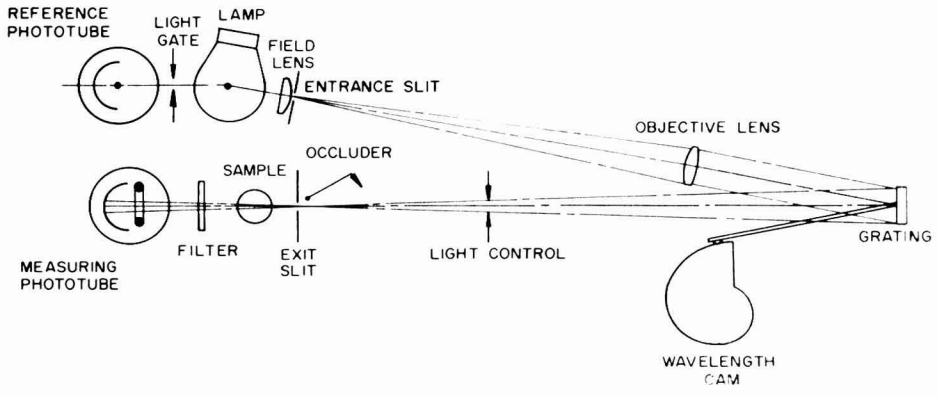

FIG. 1. Photoelectric colorimeter (Courtesy Bausch and Lomb Co.).

(mg%) glucose. The glucose levels of the unknowns can then be read directly from the standard curve.

The type of spectrophotometer used was a Spectronic 505 (Bausch and Lomb Inc., Rochester, N.Y.). This instrument has a tungsten light source that provides continuous radiation throughout the range of 320 to 800 mμ. This instrument operates on the split beam principle. The intensity of the two beams are compared and the radio is recorded [T(or OD)] on a recorder chart attached to a revolving drum. The wavelength selector can be coupled with the motor revolving the recorder drum to change wavelengths automatically as the drum revolves. Instruments of this sort greatly reduce the time required to obtain a complete absorption spectrum of a solution since the wavelengths can be changed automatically as the drum rotates (see Fig. 2).

To operate this instrument, the "on-off" switch is turned to the "on" position. The lamp selection lever on the light housing is set at "tungsten" and the "emission–absorption" switch is set to "absorption." For maximum accuracy, a 30-minute warmup time is allowed before recording a sample. In the meantime, the chart paper can be inserted on the recording drum and the ink level in the recording pen can be checked. The equipment is then ready to use.

The reagent blank was placed in the rear cuvette of the sample compartment; and the sample to be read was placed in the forward cuvette.

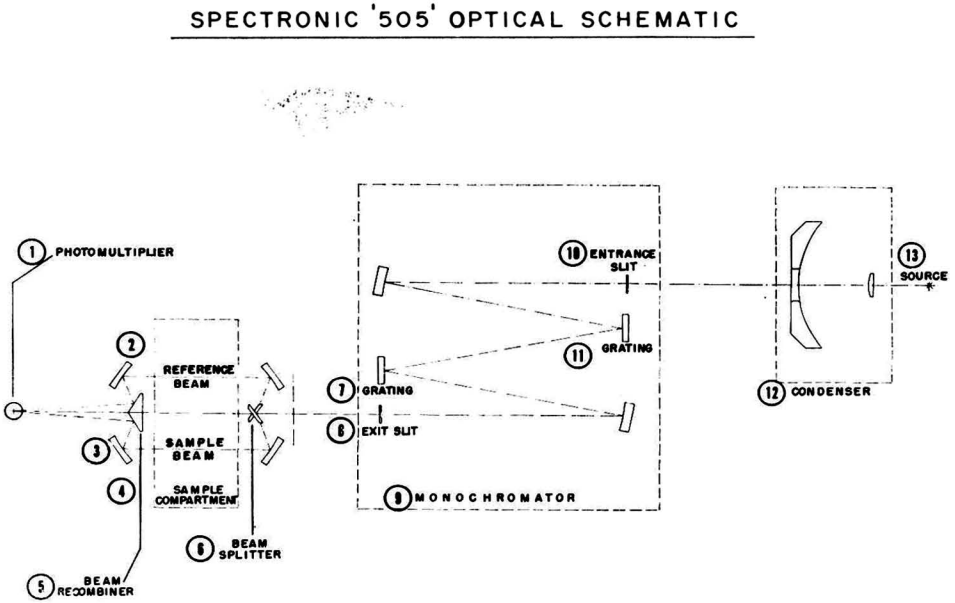

FIG. 2. Spectrophotometer (Courtesy Bausch and Lomb Co.).

This automatically subtracts the reading of the reagent blank from the reading of the sample, and eliminates the color of the reagent solutions from the color caused by the glucose in the sample.

Since glucose levels were being read for the first time on this instrument, it was necessary to scan the wavelengths to find the point of optimum absorption of the green-blue color of the glucose solution. This scanning was done with the 100 mg % glucose standard solution in the forward cuvette and the reagent blank in the rear cuvette. Scanning was accomplished by setting the wavelength selector at 320 mμ and then re-engaging the automatic drive gear. For moderately colored liquids, the instrument is run with the photometric range switch set at 100% transmittance. For very dark solutions, 10% transmittance can be used, while for very light solutions, 200% transmittance can be selected. By pressing the "record" button, one can start the drum revolving and the pen will respond to changes in the amount of light transmitted through the solution in the forward cuvette. A graph is drawn on the chart paper on the recording drum and, from it, it was possible to determine that the point of maximum absorption was 490 mμ, the same wavelength as was found to be optimum on the other colorimeter. A reproduction of the absorption graph is shown in Fig. 3.

The wavelength selector was set at 490 mμ, the drive gear was disengaged from the wavelength selector and all further reading was done at

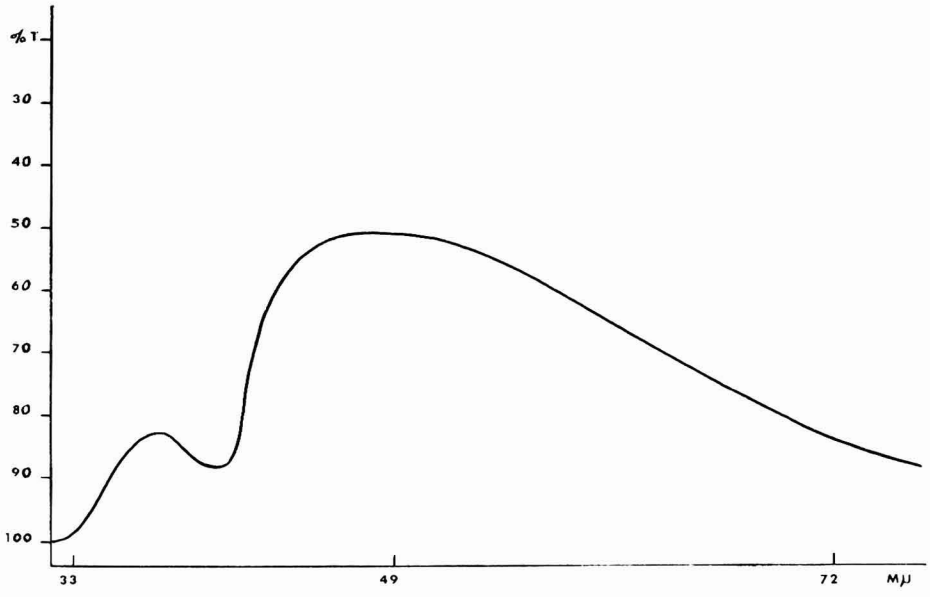

FIG. 3. Glucose standard absorption curve.

490 mμ. The readings were made when solutions to be analyzed were placed in the forward cuvette while the rear cuvette still held the reagent blank solution. By pressing the "record" button, the recorder drum was set in motion and the recorder pen moved up the chart paper in a straight line to a point that corresponded to the amount of light that was being transmitted through the forward cuvette. Thus, a series of readings were made where the pen recordings corresponded to the density of color in the standard solutions (see Fig. 4). The standard curve of the glucose solutions could then be drawn by plotting these readings against the concentrations of glucose that the solutions represented (see Fig. 5). The unknown glucose solutions were determined from the standard curve.

A color-comparator block was made by boring two rows of holes with three holes in each row in the top of a rectangular piece of wood 3 × 5 × 2.5-inches (*2*). These holes stop short of penetration through the bottom of the block. The paired holes were as close together as possible and just large enough to hold standard Pyrex test tubes. Smaller holes were then bored perpendicularly to these, an inch from the bottom, passing completely through both sides of the box and through the other holes (see Fig. 6).

A test tube containing an unknown glucose solution was placed in the middle of the front row of the three holes. The standard solutions

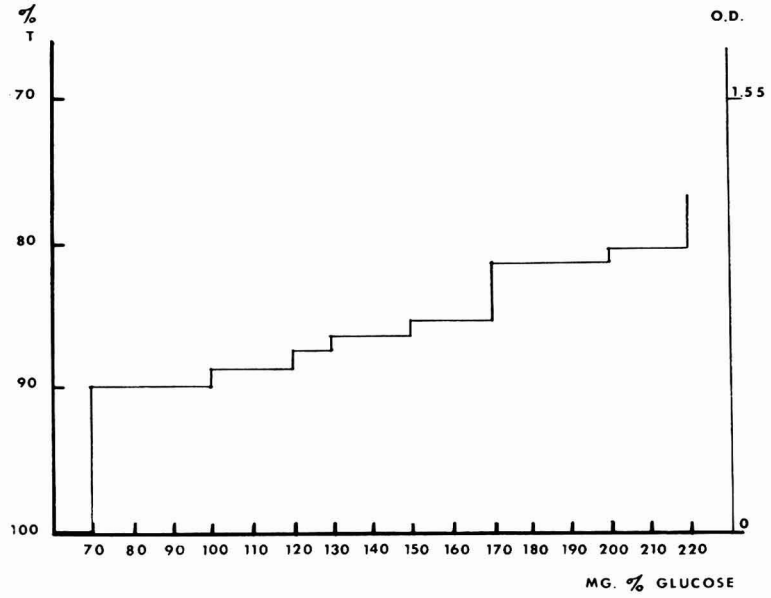

FIG. 4. Pen readings of glucose standard solutions.

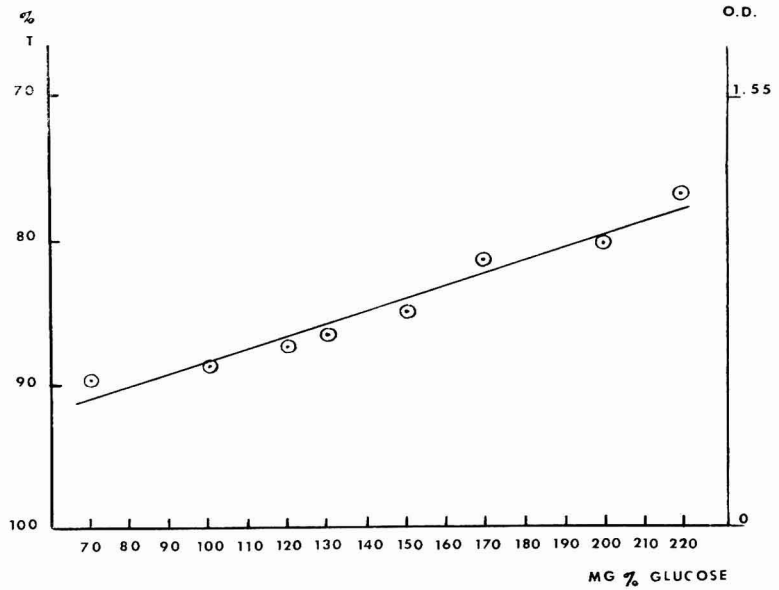

FIG. 5. Standard curve for glucose standards.

were placed in separate test tubes. A pair of standard tubes were placed in the outer two holes of the comparator block in the following sequence:

(mg %)		(mg %)
70	and	100
100		120
120		130
130		150
150		170
170		200
200		220

A white light source was placed behind the comparator block at the level of the small holes and the readings were nearest in depth of the color to the unknown were selected in this way and the amount of difference between the nearest standard and the unknown was approximated. The glucose (mg %) in the unknown solutions was approximated in this manner.

If an unknown solution possesses a color of its own, untreated samples of the unknown can be placed in the second row of holes immediately behind the two standards so that this extraneous color can be considered when comparing the colors of the standards to the unknown. When this procedure is necessary, a tube of distilled water is placed be-

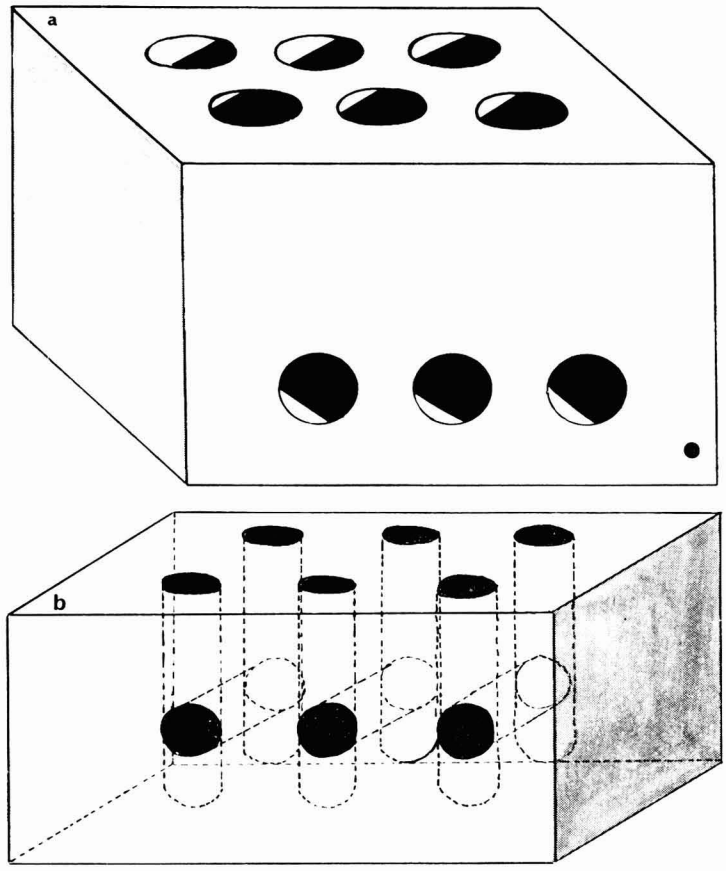

FIG. 6. Color-comparator block: (a) exterior appearance; (b) details.

hind the tube containing the unknown. If the unknown solution has no color except that developed by the testing reagents, the second row of holes is left empty.

EXPERIMENTAL RESULTS

The normal blood glucose level of the mouse is around 138 mg %. Table 1 shows that all of the insulin-treated experimental mice were hypoglycemic.

The results obtained by the three methods compared well with one another, although the color-comparator block readings were not as accurate as those obtained by the other two methods (Tables 1 and 2). Greater accuracy could have been obtained with the comparator block had a greater number of standards been prepared with smaller intervals between any two standards.

TABLE 1

COMPARISON OF THE MOUSE BLOOD GLUCOSE LEVELS (mg %) BY THE THREE METHODS

Unknown solution no.	(mg %)		
	Spectrophotometer	Photo-electric colorimeter	Color-comparator box
1 (control mouse)	130	130	130
2	68	68	70
3	92	92	100
4	110	110	115
5	80	80	90
6	102	102	100

Other laboratory animals could have been used to demonstrate microanalytical techniques and the use of analytical instruments. The normal blood glucose levels for some of these animals are:

	(mg %)
Chick	198
Hen	179
Ferret	95
Frog (*Rana pipiens*)	0.04–0.07
Mackerel	63
Rabbit	136
Toadfish	15
Turtle	76

Educational Implications

The microanalytical techniques proved no barrier to proper performance of the tests attempted. The attention to detail in measuring and transferring the smaller amounts of material soon became routine.

TABLE 2

COMPARISON OF THE HUMAN GLUCOSE TOLERANCE CURVE BY THE THREE METHODS

Time (hour)	(mg %)		
	Spectrophotometer	Photo-electric colorimeter	Color-comparator box
Fasting level			
0.5	103	103	105
1	132	132	135
1.5	93	93	95
2	102	102	110

It is felt that experiments such as those outlined in this paper could be introduced as early as the junior year in high school. With this in mind, the following suggestions are included for the use of high school instructors wishing to introduce microanalytical techniques into the laboratory experiences of their students.

Adaptations of Methods for High School Use

The authors of this article realize that all school systems will not have the exact equipment used in this article. They would like to suggest that comparisons of any equipment available provide a worthwhile learning experience since it teaches students the fact that errors in methods and techniques are still the greatest source of false results. No amount of expensive instrumentation can correct an error in the measurement of the first sample of a material to be tested. Many times an older and, perhaps less exact, instrument will give fairly accurate readings if precision and care are exercised in the preparation of the material to be analyzed.

In those school systems where neither the students themselves nor an animal may be used for small experiments such as outlined in this article, the authors would like to suggest that the following fluid be used in place of blood: 8.5 g of sodium chloride; 70.0 g of albumen (egg or other); dissolve in distilled water and make to 1 liter. "Blood" glucose solutions may be made up from this solution by placing weighed amounts of glucose (from 50 to 250 mg) in 100-ml volumetric flasks and diluting to the proper volume with the sodium chloride–albumen solution. Food dye can be added (red for whole blood or yellow for plasma) to give the resulting solutions a more realistic appearance.

ACKNOWLEDGMENT

The authors express their thanks to Dr. Jean Lane of the Art Department of Jersey City State College for her help with diagrams. Figures 1 and 2 are reproduced through the courtesy of Bausch & Lomb.

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MICROCHEMICAL JOURNAL **16**, 68–76 (1971)

Microprocedure for Serum Triglyceride Estimation

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Received August 27, 1970

The Van Handel and Zilversmit method (*12*), as modified by Carlson and Wadström (*3*), which measures the formaldehyde produced by oxidation of liberated glycerol is a commonly used procedure for triglyceride estimation in serum. Reproducibility is difficult with this method because of the fact that the procedure is time consuming and elaborate. Procedures which employ the condensation of triglycerides with hydroxylamine and estimation of the hydroxamic acids as the ferric complex, yield results which are elevated, due to interference from other esters (*1*). The fluorometric procedure, using the condensation of acetyl acetone with ammonia and the formaldehyde liberated, also yields elevated results, as practiced (*4, 8*). This is especially true when the patient shows an elevated glucose level.

The enzymatic procedures proposed are specific for glycerol but do not solve, in a convenient manner, the problem of extracting the triglyceride free of other substances and hydrolysis to liberate the glycerol (*5, 6*). Other procedures such as by gas chromatography (*9, 13*) and thin-layer chromatography (*10, 14*) have not been shown to be practicable for quantitation, in the routine laboratory. The nephelometric procedures are unreliable (*2*).

With the above experiences in view, it was decided to explore the possibility of modifying the method of Carlson and Wadström so that it could be made simple and practical for the routine laboratory on small initial samples.

The Folch *et al.* (*7*) procedure for the extraction of triglycerides is effective but requires the washing out of the methanol, usually in separatory funnels. This was eliminated by using a direct method of extraction into chloroform in the presence of 1 *N* H_2SO_4. This technique has been successfully used for cholesterol extraction for many years (*15*). This system had the added advantage that water-soluble substances

[1] Supported in part by the Morris Natelson Research Fund.

such as glucose, which interfere with the test, are removed. Attempts to use a method described, which extracts the triglycerides with alcohol, and removes phospholipids with a $Ca(OH)_2$, Zeolite, and Lloyd's reagent mixture failed, when high glucose levels were present in the serum (*11*). Glucose was not completely removed in this procedure.

The method of hydrolysis was studied and conditions were found for quantitative hydrolysis. One of the problems which presented itself was the removal of fatty acids after hydrolysis, to avoid turbidity. In the original procedure, this required acidification and evaporation to dryness, addition of ether, and extraction of the fatty acids with petroleum ether. This problem was solved by adding barium hydroxide to the KOH solution during hydrolysis. On acidification with sulfuric acid, the barium sulfate formed, absorbs the fatty acids which can now be separated by centrifugation. This saves considerable time in the procedure.

The final procedure developed, uses 100 μl of serum. Smaller quantities are also readily used by a slight modification of the procedure. The triglycerides are extracted directly into chloroform in a test tube. Silica gel removes the phospholipids from the chloroform extract. An aliquot of the chloroform extract is evaporated to dryness. Alcoholic KOH, containing barium hydroxide, is used to hydrolyze the esters. Addition of sulfuric acid serves to precipitate the fatty acids, adsorbed to the barium sulfate. These are removed by centrifugation. The color is then developed by oxidation of the glycerol, in the supernatant, to formaldehyde.

MATERIALS AND METHODS

Reagents

Chloroform. Analytical reagent grade.

Sulfuric acid (4 N). Add 107 ml of conc H_2SO_4 analytical reagent, to approx 600 ml of water. Make up to 1 liter. Stable for months. Dilute fourfold to make 1 *N* H_2SO_4.

Silicic acid. Heat silicic acid (Mallinckrodt, 100 mesh powder, $SiO_2 \cdot H_2O$) in a flat dish in a 100°C oven for 2 hours. Allow to cool in a vacuum desiccator and store in a tightly stoppered bottle. Keep in a desiccator over sulfuric acid.

Ethanol. Absolute, analytical reagent grade.

Potassium hydroxide–barium hydroxide solution. Mix equal volumes of (a) and (b) just before use.

(a) Potassium hydroxide (4 *N*): Dissolve 22.4 g of KOH, analytical reagent in water and make up to 100 ml. Stable if kept in a tightly closed polyethylene bottle.

(b) Barium hydroxide, saturated: Shake approximately 10 g of

$Ba(OH)_2 \cdot 8H_2O$ analytical reagent with 100 ml of water in a polyethylene bottle. Stable if prevented from absorbing CO_2.

Sodium periodate (0.02 M). Dissolve 428 mg of $NaIO_4$(anhydrous) analytical reagent, in water and make up to 100 ml. Stable in the refrigerator for at least 1 month.

Sodium arsenite (0.2 M). Dissolve 2.6 g of sodium meta-arsenite ($NaAsO_2$), analytical reagent, and make up to 100 ml with water. Stable at room temperature for at least 2 months.

Chromotropic acid reagent. Add 300 ml of conc H_2SO_4, analytical reagent, to 150 ml of water, and cool to room temperature. Dissolve 1 g of chromotropic acid (4,5-dihydroxy-naphthalene-2,7-disulfonic acid, disodium salt, Eastman Kodak) in 100 ml of water and filter. Add the diluted sulfuric acid, slowly, to the chromotropic acid solution. Keep refrigerated in a glass stoppered, dark colored bottle. Stable for 2 months.

Tristearin standard (2.5 mg/100 ml). Dissolve 250.0 mg of tristearin (Sigma, highest purity) in chloroform and make up to 100 ml. Dilute 1 ml of this stock standard to 100 ml with chloroform (working standard). The solution is stable if evaporation of chloroform is prevented.

Glycerol standard (0.5 mg/100 ml). Dissolve 526 mg of glycerol (reagent grade, Mallinckrodt, 95%) in water, and make up to 1 liter. Dilute 1 ml of the stock solution with 1 *N* sulfuric acid to 100 ml for the working standard. Stable for 2 weeks in the refrigerator. This standard is not the primary standard. It is used daily to check the efficacy of the reagents for oxidation and development of color.

Method

To 100 μl of serum in a 15-ml centrifuge tube, fitted with a ground glass stopper, add 4 ml of 1 *N* H_2SO_4 and 4 ml of chloroform. Stopper tightly and shake on the shaking machine for 10 minutes. Centrifuge and aspirate the aqueous top layer and the protein button (middle layer). Filter the chloroform layer through a filter paper (4-cm diameter, Whatman no. 40) into a second centrifuge tube that contains 200 mg of dried silicic acid. Shake for 10 minutes on the shaking machine. Centrifuge for 3 minutes at 2000 rpm in an International no. 1 centrifuge.

Evaporate a 2-ml aliquot of the chloroform solution to dryness in a calibrated centrifuge tube. This is done by placing a rack of test tubes in a stainless steel pan containing lukewarm water, in the hood, and raising the temperature gradually to 75–80°C with a Bunsen burner. When the chloroform is almost all evaporated, the temperature is raised to boiling. In another calibrated centrifuge tube place 2 ml of a 2.5 mg/100 ml tristearin solution in chloroform and evaporate to dryness. This is the standard.

Dissolve the residue in 0.4 ml of ethanol, add 0.1 ml of KOH–$Ba(OH)_2$ solution and mix well. Cap the tubes with loose Teflon caps. Incubate for 30 minutes in a 75–80°C water bath. Set up a blank using 0.4 ml of ethanol and 0.1 ml of the KOH–$Ba(OH)_2$ solution. Treat as for the unknown. This is the blank for the unknown and the tristearin. Cool the tubes to room temperature and make up to the 1-ml mark with 4 *N* H_2SO_4. Mix well, allow to stand for 10 minutes, and centrifuge.

To 0.5-ml aliquots of the supernatants, to 0.5 ml of the 0.5 mg/100 ml glycerol standard, and to 0.5 ml of 4 *N* H_2SO_4 for the glycerol standard blank, add 0.1 ml of sodium periodate solution. Mix, and let stand for 10 minutes. Add 0.1 ml of arsenite solution to all tubes and mix. Add 3 ml of chromotropic acid reagent to each tube. Mix well, and heat in a heating block, set to 105–110°C, for 30 minutes. Cool to room temperature and read the color at 575 nm (no. 56 filter in the Klett-Summerson colorimeter) using water to zero the instrument. A control serum (e.g., Metrix) with a known triglyceride content is run through the same procedure.

Calculations

With the glycerol standard:

$$\frac{\text{Abs. unknown} - \text{Abs. blank}}{\text{Abs. glycerol} - \text{Abs. glycerol blank}} \times 95 = \text{mg of triglycerides/100 ml.}$$

With the tristearin standard:

$$\frac{\text{Abs. unknown} - \text{Abs. blank}}{\text{Abs. tristearin} - \text{Abs. blank}} \times 98 = \text{mg of triglycerides/100 ml.}$$

RESULTS

The proportionality formula as used in the calculations is based on the observations shown in the line of best fit drawn in fig. 1. It is to be noted that the Beer-Lambert law is obeyed. The factors are obtained by first determining the amount of serum which finds its way into the final tube. In the procedure, 100 μl of serum is used. One half aliquot is then taken for hydrolysis. Subsequently, half of this is taken for color development. The color is then developed on 25 μl of serum.

It is assumed that the average molecular weight of the triglycerides is 875. Glycerol has a molecular weight of 92. The 0.5 mg/100 ml glycerol standard is therefore equivalent to an $875/92 \times 0.5 = 4.755$ mg/100 ml of triglycerides. In the procedure, 0.5 ml of the glycerol standard and 25 μl of the serum found its way into the final tube. This factor $0.5/0.024 = 20$ is the factor needed for correction for differ-

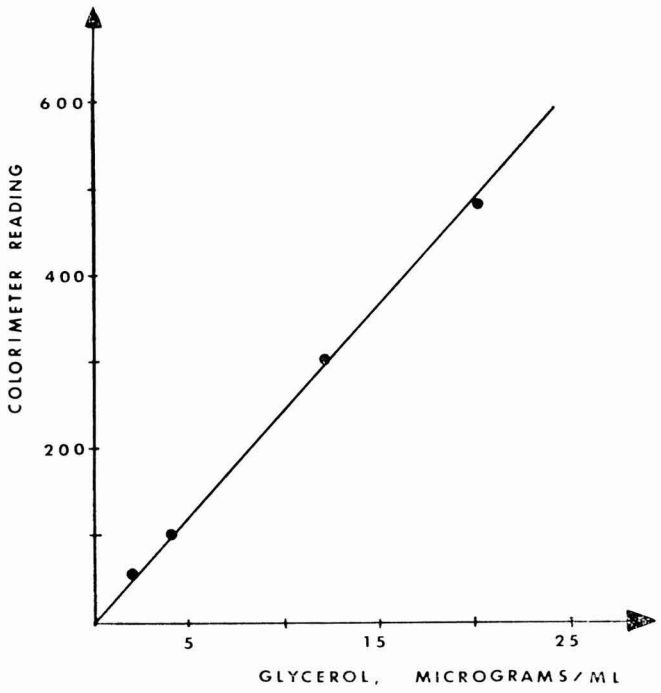

FIG. 1. Plot of glycerol concentration vs Klett-Summerson colorimeter readings (absorbance × 500) using the procedure described.

ences in volume of the serum and standard. The factor then becomes 4.755 × 20 = 95.1. This standard is useful for checking the reagents used in color development. Its exact value is calculated against the tristearin standard.

Using the tristearin standard, 2 ml of 2.5 mg/100 ml solution is evaporated. Also 2 ml of chloroform containing the extract from 50 μl of serum is evaporated in the same way. The factor is then, 2/0.05 × 2.5 = 100. However, tristearin has a molecular weight of 891.45 as compared to 875, generally accepted as the average molecular weight of the triglycerides. The factor then becomes 875/891.45 × 100 = 98.1. The tristearin standard is used as the primary standard.

A study of the hydrolysis procedure, using tristearin as the test substances, indicated that approximately 20 minutes are required for complete hydrolysis. Tristearin was chosen since it is the most difficult to hydrolyze, of the triglycerides. In the procedure, 30 minutes is recommended.

Using this procedure, Table 1 indicates that the results on a human control serum are reproducible to approximately ±9% (±2 SD). In

TABLE 1

STUDY OF THE REPRODUCIBILITY OF THE TRIGLYCERIDES METHOD

Specimen	No. of test	Mean (mg/100 ml)	SD
Metrix [a] Lot no. 5419	12	94	±4.75

[a] Triglyceride content, method of Van Handel and Zilversmit: 101 mg/100 ml, SD ± 10; Kessler and Lederer: 107 mg/100 ml, SD ± 11.

our experience, this was more reproducible than either the Van Handel–Zilversmit method, or the Kessler–Lederer procedure.

The efficacy of the absorbance of phospholipids, from the chloroform extract, was measured by dissolving 100 mg of lecithin in 100 ml of chloroform. One ml of his solution was evaporated to dryness and digested, 44 μg of phosphorus being recovered. This is close to the theoretical amount of 44.4 μg. Two ml of the solution was then shaken with 200 mg of silica gel, as in the procedure. One ml of the supernatant was evaporated to dryness and the total phosphorus content was determined. No measurable lipid phosphorus was detectable in the chloroform residue, after silica gel adsorption.

The distribution of tristearin levels were studied, by this procedure, on 32 apparently normal, fasting adults, taken at random. When the data was fitted to a curve of distribution, a log normal distribution most closely approximated the results. The normal range, as determined by this method was then 52–141 mg/100 ml (Table 2). This compares with a range of 29 to 134 mg/100 ml observed by Carlson and Wadström with the more elaborate procedure.

Table 3 lists the results obtained by extracting with 4 ml of chloroform containing 200 μg of tristearin. Twenty recovery experiments were run, in this manner. As shown in Table 3, recovery was in the range of ±15% (±2 SD).

DISCUSSION

This study comprises the adaptation of two basic changes in the Carlson–Wadström method for triglycerides. These include changes in the method of extraction and in the procedure for removal of fatty acids.

In the Folch method of lipid extraction, the serum is heated with methanol and chloroform, both of which are volatile liquids. The protein is filtered off. The methanol is then washed out with water, in a separatory funnel. This requires that the chloroform be made up to a known volume so that an aliquot can be taken. The alternative is to

TABLE 2

RANGE OF TRIGLYCERIDE VALUES FOUND ON FASTING SERA OF 32 HEALTHY PERSONS

Arithmetic mean (mg/100 ml)	Geometric mean (mg/100 ml)	Range [a] (2 SD) (mg/100 ml)
89	86	52–141

[a] Log normal distribution.

rinse the separatory funnel with chloroform and dry the chloroform. After removal of the drying agent, the chloroform may then be evaporated to dryness, to collect all of the lipid. This may be dissolved so that a suitable aliquot may be taken. It is apparent that this is a time-consuming procedure which requires careful attention to details if the aliquot to be taken is to be accurate.

The present method simplifies this procedure. When a mixture of dilute sulfuric acid, chloroform, and serum are shaken and centrifuged, the protein will be precipitated and held in a "button" at the interface of the two liquids. During centrifugation, the aqueous layer prevents chloroform evaporation. After aspiration of the acid and button, an accurate chloroform aliquot may be readily taken. This whole procedure takes only a fraction of the time required for the Folch extraction. As pointed out above, the procedure has been practiced routinely, for many years. It also eliminates the possibility, inherent in the Folch extraction, of methanol being carried over to the oxidation procedure resulting in the formation of formaldehyde on oxidation.

An added advantage of this procedure is that the compactness of the protein button is related, approximately, to the triglyceride level. This serves to warn the operator when high results are to be expected. With elevated triglyceride levels, the protein button is wider and with very high levels, it appears that incomplete separation of the chloroform and acid solution takes place. In this case, an equal volume of chloroform and additional acid solution is added. The tube is shaken by hand and recentrifuged. The same aliquot is taken and the results are multiplied by a factor of two. This keeps the readings in the linear range of the

TABLE 3

RECOVERY OF TRISTEARIN ADDED TO CHLOROFORM EXTRACT

No. of specimens	Added (μg)	Recovered (μg; mean)	SD
20	200	201.6	±14.6

calibration curve. Where the serum is lipemic, a 25-μ sample is used, and the factor is multiplied by four.

The fatty acids must be removed or there will be turbidity in the final solution whose absorbance is to be measured. The conventional procedure is to neutralize the alkali and evaporate off the alcohol used during hydrolysis. The residue is dissolved in water and acidified, the fatty acids being extracted with an organic solvent. Separation of the solvent is then required. This is a slow laborious process. By the addition of barium hydroxide to the alkali, the hydrolysis rate is actually accelerated, probably due to the formation of the insoluble barium soaps. This permits the precipitation of the fatty acids, adsorbed to barium sulfate.

The color is developed in the supernatant, after precipitation of the fatty acids. This contains a substantial percentage of ethanol. This does not interfere with color development procedure. Blank readings are reproducible and relatively low compared to those of the unknown. The alcohol is required since barium hydroxide solution alone or aqueous KOH solutions required prolonged heating times in order to complete the hydrolysis. Long heating times are objectionable, especially since the glycerol formed is partially destroyed. It is important to use an analytical grade of absolute ethanol. The presence of methanol or other denaturants such as pyridine will produce high blanks.

The introduction of the improvements discussed above permits the analysis to be carried out in test tubes. Sample size is 100 μl. Smaller amounts, such as 25 μl, can also be used, reducing the amount of reagent used proportionally. The over all time for this procedure permits the analysis of approximately 20 specimens in 3 hours, depending upon the equipment availability. In routine use, for many months, the blanks have remained fairly constant provided the chromotropic acid reagent was less than 2 months old. Standard readings remained remarkably constant.

SUMMARY

A microprocedure is described for estimating the triglycerides in blood serum. 100 μl are shaken with a mixture of 1 *N* sulfuric acid and chloroform. On centrifugation, the protein precipitates at the interface of the two solvents, the triglycerides dissolving in the chloroform. Phospholipids are removed with silica gel. An aliquot of the chloroform is evaporated. Hydrolysis is carried out in the presence of a mixture of KOH and $Ba(OH)_2$ solution. The barium hydroxide is added so that on acidification with dilute sulfuric acid, the fatty acids will co-precipitate with the barium sulfate, permitting their easy removal. The glycerol formed is then determined by oxidation to formaldehyde and reaction with chromotropic acid. The procedure is readily applied in the routine laboratory and is reproducible. The range found for serum triglycerides by this procedure on 32 normal adults was 52–141 mg/100 ml, with a mean value of 86 mg/100 ml of serum.

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MICROCHEMICAL JOURNAL 16, 77–86 (1971)

Sources of Error in the Mercury(I) Titration of Low Concentrations of Hexacyanoferrate(III) In Alkaline Iodide Medium

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Received September 3, 1970

Satisfactory results have been reported in the mercury(I) perchlorate potentiometric or visual titration of approximately 0.01 *M* hexacyanoferrate(III) in sodium hydroxide–potassium iodide medium (*1*). Attempts were, therefore, made to apply the method to the determination of hexacyanoferrate(III) at submillimolar concentrations. Amperometric end point location was used, because this technique was known to give satisfactory results in the mercury(I) titration of low concentrations of iron(III) (*12*), copper(II) (*9*), or iodine (*10*). Initial results were indeed promising; a set of 30 titrations of 10^{-4} *M* hexacyanoferrate(III) in 1 *M* sodium hydroxide–0.4 *M* potassium iodide gave slightly high hexacyanoferrate(III) results that were precise to $\pm 2\%$. However, strict adherence to timing and to other points of technique was found to be essential.

Further examination showed that the results were quite sensitive to the composition of the solution being titrated (*13*). Sensitivity to the concentrations of alkali and of iodide is not very important, because these concentrations can easily be kept essentially constant. However, when this was done, the percentage error in the determination of hexacyanoferrate(III) changed markedly with the concentration of this ion. The causes did not seem to be in the use of an amperometric technique, because this technique permits the precise and accurate mercury(I) titration of low concentrations of hexacyanoferrate(III) in acid thiocyanate medium (*13*).

Side reactions that pass unnoticed in quick, high-concentration titrations may become significant causes of error in titrations that are performed more slowly. The accurate amperometric mercury(I) redox titration of a substance at submillinormal concentrations is interpreted graphically and takes from 15 to 20 minutes. The present work was carried out in an attempt to account for the peculiar results observed in

the titration of low concentrations of hexacyanoferrate(III) in alkaline iodide medium.

MATERIALS AND METHODS

Equipment. Currents were measured at a rotating platinum electrode (RPE) that was driven at 600 rpm by a Sargent synchronous rotator. All potentials are with respect to the saturated calomel electrode (SCE), which had a mercury pool area of approximately 14 cm^2 and made contact with the cell contents through a wide salt bridge filled with saturated potassium nitrate—3% agar agar gel. To avoid actual contact with the solution under test, the extremity of the salt bridge dipped into sodium hydroxide solution contained in a fritted-glass junction tube that itself dipped into the test solution (*11*). This solution, in a 150-ml beaker that served as a cell, was maintained at 25.00 $\pm$ 0.05°C, unless otherwise specified.

A Leeds & Northrup Type E Electrochemograph with the potential setting kept at zero was used for all measurements of current. The sensitivity of the RPE(A) used in most of the work was 93.4 μA/mmole of hexacyanoferrate(III)/liter, measured at zero potential in deoxygenated 0.5 *M* potassium thiocyanate–0.01 *M* perchloric acid at 25.0°C. A few runs were made with the much less sensitive ($\sim$6 μA/mmole of hexacyanoferrate(III)/liter) RPE (B). Once a day, the RPE was rotated in concentrated nitric acid for 30 seconds, then thoroughly rinsed, and lightly mopped with filter paper. The residual current then remained low and essentially constant throughout the investigation.

Small volumes of hexacyanoferrate(III) and of other solutions were measured by Gilmont 2-ml and 0.2-ml microburets. A line-operated timer that could be read to 0.1 second was used to measure time intervals.

Reagents. All solutions were prepared from analytical-grade reagents and freshly distilled water. The stock potassium hexacyanoferrate(III) solution was stored in the dark and standardized iodometrically (*3*) every 4 weeks. The normality remained at 0.1001 $\pm$ 0.0003 throughout the investigation.

General procedure. Fifty-ml portions of each potassium iodide solution and sodium hydroxide solution were transferred to the thermostatted cell and nitrogen was bubbled through the solution for 5 to 10 minutes. After temperature equilibration had occurred, the circuit was temporarily closed while the "residual current" was read. Hexacyanoferrate(III) solution was then injected while the solution was stirred. The timer was then started at once and the "zero time current" ($t = 0$) was observed after temporarily closing the circuit.

Current readings were taken for intervals of 100 second each. To do

this, the circuit was closed 5 second before the desired reading point and opened 5 second after this point. The "100-second" reading is actually the average over the 10-second span 95 to 105 seconds. The "200-second" and later readings were obtained in a similar manner.

RESULTS AND DISCUSSION

Preliminary experiments. Observations made at room temperature confirmed or established the following facts.

(i) Solutions of sodium hydroxide, potassium iodide, or potassium hexacyanoferrate(II), either separately or mixed, gave essentially zero current (i.e., residual current only).

(ii) Provided that the current was read immediately after the injection of potassium hexacyanoferrate(III) into 1 *M* sodium hydroxide–0.4 *M* potassium iodide, a linear cathodic current–hexacyanoferrate(III) curve was obtained (*13*). A typical curve, shown at A in Fig. 1, was constructed by taking separate portions of alkaline potassium iodide solution and injecting the appropriate amount of 0.1 *M* hexacyanoferrate(III) just before reading the current.

(iii) The current given by 1×10^{-4} *M* hexacyanoferrate(III) in 1 *M* sodium hydroxide–0.4 *M* potassium iodide fell to about one-half the initial value in less than 30 min.

(iv) The injection of iodine, to a concentration of 1×10^{-4} *N*, to 1

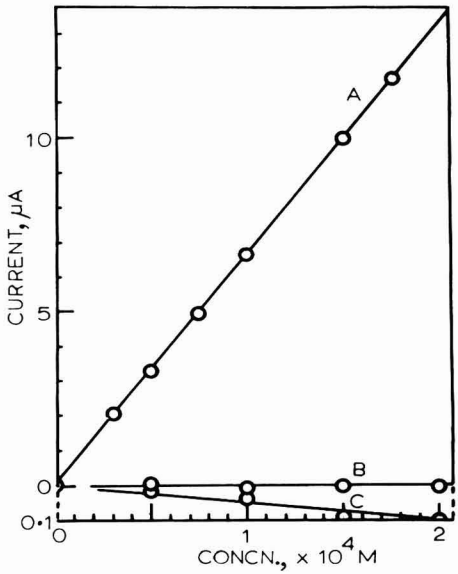

FIG. 1. Current-concentration relationships in 1.0 *M* NaOH–0.4 *M* KI. RPE (A): Curve A, $K_3Fe(CN)_6$; B, $Hg(ClO_4)_2$ or $K_4Fe(CN)_6$; C (note expanded ordinate), $Hg_2(ClO_4)_2$.

M sodium hydroxide–0.4 *M* potassium iodide, gave a surge of cathodic current. This current decayed in less than 30 seconds. A second addition of iodine caused qualitative repetition of the effect. No current increase was observed in experiments where potassium iodate was added in place of iodine.

(v) The addition of mercury(II) to alkaline iodide solution caused no increase in current. However, mercury(I) gave a small irregular *anodic* current in alkaline iodide solution (Fig. 1, curves B and C).

Rate of current decay in hexacyanoferrate(III)–sodium hydroxide--potassium iodide solutions. Runs were made in 1 *M* sodium hydroxide–0.4 *M* potassium iodide solution that contained hexacyanoferrate(III) at a low initial concentration c_0. The concentration c of hexacyanoferrate(III) remaining after elapsed time t was calculated from the current at time t and the calibration curve A, fig. 1. Plots of c or log c against t were nonlinear. Figure 2 shows the plots of $1/c$ for various values of c_0. For runs of approximately one half-life, these plots are linear. The reaction thus might be of second order with respect to the concentration of hexacyanoferrate(III). Further runs made for one half-life at 10, 15, 20, and 30°C ($c_0 = 1 \times 10^{-4}$ *M* in all cases) gave similar plots.

The run (25°C) with $c_0 = 2 \times 10^{-4}$ *M* was allowed to continue over three half lives. The $1/c$–t plot for the entire run (Fig. 2, curve C) is definitely curved; apparent linearity is restricted to the first half-life ($t_{1/2}$) of 1530 sec. The second and third half-lives for this extended run were 2130 and 3500 second, respectively. The corresponding half-lives

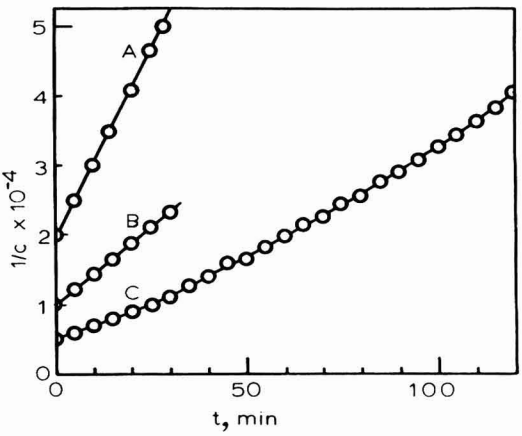

FIG. 2. Reciprocal concentration–time curves of $K_3Fe(CN)_6$ in 1.0 *M* NaOH–0.4 *M* KI: Curve A, $c_0 = 5 \times 10^{-5}$ *M*; B, $c_0 = 1 \times 10^{-4}$ *M*; C, $c_0 = 2 \times 10^{-4}$ *M*.

for short-term runs that were started with $c_0 = 1 \times 10^{-4}$ *M* and $c_0 = 5 \times 10^{-5}$ *M* (curve A) were 1350 and 1140 second, respectively.

If the reaction is really of second order with respect to hexacyanoferrate(III), the doubling of c_0 should cause the halving of $t_{1/2}$. In fact, for the short-term runs, $t_{1/2}$ becomes *larger* as c_0 is increased. This, together with the fact that the total time for three half-lives in the extended run is much longer than the sum of the $t_{1/2}$ values for the short-term runs with $c_0 = 2 \times 10^{-4}$, 1×10^{-4}, and 0.5×10^{-4} *M*, respectively, suggests that the reaction is being hindered by buildup of reaction products.

The reaction between hexacyanoferrate(III) and iodide ions has been extensively studied (*2*). This earlier work refers to hexacyanoferrate(III) concentrations of approximately 0.01 *M* or greater in acid, neutral, or faintly alkaline solution. Under these conditions, the reaction is of first order with respect to hexacyanoferrate(III) and of second order with respect to iodide ion, and is inhibited by hexacyanoferrate(II) ion. Sets of short-term runs were made with $c_0 = 1 \times 10^{-4}$ *M*, but with variable concentrations of sodium hydroxide and of potassium iodide. For [NaOH] = 1.0 *M* and [KI] = 0.2 to 0.8 *M*, the results imply that the reaction is of second order with respect to iodide even when the solution is strongly alkaline. For [KI] = 0.4 *M* and [NaOH] = 0.5 to 2.0 *M*, the apparent reaction order with respect to hydroxide ion concentration was substantially less than one.

The hexacyanoferrate(III) current decays even when iodide is omitted from the medium. However, Fig. 3 shows that the decay rate is

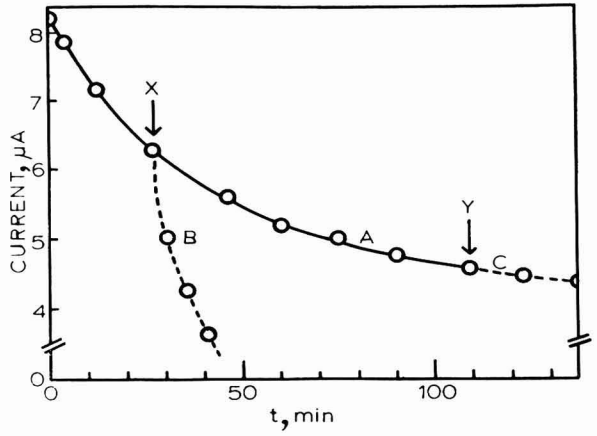

FIG. 3. Decay of $K_3Fe(CN)_6$ current in 1.0 *M* NaOH. RPE (A); $c_0 = 1 \times 10^{-4}$ *M*: Curve A, no additives; B, made 0.4 *M* in KI at point X; C, as A, but made 1×10^{-4} *M* in KCN at point Y.

greatly increased by restoring the iodide ion, and that neither the current nor the decay rate is affected by the addition of cyanide at concentrations equal to c_0. The addition of iodine or of potassium iodate at concentrations equal to c_0 had no detectable effect upon the rate of hexacyanoferrate(III) current decay in 1.0 *M* sodium hydroxide–0.4 *M* potassium iodide solutions.

The poor current stability exhibited by hexacyanoferrate(III) in alkaline iodide media contrasts strikingly with the good stability in 0.01 *M* perchloric acid–0.5 *M* potassium thiocyanate. In this acid thiocyanate medium, finally chosen for the actual titration of submillinormal concentrations of hexacyanoferrate(III), the fall of current in 30 minutes does not exceed 5% and is usually much less (*13*).

Sources of error in the titration of hexacyanoferrate(III) in alkaline iodide media. Attempts to interpret the effects observed in the mercury(I) amperometric titration of submillimolar concentrations of hexacyanoferrate(III) are rendered difficult by facts such as the following: (i) with four likely primary reactants, the overall chemistry is complicated; (ii) the medium that gives the least unsatisfactory results has a high ionic strength, so that standard equilibrium and other constants can be used qualitatively only; (iii) measurements were made at the RPE. Platinum is known to have a catalytic effect upon the hexacyanoferrate(III)–iodide reaction (*8*).

Although the equation,

$$2Fe(CN)_6^{3-} + 2I^- = 2Fe(CN)_6^{4-} + I_2, \qquad (1)$$

represents the overall effect in acid and in essentially neutral solutions, this situation cannot hold under strongly alkaline conditions. Iodine that would be produced as in Eq. (1) then passes rapidly into hypoiodite and iodide ions.

$$I_2 + 2OH^- = IO^- + I^- + H_2O. \qquad (2)$$

Hypoiodite then disproportionates at a measurable rate (*6*).

$$3IO^- = IO_3^- + 2I^-. \qquad (3)$$

Hypoiodite, but not iodide, iodate, nor hexacyanoferrate(II), gives a cathodic current at a potential of zero *(5)*. However, if reaction (3) is rapid compared with reaction (1), the hypoiodite current may be expected to make but little contribution to the total limiting current. A comparison of the results of preliminary experiments (iii) and (iv) indicates that, under prevailing experimental conditions, this expectation is realistic. The current should, therefore, provide a direct measure of the

concentration of hexacyanoferrate(III), the only obvious nonmercury species that is electroactive under the titration conditions.

In the mercury(I) titration of hexacyanoferrate(III) in alkaline iodide media, Stock and Merrer (*13*) obtained low recoveries of hexacyanoferrate(III) when the concentrations of hydroxide, iodide, and hexacyanoferrate(III) were high, high, and low, respectively. The present work indicates that such results are to be expected. An increase in the concentration of either hydroxide or iodide will cause the current to decay faster. Because this effect appears to be of second order with respect to iodide ion, the titration error should be quite sensitive to changes in concentration of this ion. This conclusion may be illustrated by the results of the titration of 1×10^{-4} *M* hexacyanoferrate(III) by "procedure A" (*13*). The average error in the "optimum" medium 1.0 *M* sodium hydroxide–0.4 *M* potassium iodide was +0.2%. The error changed to about −7% when the sodium hydroxide concentration was increased by one half. With the sodium hydroxide concentration kept at 1.0 *M*, the error rose to about −13% when the potassium iodide concentration was increased by one half.

In 1.0 *M* sodium hydroxide–0.4 *M* potassium iodide, Stock and Merrer (*13*) found titration errors of − 11 and −25% when the concentrations of hexacyanoferrate(III) were 2×10^{-5} and 1×10^{-5} *M*, respectively. At these low concentrations, current decay occurs rapidly, presumably because the concentration of hexacyanoferrate(II) does not become high enough to exert any significant retarding action upon the reaction represented by Eq. (1). It is possible that the use of a sodium hydroxide–potassium iodide–potassium hexacyanoferrate(II) medium would minimize the tendency towards large negative errors.

High hexacyanoferrate results were found when the concentrations of hydroxide or iodide were low, or when the concentration of hexacyanoferrate(III) was high (*13*). Such conditions decrease the relative rate of loss of hexacyanoferrate(III), but even a decrease to zero could not cause *positive* hexacyanoferrate errors. The occurrence of such errors suggests that the mercury(I) perchlorate titrant is being destroyed by a process other than direct or indirect reaction with the titrand. Stock and Merrer (*13*) proposed that this destructive process is the disproportionation of the titrant into mercury(II) oxide and metallic mercury. Such an effect may become significant at low iodide concentrations, especially when the titrand concentration is high. The larger additions of titrant then made can produce high local concentrations that lead to precipitation before the disproportionation is arrested by the mixing action of the stirrer. The occurrence of positive errors at a potassium iodide con-

centration as great as 0.4 M suggests that an additional or alternative titrant-destroying process is at work.

The equilibrium constants (25°C) for the reactions,

$$Fe^{n+} + 6CN^- \rightleftharpoons Fe(CN)_6^{(n-6)+}, \quad (4)$$

have been calculated to be 10^{24} and 10^{31} for $n = 2$ and $n = 3$, respectively (*7a*). Assuming that these values hold under the present experimental conditions, the concentration of cyanide ion in 1×10^{-3} M hexacyanoferrate(III) or hexacyanoferrate(II) will be approximately 2×10^{-5} or 2×10^{-4} M, respectively. These conditions do not apply to acid solutions, in which cyanide exists almost entirely as HCN.

The solubility product of mercury(I) cyanide is only 5×10^{-40} (*7b*). The maximum permissible concentration, c_{Hg}, of mercury(I) ion in the presence of 1×10^{-3} M hexacyanoferrate(III) is, therefore, 1×10^{-30} M. As hexacyanoferrate(III) is converted by redox titration into hexacyanoferrate(II), c_{Hg} will fall to 1×10^{-32} M.

By use of the overall formation constant β_4 of HgI_4^{2-} (*7c*) and the standard potentials of the Hg^{2+}/Hg_2^{2+} and the Hg^{2+}/Hg couples (*4*), the value $K = 2 \times 10^{28}$ is calculated for the equilibrium,

$$[HgI_4^{2-}]/[Hg_2^{2+}][I^-]^4 = K. \quad (5)$$

Equation (5) then permits calculation of a second maximum permissible concentration, c'_{Hg}, of mercury(I), where cyanide ion is supposed to be absent, so that c'_{Hg} is controlled by the concentration of hexacyanoferrate actually titrated.

Suppose that 1×10^{-3} M hexacyanoferrate(III) in 0.4 M iodide medium is being titrated with mercury(I), and that the titration is half completed. Then $[HgI_4^{2-}] \simeq [Fe(CN)_6^{4-}] = 5 \times 10^{-4}$ M, so that $c'_{Hg} \simeq 1 \times 10^{-30}$ M. Because c_{Hg} begins at about this value and becomes smaller during the titration, destruction of titrant by formation of $Hg_2(CN)_2$ is to be expected. If $1 \times 10^{-3}M$ hexacyanoferrate is being titrated in 0.1 M (instead of 0.4 M) iodide medium, c'_{Hg} has the larger value of 3×10^{-28}. Cyanide interference, and hence increased positive error in the determination of hexacyanoferrate(III) should, therefore, be favored by lowering the concentration of iodide ion.

For $1 \times 10^{-4}M$ hexacyanoferrate(III) in 0.4 M iodide medium, the calculated c_{Hg} values are 1×10^{-28} and 1×10^{-30} M, at the beginning and at the end of the titration, respectively. The approximate value of c'_{Hg} at the midpoint of the titration of 1×10^{-4} M hexacyanoferrate(III) is calculated to be 1×10^{-31} M. This is *smaller* than either of the cHg values of 1×10^{-28} M (beginning) and 1×10^{-30} M (end) for an initial hexacyanoferrate(III) concentration of 1×10^{-4} M. Consequently,

destruction of titrant by formation of $Hg_2(CN)_2$ should not occur, and any positive errors in the titration of 1×10^{-4} *M* hexacyanoferrate(III) should not be greater than average experimental error. The titrimetric results of Stock and Merrer *(13)* support the conclusions drawn from these calculations.

The deliberate addition of cyanide ion to a titration system in which cyanide ion interference is already occurring should increase the magnitude of this interference. Figure 4 shows a composite curve obtained by successively titrating (at room temperature) three portions of 1×10^{-3} *M* hexacyanoferrate(III) in 1.0 *M* sodium hydroxide–0.4 *M* potassium iodide. To standardize the effect of current decay with time, the hexacyanoferrate(III) was not injected until just before a particular portion was titrated. The current was then allowed to decay briefly, so that all titrations could be started at the same initial current. Titrations were performed at the same speed and with small equal titrant additions. The titration of the first portion was conducted normally. Potassium cyanide, to give a concentration of 2×10^{-4} *M*, was injected into the other two portions, at stages in the titration that are indicated by the arrows. This comparatively large concentration was used to emphasize the change in slope of the curve (and the corresponding increase in the amount of hexacyanoferrate found) that can be caused by the presence of cyanide ion.

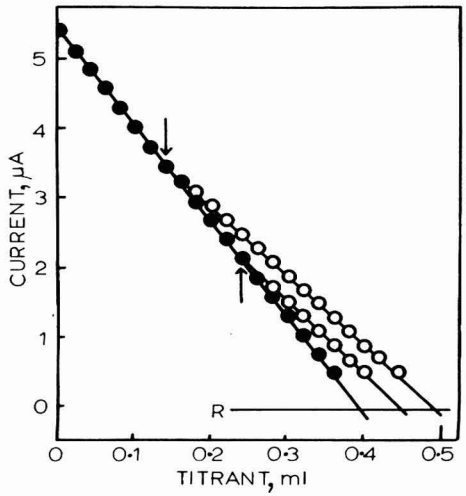

FIG. 4. Titration of 50-ml portions of initially 1×10^{-3} *M* $K_3Fe(CN)_6$ in 1.0 *M* NaOH — 0.4 KI with 0.096 *N* (= 0.048 *M*) $Hg_2(ClO_4)_2$ · RPE (B): (●) without addition of KCN; (○) after addition of KCN at points indicated by the arrows; R is the residual current line.

SUMMARY

Although submillimolar concentrations of hexacyanoferrate(III) in acid thiocyanate medium can be accurately titrated amperometrically with mercury(I) perchlorate, the titration is unsatisfactory when carried out in alkaline iodide medium. The decay of hexacyanoferrate(III) current in alkaline iodide medium and the effect of cyanide ion on the titration have been examined. The results provide an explanation for positive and negative titration errors that are encountered when hexacyanoferrate(III) is titrated in this medium.

ACKNOWLEDGMENT

This work was carried out with the partial support of grants from the University of Connecticut Research Foundation.

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MICROCHEMICAL JOURNAL 16, 87–93 (1971)

Determination of Silver with the Thiosemicarbazone of picoline-2-aldehyde

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Received September 6, 1970

INTRODUCTION

It is well known that the thiol group has a particular affinity for silver. Dithizone and dimethylaminobenzalrhodanine are frequently used as reagents in the determination of silver. A review of existing methods is given by Sandell (*3*). All methods, other than the extraction technique using dithizone, rely on the formation of a suspension. The method proposed below has the advantage of being a rapid, solution method, useful in the range 0–1.0 μmole of silver. Since ethylenediaminetetraacetic acid disodium salt (EDTA) forms a weak complex with silver it is used as a masking reagent in the determination.

MATERIALS AND METHODS

Apparatus

All photometric measurements were made using the Cary 14 recording spectrophotometer (Applied Physics Corporation, Monrovia, California). 1.00-cm cells were used throughout. An Orion Model 801 pH meter was used for the determination of pH.

Preparation of the Reagent

Dissolve 4.55 g of thiosemicarbazide in 200 ml of ethanol. Heat to boiling and continue heating until complete solution is obtained. Add 5.03 g of picoline-2-aldehyde, dissolved in 20 ml of ethanol, and reflux for 0.5 hour. Allow to cool to room temperature in the dark. Filter the crystals, wash with ethanol and dry at 100°C for 10 minutes. Crude yield is 7.4 g (82%). Product is recrystallized twice from boiling ethanol forming white needles, mp 205–205.5° C (*1*).

The product was analyzed with the following results: 46.90%C; 4.67% H; 30.75% N; 18.00% S; required for $C_7H_8N_4S$: 46.64% C; 4.47% H; 31.09% N; 17.79% S.

Reagents

Aqueous 2.000×10^{-4} *M* solutions of reagent and silver nitrate were used throughout. Other metal ions used were as 0.20 *M* aqueous nitrate or sulfate stock solutions. Each stock solution was quantitatively checked by titration with EDTA (*4*). Aqueous anion stock solutions were also 0.20 *M*. The pH was adjusted by means of the following buffers: 0.50 *M* perchloric acid and 1.00 *M* hexamine + 0.50 *M* sodium perchlorate for 1.10–2.18 and 4.05–6.45; 0.50 *M* sodium hydroxide and 0.50 *M* formic acid + 0.50 *M* sodium perchlorate for 2.32–3.99; 0.50 *M* perchloric acid and 0.125 *M* sodium borate for 7.88–9.01; 0.50 *M* sodium hydroxide and sodium borate for 9.26–9.80; 0.50 *M* sodium hydroxide and 1.00 *M* hexamine + 0.50 *M* sodium perchlorate for 10.93–12.95; 1.125 *M* sodium acetate and 0.50 *M* acetic acid for 5.48 (*2*). 0.10 *M* EDTA disodium salt was used for masking purposes. All chemicals used were either "Analar" products of B.D.H. (Canada) or from the Aldrich Chemical Company.

Determination of Silver

The following solution is prepared: 100.0 ml of 1.125 *M* sodium acetate and 25.0 ml of acetic acid are diluted to 250.0 ml in a volumetric flask. 5.0 ml of this solution and 5.0 ml of 0.10 *M* EDTA are added to a sample containing 0–1.0 μmoles of silver. The pH should be 5.3–5.6. Add 5.0 ml of 2.000×10^{-4} *M* reagent solution, dilute to 25.0 ml in a volumetric flask and mix well. The absorbance is measured at 316 n*m*. Construct a calibration curve for the given range of silver concentrations under the same conditions. A straight line should be obtained.

RESULTS AND DISCUSSION

Sensitivity and Reproducibility of the Determination

The sensitivity is expressed in the form of effective molar absorbitivity for silver corresponding to the linear part of the dependence between relative absorbance (blank minus sample solution) and the total concentration of the metal. The effective molar absorbtivity is found to be 13,000 cm^2/mmole at pH 5.48.

Multiple analyses of a series of solutions containing 1.0 μmoles of silver were performed. The precision of the absorbance measurement for silver is ±0.782% (relative SD). This corresponds to 0.0051 μmoles of silver.

Spectral Characteristics

Typical spectral curves are shown in Fig. 1 for the ligand and the complex.

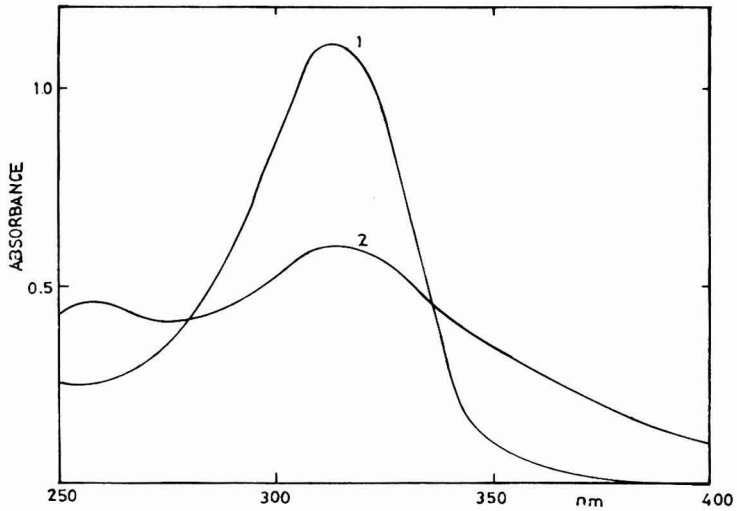

FIG. 1. Absorbance spectra of picoline-2-aldehyde thiosemicarbazone (1) and the silver complex (2) at pH 5.48; $c_L = c_M = 4.00 \times 10^{-5}$ *M*; $c_{EDTA} = 0.02$ *M*.

Influence of Acidity and Adherence to Lambert-Beer Law

The dependence of the absorbance of the reagent and complex on pH is shown in Fig. 2. These curves show that the best pH for complexation is in the region 5.0 to 6.0. Figures 3 and 5 show that the Lambert-Beer Law is obeyed for both reagent and complex.

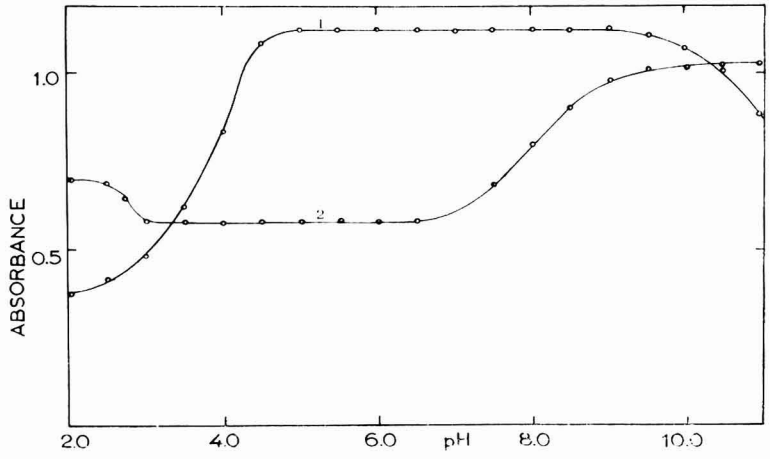

FIG. 2. The pH dependence of picoline-2-aldehyde thiosemicarbazone (1) and the silver complex (2) at 312 nm; $c_L = c_M = 4.00 \times 10^{-5}$ *M*; $c_{EDTA} = 0.02$ *M*.

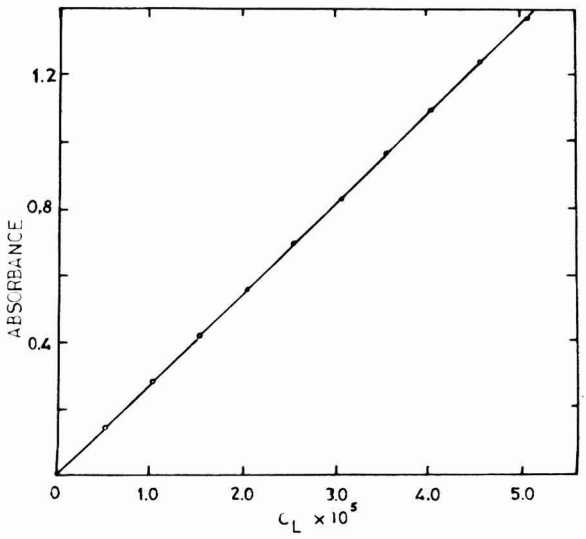

FIG. 3. Dependence of absorbance on the concentration of picoline-2-aldehyde thiosemicarbazone: $c_{EDTA} = 0.02\ M$.

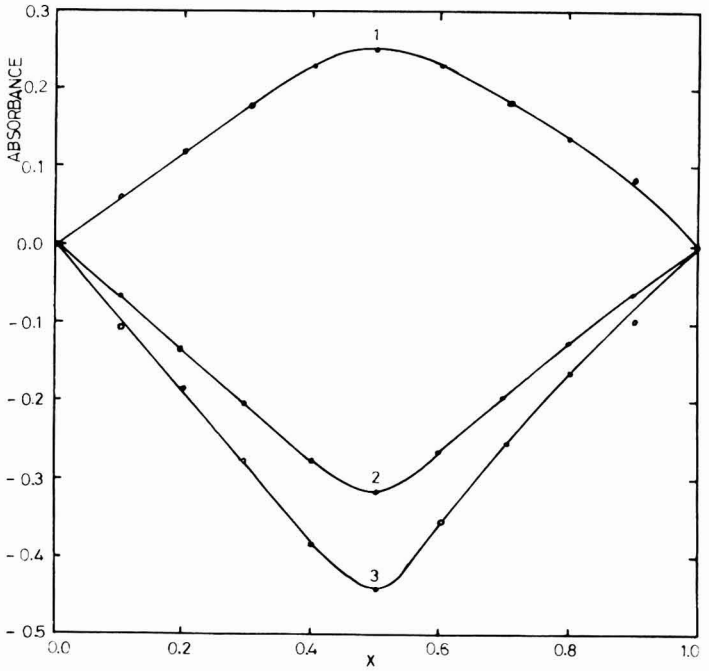

FIG. 4. Continuous variation in isomolar solutions at pH 5.49; $X = c_M/(c_M + c_L)$, $c_M + c_L = 6.00 \times 10^{-5}\ M$: (1) at 350 nm, (2) at 320 nm, (3) at 316 nm; $c_{EDTA} = 0.02\ M$.

Stability of Reagent and Complex

Using stock solutions of 2.0×10^{-4} *M* of reagent and silver it was found that at pH 5.5, under conditions of the determination, the absorbance was 1.110 ± 0.002 for the reagent and 0.589 ± 0.002 for the complex up to 3 hours. An aqueous solution of the reagent, if stored in the dark, is stable for up to 3 months.

Structure of the Complex

The ratio of metal to reagent in the complex formed was investigated by the method of continuous variation in isomolar solutions and by the mole ratio method. The corrected curves are given in Figs. 4 and 5. A ratio of 1:1 was found in both cases.

Effect of Foreign Ions on the Determination of Silver

The effect of 21 cations and 12 anions on the determination of silver was investigated. The limiting value of the concentration of a foreign ion was taken as that which caused an error of $\pm$ 5% in the absorbtion of the complex. The results are summarized in Table 1. For each for-

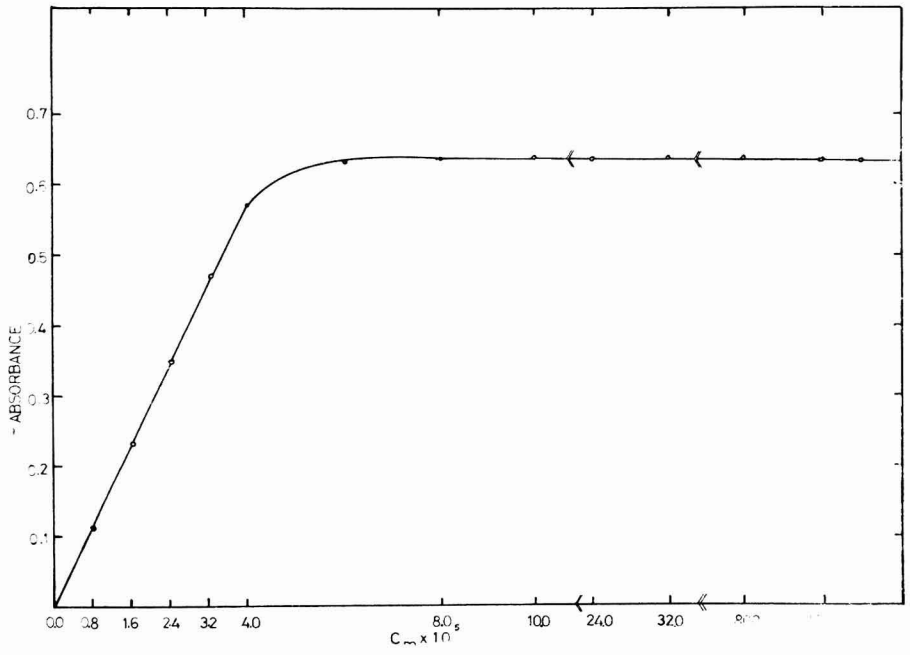

FIG. 5. Dependence of absorbance on the concentration of silver at 315 nm: $c_L = 4.00 \times 10^{-5}$ *M*; $c_{EDTA} = 0.02$ *M*.

eign ion the reagents were added in the following order: 5.0 ml of 0.1 *M* EDTA; 5.0 ml of the sodium acetate acetic acid buffer; 5.0 ml of 2.00×10^{-4} *M* silver nitrate solution; and 5.0 ml of the foreign ion. The above mixture was allowed to stand for 3 minutes; and then 5.0 ml of 2.00×10^{-4} *M* reagent were added. The absorbance was measured at 316 nm. The actual amount of silver found was calculated from the Lambert-Beer curve.

SUMMARY

Picoline-2-aldehyde thiosemicarbazone forms a stable complex with silver in the pH range 5.0 to 6.0. The complex has the metal:ligand ratio of 1:1. Complexation with silver at pH 5.5 in the presence of EDTA allows a selective determination to be performed. Of 21 cations studied, 1-fold molar ratio of mer-

TABLE 1

DETERMINATION OF SILVER [d] IN THE PRESENCE OF FOREIGN IONS

	Silver found (μmole)				Silver found (μmole)		
Cation	—[a]	—[b]	—[c]	Anion	—[a]	—[b]	—[c]
Cobalt(III)	1.011	1.029	1.081	Fluoride	0.995	0.996	0.995
Copper(II)	0.952	0.772	—	Chloride	0.985	0.631	—
Nickel	0.982	0.991	1.026	Bromide	0.480	—	—
Mercury(II)	0.686	—	—	Iodide	0.449	—	—
Bismuth	1.003	0.974	0.760	Cyanide	0.897	0.500	—
Lead	0.992	0.992	0.944	Sulfate	1.001	1.009	0.997
Chromium	0.981	0.991	0.884	Phosphate	1.001	0.997	0.983
Iron(II)	0.804	—	—	Oxalate	0.980	0.970	0.971
Iron(III)	0.768	—	—	Tartrate	1.035	0.975	0.935
Zinc	0.987	1.000	0.983	Citrate	0.970	0.966	0.966
Manganese	0.995	1.008	1.008	Chlorate	0.958	0.970	0.941
Cadmium	1.005	1.018	1.026	Molybdate	0.960	0.939	0.740
Hafnium [e]	0.960	0.648					
Lanthanum	0.991	0.991	0.936				
Thorium	0.981	0.942	0.884				
Uranium	0.914	0.598	—				
Titanium	0.617	—	—				
Scandium	0.942	0.706	—				
Vanadium	1.010	0.961	—				
Strontium	0.994	0.994	0.985				
Yttrium	1.000	0.970	0.970				

[a] 1 μmole of foreign ion present.

[b] 10 μmole of foreign ion present.

[c] 100 μmole of foreign ion present.

[d] In each case 1.00 μmole of silver nitrate was present.

[e] The stock solution was boiled prior to use to destroy any polynuclear hydroxo-complexes.

cury(II), 2-fold molar ratios of titanium and uranium, 5-fold molar ratios of copper (II) and hafnium and 8-fold molar ratio of scandium caused interference in the determination of 1.0 μmole of silver. Amongst 12 anions, a 1-fold ratio of bromide, iodide, and cyanide; 3-fold molar ratio of chloride; and 50-fold molar ratio of molybdate caused low results.

ACKNOWLEDGMENTS

One of us (BWB) expresses appreciation to the National Research Council of Canada for financial support.

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MICROCHEMICAL JOURNAL **16**, 94–112 (1971)

Identification of Several Ions by Nonaqueous Solutions of Tin(II) Chloride [1]

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Received October 7, 1970

Tin(II) chloride has many advtantageous properties for use as a reducer solution. Its redox potential, $E^0 = 0.14$ vol, is relatively low, and therefore it can reduce a great number of substances and it is also useful for catalyzing many procedures.

It has more advantages than other substances of this type, because it can be transformed in a uniform product of oxidation. This is tin(IV) chloride, which cannot go on reacting because the tin has no more elevated degree of oxidation.

Another characteristic of this reagent is that it reacts with most substances at room temperature in an instantaneous form. But, it is well known that tin(II) chloride solutions undergo a rapid oxidation when they are stored without special precautions. The oxidation takes place in an acidic medium according to this equation:

$$2Sn^{2+} + 4H^+ + O_2 \rightleftharpoons 2Sn^{4+} + 2H_2O,$$

and in an alkaline medium according to this reaction:

$$2SnO_2H^- + \tfrac{1}{2}\, O_2 \rightleftharpoons SnO_3H^-.$$

To avoid this inconvenience many investigators have researched how to stabilize the tin(II) chloride solutions.

With this work, it is started the research of stable tin(II) chloride solutions to be used as reagents in qualitative inorganic analysis.

Miyamoto (*8*) has studied the tin(II) oxidation velocity by passing an air draught across it. He found that the temperature and velocity of the air draught has little influence. He also demonstrated that oxidation velocity is the maximum in an alkaline medium 0.2 n and it increases with time (*9*).

[1] Paper presented at the VIth International Symposium on Microtechniques held at Graz, Austria September 7–11, 1970.

Filson and Walton (*4*) found that the oxidation, which takes place in tin(II) chloride solutions, within certain limits, depends on the increasing acid concentration; and it is directly proportional to hydrogen ion concentration between pH 0.20 and 0.25.

Haring and Walton (*5*) have studied the influence of temperature and they found that it has very little influence in oxidations of tin(II) chloride solutions, because although the oxidation velocity increases, oxygen solubility diminishes. The same authors assert that ultraviolet light catalyzes the oxidation.

Lachman and Tomkins (*7*) have made a study about velocity curves of tin chloride oxidation in solution with the total variation of chloride, hydrogen and tin(II) concentrations. They found that oxidation velocity is proportional to the square root of $HSnCl_3$ concentration.

It is usual to add some metallic tin to the solution to get it dissolved, so that it can compensate the partial oxidation of tin(II). But, it is logical that this procedure is not useful if the solution is employed in quantitative form, because in most cases more metallic tin is dissolved in the solution than is oxidized, for that reason, the concentration of the solution changes a great deal causing turbidity and precipitates in the solution.

Szabó and Sugar (*12*) proposed a general method for use tin(II) chloride solution in oxidation–reduction volumetric titrations experimenting this application to many substances. Thanks to the using of a special automatic apparatus, tin(II) chloride solution can be preserved in carbon dioxide atmosphere for 2 months. The normality of this solution changes very little in this condition.

Shah (*10*) has studied the behavior of metallic tin dissolved in glacial acetic acid, in the presence and absence of water and its application in quantitative analysis and he demonstrated that presence of air does not interfere in the solutions and the error is not more than 1%.

Bilii and Okhrimenko (*3*), confirming the works by Haring and Walton, stated that ultraviolet light produces an oxidation in the tin(II) chloride solution by an acid agent and this oxidation is directly proportional to the quantity of chloride and hydrogen ions presents in it.

Barinska and Richelk (*2*) suggested the use of very strong acidulated solutions with hydrochlorhydric acid so that the oxidation was less, but in practice this method has not had satisfactory results.

There are a lot of negative catalysts which have been proposed to retard tin(II) oxidation in solution: picric acid, quinones, sugars, sodium arsenite, alcohols, and polyalcohols.

Some years ago, Sletten and Bach (*11*) proposed that the methods suggested by the "Association of American Soap and Glycerine Prod-

ucers" for the preparation of tin(II) chloride in glycerin be used instead of those methods previously used in mineral acids; it could give good results for absorptiometric determination of orthophosphates.

Henriksen (*6*) stated that, by polarographic analysis, tin(II) chloride solutions in glycerine preserved during 5 months at room temperature shows only 16% tin(II) ions oxidized to tin(IV); in spite of having taken few cautions to protect them from light and air.

Arribas *et al.* (*1*) stated that tin(II) chloride solutions in mixed solvents glycerin–ethanol (3:1), are much more stable when exposed to light and atmospheric oxidation than commonly used solutions in hydrochlorhydric acid, and nevertheless their reducing properties have not been lost.

Taking as a base what was proposed in these last works, tin(II) chloride solutions have been prepared in different organic solvents such as polyalcohols or glycolic ethers:

I. Mixture glycerin-ethanol (3:1).

II. Ethyleneglycol $HO–CH_2–CH_2–OH$.

III. Diethyleneglycol $HO–CH_2–CH_2–O–CH_2–CH_2–OH$.

IV. Triethyleneglycol $HO–CH_2–CH_2–O–CH_2–CH_2–O–CH_2–CH_2–OH$.

The three last solvents have the advantage they are much more fluid than glycerin and entirely colorless.

The solution of the salt is in all of them a little slow, but it can be well solved producing a perfectly limpid liquid in all of them. Moreover, it has been proved that these solvents have no oxidant effects appreciated in the tin(II) taking special care to keep them in amber colored bottles because light catalyzes oxidation. It's also convenient to keep the bottles tightly closed because these solvents are very hygroscopic and they easily absorb the moisture from the ambient air.

In the first place, a comparative study of stability was made on these solutions in different organic solvents and in a medium of hydrochlorhydric acid water.

In the second place, we studied the reactions for identification of ions which are based on the use of tin(II) chloride solutions as a reducer.

The identification test reactions were made on drop-plates, on filter paper, and in glass test tubes for the three scales: Macro-, semimicro-, and micro scale.

A study was made in all of identification limits reached by determining in which tests and for which ions these limits are better than the ones which have been obtained up to the present and which of them remain in the same state, instead of utilizing tin(II) chloride solutions in an aqueous hydrochlorhydric medium.

Starting from the data found in these tests, the sensibilities are represented by diagrams determining the zone in which the reaction occurs.

In the determination of stability of solutions of tin(II) chloride in glycolic ethers; diethylen glycol, triethylen glycol, and the glycol: ethyleneglycol ahead of the mentioned salt in aqueous hydrochlorhydric solution, advantages are: the mentioned glycols are good stabilizers of tin(II) chloride; all of them give colorless and transparent solutions; and they do not produce appreciably oxidant effects on the tin(II).

Use of these solutions for the identification of ions of mercury(II), bismuth(III), molybdenum(VI), tungsten(VI), tellurium(IV), and arsenic(III) gives to us a complete and satisfactory result, improving the sensibility to the reactions of identification in the most part of them, for what, the solutions of tin(II) chloride in glycolic solvents before mentioned are recommended as reducer reagents in the analytical identification of inorganic ions.

We found in our work that tin(II) chloride solutions in organic solvents are stable without alteration in concentration for about 2 months. For longer periods, diethylene and triethylene glycol solutions are more stable (Figure 1); in 160 days they varied only 10% in concentration. On the contrary, the increase of the slope in the Fig. 2 shows the rapid oxidation of hydroclorhydric solutions of tin(II), that in 15 days lost 86.3% of the initial concentration.

MATERIALS AND METHODS

The dihydrated salt of tin(II) chloride in a pure state was used for analysis. To the theoretical quantity of salt was added 750 ml of sol-

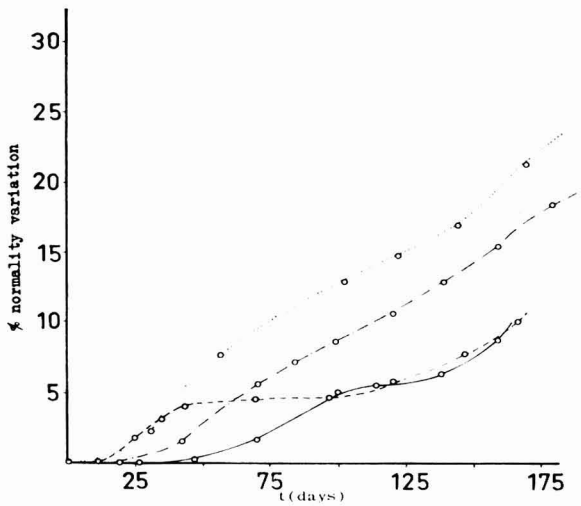

FIG. 1. Normality variation of tin(II) chloride solution in: glycerin–ethanol (3:1) (—.); ethyleneglycol (. . .); diethyleneglycol (- -); and triethyleneglycol (—) in function of time.

vents: glycerin, ethyleneglycol, diethyleneglycol, and triethyleneglycol, respectively all reagents for analysis, making this addition at room temperature and allowing it to stand until total solution. It should not be shaken, because air bubbles remain firmly retained and they can oxidate part of the tin(II) which remains in its contour.

The volume is made up to 1 liter with ethanol when glycerin is used as solvent; and with the proper solvent in the other cases, and homogenizing it, at the end.

Owing to the fact that in the titration of tin(II) chloride solution with potassium ferrocyanide solution in an alkaline medium of sodium carbonate at room temperature the ion ferrocyanide does not act on the organic solvents used, it was chosen for titration of tin(II) in organic solvents.

Athough the ferrocyanide ion can serve us as autoindicator, the titration was made by potentiometric method, using a platinum electrode as an indicator and one saturated calomel, as a reference electrode.

In the four solutions of tin(II) chloride solutions have been periodically tested their concentration with the purpose of studying their stability and compare it in front of tin(II) chloride solution in hydrochlorhydric acid-water.

Figure 1 shows the percentage of variation in normality in function of time (days), for tin(II) chloride solutions in several organic solvents. Figure 2 shows the same variation for tin(II) chloride solutions in aqueous hydrochlorhydric acid.

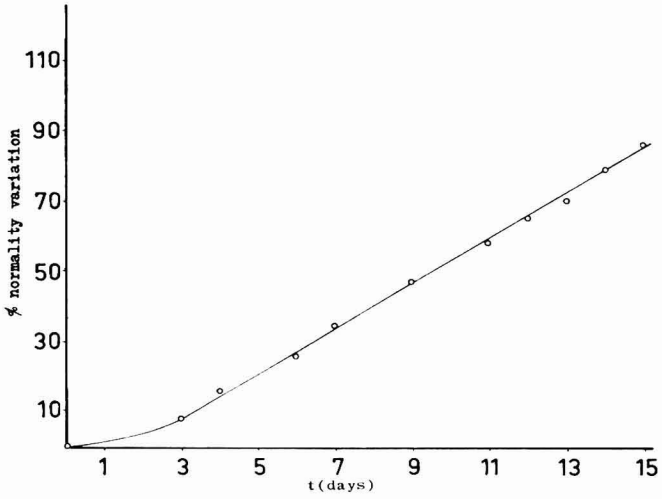

FIG. 2. Normality variation of tin(II) chloride solutions in aqueous hydrochlorhydric acid in function of time.

Tin(II) chloride solutions in organic solvents have been used for the identification of metallic cations. For their study it has been utilized the sensibility diagrams which consist of plain representations in which they express the variations of identification limit in function of the variations in the relative concentrations of strange substances or of the proper solvent in the initial solutions which is tested. Any sort of diagram must be referred to the system which is being tested before adding of reagent.

In general, any system in solution which is subjected to analytical reaction can be considerated to be or formed by a substance which is being investigated A and the solvent B which are found into the mixture A + B in relative concentrations C_A and C_B. These values agree with: $pA = -\log C_A$; $pB = -\log C_B$.

Here by that any solution which can be subjected to an analytical reaction can be represented by a point. The inclined lines towards 45° angle, respecting to abscises axle represent the geometrical place of different solutions which are found into the identification limit.

Thus, when $pB = 0$, the pA gives us the concentration limit, because it is the quantity a of substance A in volume unity in the solvent. Therefore, a, expressed in micrograms, is the perceptibility limit or identification for the substance A.

So, from the practical point of view, it is not the same that a small quantity of substance contained in a few mililiters or in few liters can be recognized; the concentration limit, has been defined as the slightest quantity of appreciable substance for volume unity, expressed by the logarithm with the sign changed.

$$pD = -\log\left(\frac{(\text{identification limit in } \mu g \times 10^6)}{ml}\right)$$

MERCURY(II) WITH TIN(II) CHLORIDE IN ORGANIC SOLVENTS

Because of the normal potential of the system $Hg^{2+}/Hg = 0.86$ vol. the ion Hg^{2+} is easily reduced to metal with numerous metals with which the mercury is amalgamated.

One of the most useful reactions for identification of mercury(II) ion is that based on the reduction of this ion by tin(II) to produce a white, black-brown, or grey precipitate of mercurious chloride, metallic mercury, or a mixture of both, respectively, according to the same quantities and abundance of reagents.

Reagents

Mercury(II) solution, prepared with mercury(II) nitrate crystallized and titrated with a standard solution of sodium chloride using sodium

nitroprussiate as an indicator of equivalence point. More dilute solutions were prepared by successive dilutions.

Tin(II) chloride solution, prepared to 5% starting from $SnCl_2 \cdot 2\ H_2O$ p.a. in various organic solvents: ethyleneglycol, diethyleneglycol, triethyleneglycol, and glycerin–ethanol (3:1), respectively; and starting from these, more dilute solutions were prepared.

Procedure

a. On filter paper. On a filter paper, place 1 drop of mercury(II) solution and 1 drop of tin(II) chloride solution. A brown-black spot of metallic mercury appears. An identification limit of 1.83 μg of Hg was obtained; pD = 4.2.

b. In macroscale. In a 16 cm length and 1.5-cm diameter glass test tube place 5 ml of mercury(II) solution, then, add 20 drops of tin(II) chloride solution, and shake lightly to make the sample homogeneous. A black-brown precipitate appears. In this case, the identification limit was 305 μg of Hg; pD = 4.2.

c. In semimicroscale. In a glass test tube of the same dimensions as (b), place 2.5 ml of mercury(II) solution, then, add 10 drops of tin(II) chloride solution and shake lightly to make the sample homogeneous. An identification limit of 152.5 μg of Hg was obtained; pD = 4.2.

d. In microscale. Using the same technique as (c), place in a (10-cm length and 1-cm diameter) glass test tube 1 ml of mercury(II) solution and 5 drops of tin(II) chloride solution. An identification limit of 61 μg of Hg was obtained; pD = 4.2. In all cases, the same results were obtained using 4, 3, 2, and 1% solutions of tin(II) chloride in organic solvents (see Fig. 3).

MOLYBDENUM(VI) WITH TIN(II) CHLORIDE IN ORGANIC SOLVENTS

The molybdenum is so much acid that it's predominantly found forming anions like molybdate, which in solution becomes a yellow color by addition of thiocyanate solution and hydrochlorhydric acid.

If a solution which contains molybdenum(VI) we add a small quantity of reducer agent solution, the molybdenum passes to five in valence forming with the thiocyanate various soluble complexes red-orange or colorless.

$$Mo^{5+} + m SCN^- \rightarrow Mo(SCN)_m^{5-m} \text{ colorless,}$$
$$Mo(SCN)_m^{5-m} + (5-m)\ SCN^- \rightarrow (SCN)_5Mo \text{ red.}$$

An excess of thiocyanate solution can produce weakness in color; it is probably due to a formation of a less colored complex whose formula is: $Mo(SCN)_6^-$.

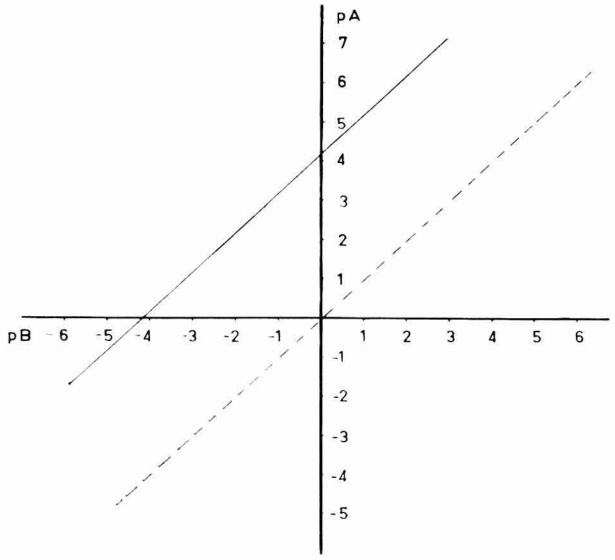

FIG. 3. Sensibility diagram for detection of mercury(II) with tin(II) chloride in glycerin–ethanol (3:1), ethyleneglycol, diethyleneglycol, and triethyleneglycol. pA = −log g of Hg/ml; pB = −log ml of H_2O.

Reagents

Molybdenum(VI) solution, standard solution prepared by solution of 75 mg of molybdenum oxide, previously purified by sublimation to 780 degree C in a quartz tube, in a slight excess of sodium hydroxide and diluted to 500 ml acidulating previously with hydrochlorhydric acid. This solution contains 100 μg of Mo/ml.

Potassium thiocyanate solution, aqueous solution of 10% potassium thiocyanate.

Tin(II) chloride solution, prepared to 5% as above.

Procedure

a. On filter paper. On a filter paper previously moistened with hydrochloric acid (1.1), place 1 drop (0.03 ml) of molybdenum solution, adding 1 drop of potassium thiocyanate solution, and 1 drop of tin(II) chloride in an organic solvent. Then, red-orange spot of the complex formed by the thiocyanate and molybdenum appears. It is very important to insure that enough reducer is added because different colorations can appear from several degrees of oxidation in the element tested. Identification limit reach 0.06 μg of Mo; pD = 5.7.

b. In macroscale. Place 5 ml of molybdenum solution in a glass test tube, (16 cm length and 1.5-cm diameter), acidulate with 1 or 2 drops

of hydrochlorhydric acid (1:1) and add 15 drops of potassium thiocyanate solution and 10 drops of tin(II) chloride solution in organic solvent. Then a red-orange coloration appears. Identification limit reached: 2.5 μg of Mo; pD = 6.3.

c. In microscale. Place 1 ml of molybdenum solution in a glass test tube (10-cm length and 1-cm diameter) acidulate with 1 drop of hydrochlorhydric acid (1:1) and then add 5 drops of tin(II) chloride solution in organic solvent. A red-orange coloration then appears. Identification limit reached: 0.05 μg of Mo; pD = 6.3. see Figs. 4 and 5.

BISMUTH(III) WITH TIN(II) CHLORIDE IN ORGANIC SOLVENTS

The normal potential of the system $Bi^{3+}/Bi = 0.23$ vol suggests that it can be reduced to metal without much difficulty; the tin(II) chloride makes it into alkaline medium. This potential also explains that the metal can only be attacked by oxidant acids.

Alkaline stannites reduce the bismuth(III) to black metal in basic medium according the following equation:

$$2Bi^{3+} + 3Sn(OH)_3^- + 9OH^- \rightarrow 2Bi + 3Sn(OH)_6^{2-}.$$

Stannite solutions which contain big quantities of hydroxide ion can deposit black elementary tin:

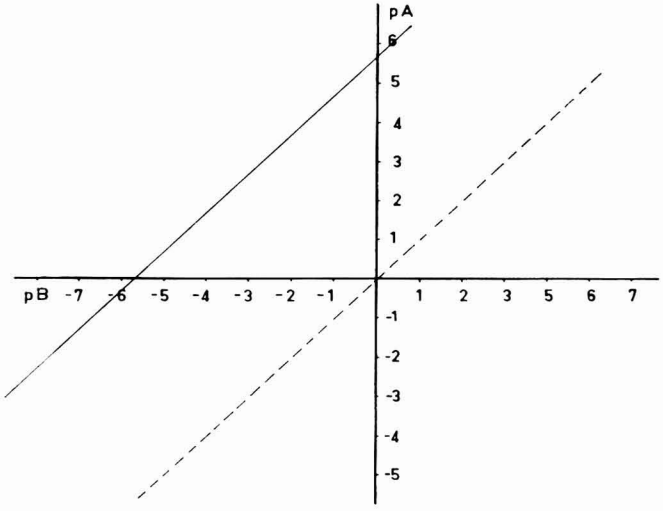

FIG. 4. Sensibility diagram for detection, on paper filter, of molydenum(VI) with tin(II) chloride in glycerin–ethanol (3:1), ethyleneglycol, diethyleneglycol and triethyleneglycol, respectively. pA = −log g of Mo/ml; pB = −log ml of H_2O.

$$2HSnO_2 + 2H_2O \rightarrow Sn + Sn(OH)_6^{2-}.$$

In presence of insufficient quantity of hydroxide they can precipitate black tin(II) oxide:

$$HSn_2O^- \rightarrow SnO + OH^-,$$

to what it can give us mistake in bismuth(III) identification.

Reagents

Bismuth(III) solution, aqueous solution of bismuth(III) nitrate standardized with EDTA solution to pH 2–3 using pyrocatechol violet as indicator.

Potassium hydroxide, aqueous solution 2 *F*.

Tin(II) chloride, prepared to 5% as above.

Procedure

a. On drop plate. On drop plate, put 1 drop of bismuth solution, lightly acidulate, add 3 drops of potassium hydroxide solution and 1 drop of tin(II) chloride solution in organic solvents. Check that the

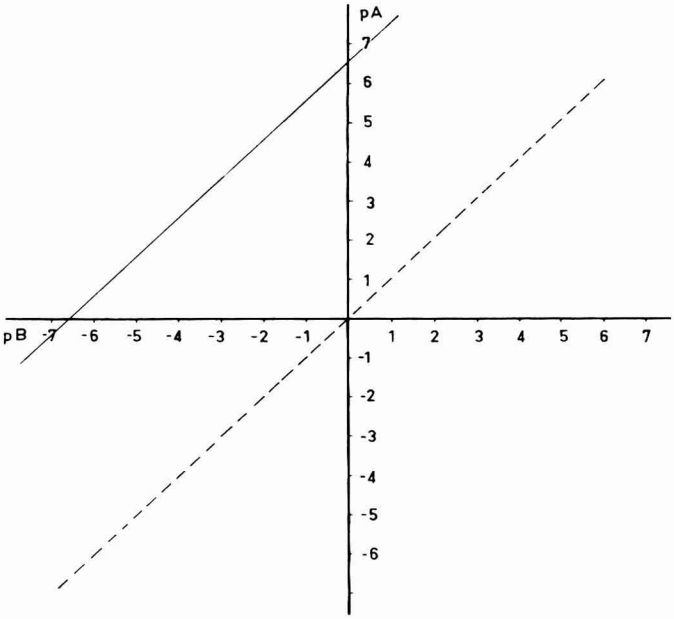

FIG. 5. Sensibility diagram for detection, in macro-, semimicro-, and microscale, of molybdenum(VI) with tin(II) chloride in glycerin–ethanol (3:1), ethyleneglycol diethyleneglycol, and triethyleneglycol, respectively. pA = −log g of Mo/ml; pB = −log ml of H_2O.

mixture remains alkaline, adding more solution of potassium hydroxide if necessary. A black precipitate of metallic bismuth is produced. Identification limit reached: 1.35 μg of Bi; pD = 4.3.

b. In macroscale. In a glass test tube (16-cm length and 1.5-cm diameter) place 5 ml of bismuth solution, add 1 ml of potassium hydroxide. Check that the mixture remain alkaline, adding more solution of potassium hydroxide if necessary. Add 8 drops of tin(II) chloride solution in organic solvents, shaking slightly to make the mixture homogeneous. A black-brown precipitate or a brown turbidity appears, according to the concentration of bismuth solution. Identification limit reached: 22.55 μg of Bi; pD = 5.3.

c. In microscale. Pour in a glass tube (10-cm length and 1-cm diameter) one ml of bismuth solution, add 0.5 ml of potassium hydroxide solution, and 3 drops of tin(II) chloride in organic solvents. Shake it to make the mixture homogeneous. A brown precipitate or turbidity is produced, according to the concentration of bismuth. Identification limit reached: 4.51 μg of Bi; pD = 5.3 (see Figs. 6 and 7).

TELLURIUM(IV) WITH TIN(II) CHLORIDE INTO ORGANIC SOLVENTS

The greatest part of detection reactions for this element are based in reducing of tellurium ions to elemental state in black color. The alka-

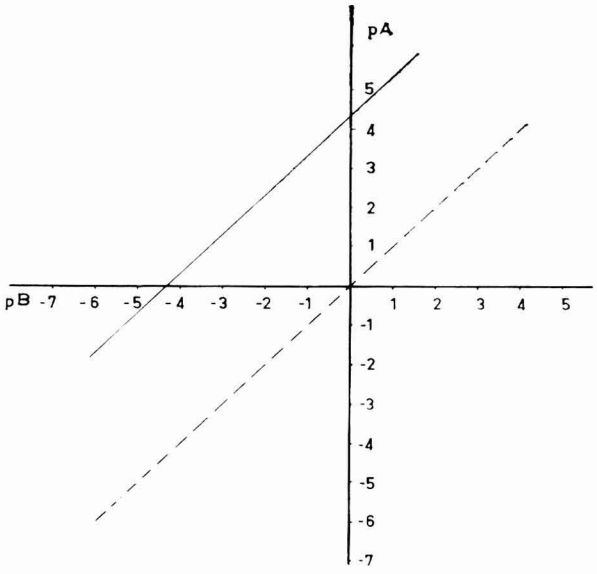

FIG. 6. Sensibility diagram for detection of bismuth(III) with tin(II) chloride on drop plate. pA = $-\log$ g of Bi/ml; pB = $\log$ ml of H_2O.

line stannites reduce the tellurites in an alkaline solution according the following equation:

$$TeO_3^{2-} + 2\ SnO_2^{-} + H_2O \rightarrow 2SnO_3^{2-} + Te + 2\ OH^{-}.$$

This produces a precipitate black, grey, or brown of metallic tellurium according your concentration.

Reagents

Tellurium(IV) solution, aqueous solution prepared by direct weighing of tellurium(IV) oxide previously purified (by dissolving in hydrochlorhydric acid. To this solution sodium hydroxide solution was added until the formation of a persistent precipitate. Immediately, add 10 ml of 1 *F* solution hydrochlorhydric acid solution, and dilute with distilled water. After warming it until bubbles appear, add, drop by drop, 10 ml of 20% aqueous urotropine solution. The precipitate is filtered after washing it with distilled water and, finally, with ethanol. The precipitate of tellurium(IV) oxide was dried to 140°C) and solution of this into 100 ml of 1 F sodium hydroxide solution and dilution to 1 volume.

Sodium hydroxide, aqueous solution to 6%.

Tin(II) chloride solution, prepared to 5% as above.

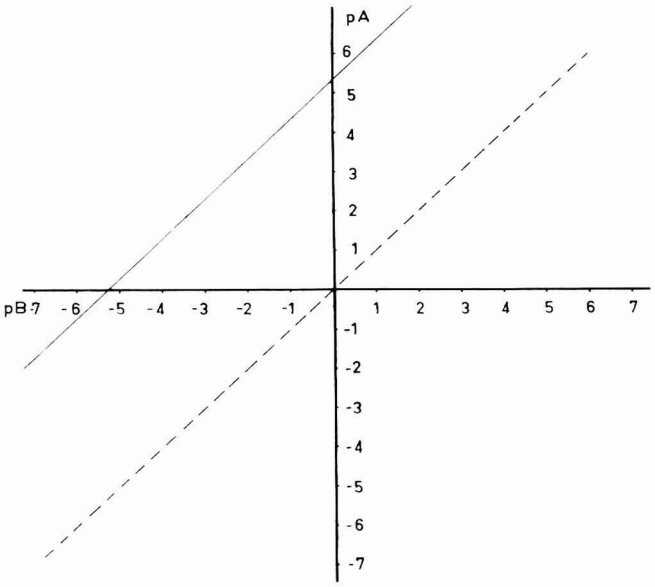

FIG. 7. Sensibility diagram for detection of macro-, semimicro-, and microscale of bismuth(III) with tin(II) chloride in glycerin–ethanol (3:1), ethyleneglycol, diethyleneglycol, and triethyleneglycol, respectively. pA = −log g of Bi/ml; pB = −log ml of H_2O.

Procedure

a. On drop plate. Put successively on a drop plate, 1 drop of sodium hydroxide solution, 1 drop of tin(II) chloride in organic solvent, and 1 drop of alkaline tellurium(IV) solution. It appears a black or grey precipitate, according to the quantity of tellurium in the sample, after 1 or 2 minutes pass. It is necessary to run a blank test. Identification limit reached: 0.38 μg of Te; pD = 4.9.

b. In macroscale. Place in a glass test tube (16-cm length and 1.5-cm diam), 10 drops of tin(II) chloride solution in organic solvent, add, drop by drop, sodium hydroxide solution until the precipitate of tin(II) hydroxide becomes dissolved, and add 5 ml of the tellurium(IV) solution. A black-brown precipitate appears. Identification limit reached, 15.60 μg of Te; pD = 5.5.

c. In microscale. Place in a glass test tube (10-cm length and 1-cm diam), four drops of tin(II) chloride solution and add some drops of sodium hydroxide solution until the tin(II) hydroxide precipitate dissolves. Add 1 ml of tellurium(IV) solution. A black-brown precipitate appears, according the tellurium concentration, after about 2 minutes. Identification limit reached: 3.12 μg of Te; pD = 5.5 (see Figs. 8 and 9).

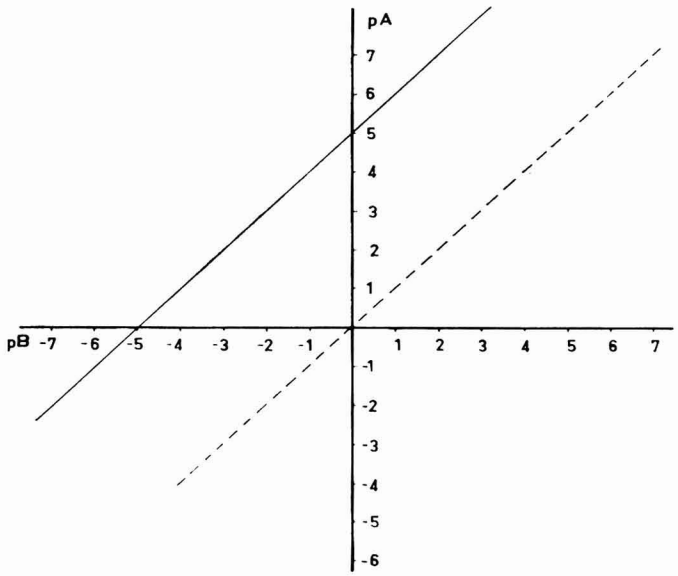

FIG. 8. Sensibility diagram for detection of tellurium(IV) with tin(II) chloride in organic solvents, on drop plate. pA = $-\log$ g of Te/ml; pB $-\log$ ml of H_2O.

TUNGSTEN(VI) WITH TIN(II) CHLORIDE IN ORGANIC SOLVENTS

Tungsten is easily reduced by tin(II) chloride solutions to tungsten(V) oxide in a colloid form or in blue precipitates. It is a sensitive reaction and it is used for identification of the element. At first, a yellow color is obtained, but, when some drops of conc. Hydrochlorhydric acid are added, and warmed, a blue precipitate soon appears.

Reagents

Tungsten(VI) solution, aqueous solution, prepared dissolving the theoretical quantity of tungsten(VI) oxide in 100 ml of sodium hydroxide solution to 2%, and dilution with distilled water to 1 liter.

Tin(II) chloride solution, prepared to 25% as above.

Hydrochlorhydric acid, conc. p. a.

Procedure

a. On drop plate. On drop plate, mix 1 or 2 drops of tungsten(VI) solution with 3 to 5 drops of 25% tin(II) chloride solution and 5 drops of conc. hydrochlorhydric acid. A blue precipitate or coloration appears

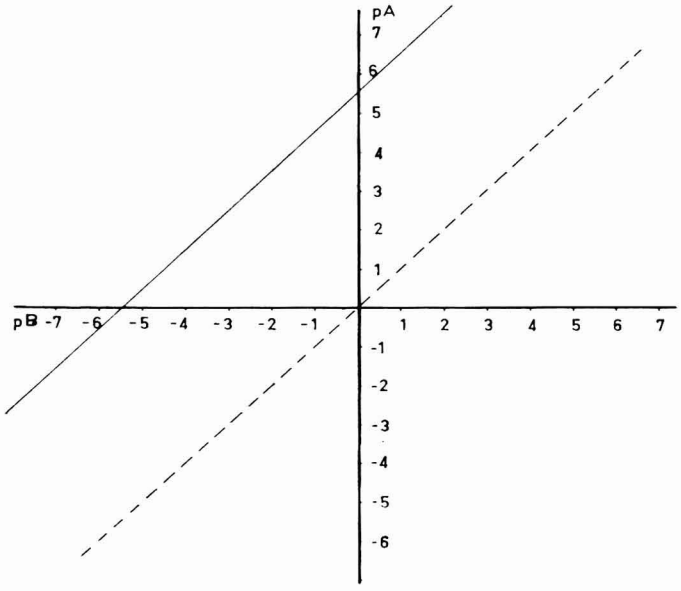

FIG. 9. Sensibility diagram for detection in macro-, semimicro-, and microscale of tellurium(IV) with tin(II) chloride in glycerin–ethanol (3:1), ethyleneglycol, diethyleneglycol, and triethyleneglycol, respectively. pA = −log g of Te/ml; pB = −log ml of H_2O.

suddenly, or after few minutes, according to the concentration of the solution. It is necessary to run a blank test. Identification limit reached: 6 μg of W; pD = 4.0.

b. In macroscale. In a (16-cm length and 1.5-cm diam), glass test-tube, place 5 ml of tungsten(VI) solution, add 1 ml of 25% tin(II) chloride in organic solvent and 1 ml of concentrated hydrochlorhydric acid. A blue precipitate appears. Identification limit reached: 250 μg of W; pD = 4.3.

c. In microscale. In a (10-cm length and 1-cm diam), glass test tube place 1 ml of tungsten solution, add 10 drops of tin(II) chloride solution in organic solvents and 10 drops of concentrated hydrochlorhydric acid. Shake it and slightly warm it. A blue precipitate appears. It is necessary to run a blank test. Identification limit reached: 100 μg of W; pD = 4.0 (see Figs. 10 and 11).

ARSENIC(III) WITH TIN(II) CHLORIDE IN ORGANIC SOLVENTS

In strongly acidified solutions with hydrochlorhydric acid, tin(II) chloride can easily reduce arsenic(III) to an elemental state, forming, a black-brown precipitate. The reduction is accelerated by warming it. This can only take place in strongly acidulated solutions, because in arsenical reactions with tin(II) chloride, the arsenic reacts in a form of

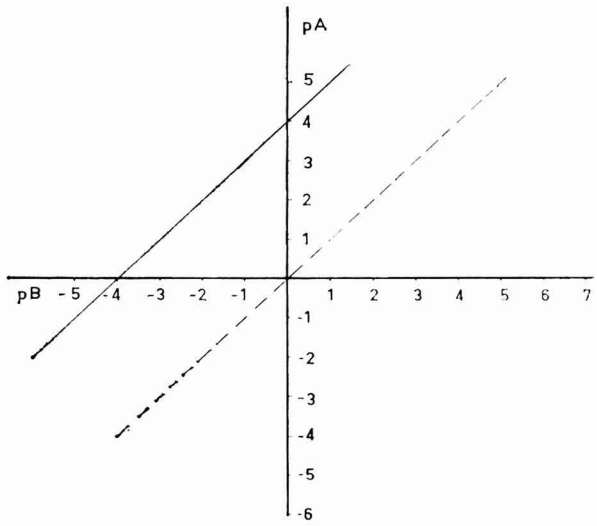

FIG. 10. Sensibility diagram for detection of tungsten(VI) on spot plate and microscale using tin(II) chloride in glycerin–ethanol (3:1), ethyleneglycol, diethyleneglycol, and triethyleneglycol, respectively. pA = −log g of W/ml; pB = −log ml of H_2O.

cation; and it is hydrolyzed in solutions which are not very acid. In the determination, only the mercury salts interfere, because the reaction is almost specific.

Reagents

Arsenic(III) solution, dissolve arsenic(III) trioxide, previously dryed to 110°C, in a beaker which contains 4 g of sodium hydroxide and 40 ml of water. After solution, dilute to 400 ml and neutralize with 1 *M* hydrochlorhydric acid in presence of phenolfphthalein. Place the slightly acidified solution in a 1000-ml volumetric flask, add 4 g of sodium hydrogencarbonate, dilute to the mark, and mix by shaking.

Tin(II) chloride solution, prepared to 5% as above.

Hydrochlorhydric acid, conc p. a.

Procedure

a. Spot test. In a (6.5-cm length and 5-mm diam), microcentrifuge glass tube place 1 drop of arsenic(III) solution, 3 drops of concentrated hydrochlorhydric acid, and 1 drop of tin(II) chloride solution. Shake slightly homogenize and warm for several minutes in a water bath. A black-brown precipitate of metallic arsenic appears. Identification limit reached: 1.1 μg of As; pD = 4.4.

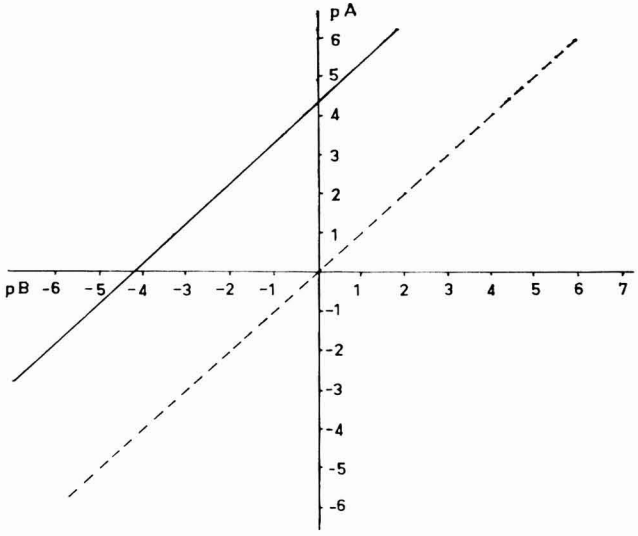

FIG. 11. Sensibility diagram for detection of tungsten(VI) in macro- and semimicroscale using tin(II) chloride in glycerin–ethanol (3:1), ethyleneglycol, diethyleneglycol, and triethyleneglycol, respectively. pA = −log g of W/ml; pB = −log ml of H_2O.

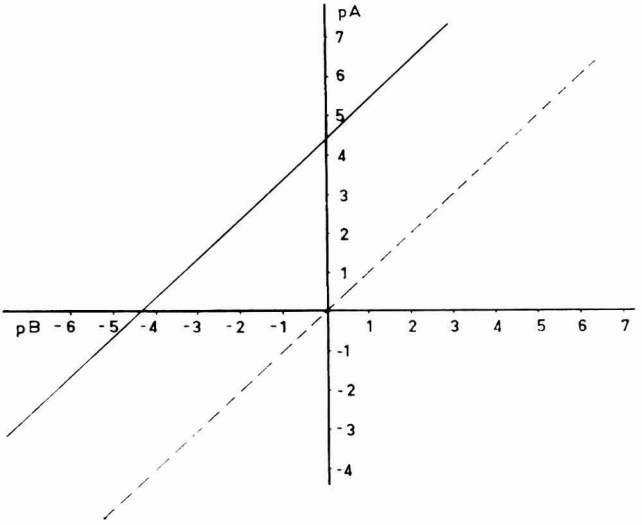

FIG. 12. Sensibility diagram for detection of arsenic(III) on spot plate using tin(II) chloride in glycerin–ethanol (3:1), ethyleneglycol, diethyleneglycol, and triethyleneglycol, respectively. pA = −log g of As/ml; pB = −log ml of H_2O.

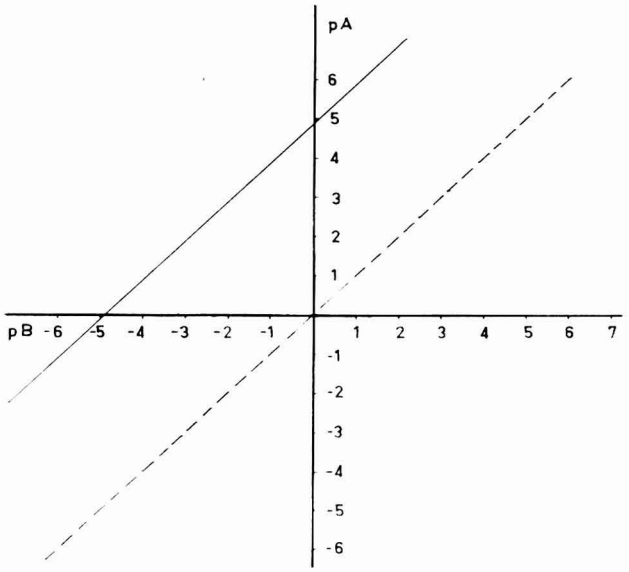

FIG. 13. Sensibility diagram for detection of arsenic(III) in macro tube using tin(II) chloride in glycerin–ethanol (3:1), ethyleneglycol, diethyleneglycol, and triethyleneglycol, respectively. pA = −log g of As/ml; pB = −log ml of H_2O.

TABLE 1

RECAPITULATION

Ion and technique	Sensibility (pD)	
	$SnCl_2$ in organic solvents	$SnCl_2$ in hydrochlorhydric acid
Mercury(II) spot test	4.2	3.3
micro and macro tube	4.2	4.2
Molybdenum(VI) spot test	5.7	5.7
macro and micro tube	6.3	5.7
Bismuth(III) spot test	4.3	4.3
macro and micro tube	5.3	4.6
Tellurium(IV), spot test	4.9	4.6
macro and micro tube	5.5	4.9
Tungsten(VI), spot test	4.0	4.0
macro and micro tube	4.3	4.0
Arsenic(III), spot test	4.4	4.4
macro tube	5.0	4.5

b. Microscale test. In a 10-cm length and 1.5-cm diam), glass test-tube place 1 ml of arsenic(III) solution, 3 ml of concentrated hydrochlorhydric acid, and 1 ml of tin(II) chloride in organic solvent. Shake slightly and warm for several minutes on water bath. A black-brown precipitate of metallic arsenic appears. It is necessary to run a blank test. Identification limit reached: 9.36 μg of As; pD = 5.0 (see Figs. 12, 13, and Table 1).

SUMMARY

Stability of glycerol-ethanolic, ethyleneglycol, diethyleneglycol, and triethyleneglycol $SnCl_2$ reagent solutions are studied comparatively ahead of classic hydrochlorhydric acid solution. The best results are obtained with diethylene and triethyleneglycol solutions.

For the first time are used nonaqueous tin(II) chloride solutions as reagents for spot test and conventional identification of mercury(II), molybdenum(VI), bismuth(III), tellurium(IV), tungsten(VI), and arsenic(III) ions with accurate results.

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MICROCHEMICAL JOURNAL 16, 113–120 (1971)

A Cooperative Study of the Determination of Lead in Milk

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Received October 10, 1970

INTRODUCTION

In view of the toxicity of lead and the importance of milk in the diet of infants and children, the lead content of market whole milk has received relatively little attention. Kasahara *et al.* (*2*) reported 20–40 μg of lead/liter for U.S. milks and 10–60 μg/liter for Japanese milks. Kehoe *et al.* (*3*) analyzed three samples and found 20 to 40 μg of Pb/liter. The U.S. Public Health Service (*7*), reporting data obtained by Murthy and Rhea, cited 40 ± 17 μg of Pb/liter for 37 market milk samples obtained in the Cincinnati area and 50 ± 25 μg of Pb/liter for 35 market milks obtained in northern and northwestern United States. Murthy *et al.* (*4*) analyzed 248 market milk samples from 59 localities scattered throughout the United States and reported an average of 49 μg of lead/liter with a range at 23–79 μg/liter.

It was anticipated that the determination of parts-per-billion concentrations of lead in whole milk would be a difficult task because of the presence of large amounts of protein. Consequently, several exploratory tests were made to evaluate various means of sample preparation. In all cases, lead was measured by a dithizone procedure. Results of these tests are given in Table 1.

These findings suggested that either lead was being lost during the course of sample preparation or that it was present in much lower concentrations than anticipated. In order to resolve the matter, a large supply of nonfat dry milk solids was obtained. The lead content of this material could be obtained readily by proven procedures and it could be reconstituted for analysis in the liquid state. Known amounts of lead were added to a portion of the reconstituted milk. The assistance of seven other laboratories was solicited who were either experienced in determining lead in milk or were known to be highly skilled in performing trace lead determinations. The laboratories were supplied with two samples and were informed that they were identical except that one

TABLE 1

EXPLORATORY SAMPLE PREPARATION TESTS

Milk brand	Sample size (ml)	Sample treatment	Pb (μg/liter)
A	250	Wet oxidation with HNO_3-$HClO_4$	3.5
	200	Acetic acid precipitation of protein, dry ashing	1.1
B	200	Acetic acid precipitation of protein, dry ashing	−0.2
C	200	Acetic acid precipitation of protein, wet oxidation	6.8
	200	Acetic acid precipitation of protein, dry ashing	−4.6
	100	Acetic acid precipitation of protein, dry ashing	9.7
D	100	Nitric acid precipitation of protein, dry ashing	6.3
	500	Nitric acid precipitation of protein, dry ashing	−3.0

contained about 50 μg of lead/quart of added lead as lead nitrate. Two of the laboratories requested and received the solid material from which the liquid samples were prepared.

This paper describes the procedure used to analyze the milks and cites the data obtained by this laboratory and the seven cooperating laboratories which also made the analyses.

MATERIALS AND METHODS

Apparatus

A Beckman Model B spectrophotometer was modified to accept special absorption cells. Cells were made by fusing the top portion of a 125-ml Squibb separatory funnel to a 10-mm Pyrex absorption cell. Samples were dried in a Thelco Model 28 mechanical convection oven with the air intake stuffed with United Air Filter Media 835 and were ashed in a Hevi-Duty muffle furnace equipped with a removable stainless steel liner and a West Instrument Corporation Gardsman Temperature Controller. Air was passed into the furnace at 1–2 liters/minute through a Gelman No. 12101 0.45-μ filter. Separatory funnels were fitted with Teflon stopcocks.

Reagents

Reagents were used without further purification. They were segregated to prevent their use for other purposes.

Ammonium hydroxide. 30% NH_3, sp gr 0.90.

Buffer solution. Dissolve in separate portions of water, 40 g of potassium cyanide (filter if necessary), 12 g of ammonium citrate, 80 g of sodium sulfite (anhydrous), and 350 ml of ammonium hydroxide. Add the other reagents to the ammonium hydroxide solution and dilute to 2 liters.

Chloroform. Passes ACS dithizone stability test.

50% Citric acid. Dissolve 500 g of citric acid in water and dilute to 1 liter.

Dithizone–chloroform solutions, 7.5 and 30 mg/liter. Dissolve the appropriate amount of dithizone (Eastman No. 3092) in chloroform and dilute to 1 liter. Store in a cool dark place. Check standard solution every 2 weeks.

20% Hydroxylamine hydrochloride. Dissolve 200 g of hydroxylamine hydrochloride in water. Dilute to 1 liter.

Nitric acid. 70% NHO_3, sp gr 1.42.

1.6% Nitric acid. Add 16 ml of 70% nitric acid to water and dilute to 1 liter.

Perchloric acid. 70% $HClO_4$.

10% Potassium cyanide. Dissolve 100 g of fresh KCN in a small amount of water and filter if necessary. Dilute to 1 liter.

Thymol blue indicator. Dissolve 0.219 g of water-soluble thymol blue in water and dilute to 500 ml.

Procedure

Transfer 200-ml samples to 250-ml beakers, add 4 ml of nitric acid and stir with a stirring rod. Leave the rod in the beaker. Place the beaker in the drying oven at 100° and, with occasional stirring to break the crust, heat until dry. Transfer the beaker to the cold muffle furnace. Raise the temperature in 50° increments until smoking begins. Hold this temperature until smoking subsides. Increase the temperature by 25 to 50° and hold this temperature until smoking again decreases. Raise the temperature by 25 to 50° and continue in this fashion until the smoking has stopped and a final temperature of 480° is reached. Hold this temperature for 12 to 15 hours. Cool the furnace to near room temperature. Remove the beakers, cover with watch glasses, add 1–2 ml of water, 5 ml of nitric acid, and 5 ml of perchloric acid. Place on a hot plate at about 375°, heat to fumes of perchloric acid, and cool. Add 2 ml of nitric acid and 50 ml of water. Warm to dissolve the salts. Transfer the solutions to 250-ml separatory funnels.

Add 2 ml of 20% hydroxylamine solution, 20 ml of 50% citric acid solution and 5 drops of thymol blue indicator. Add ammonium hydroxide to the color change (pH 9–9.5), 5 ml of 10% potassium cyanide solution, and 5 ml of dithizone solution (30 mg/liter). Shake for 15 seconds. Rinse the stopper with water and drain the chloroform layer into 125-ml separatory funnels containing 25 ml of 1.6% nitric acid solution. Rinse the stopcock plug with chloroform. Repeat the dithizone extraction until the chloroform layer retains the original dithizone color. Shake the 125-ml separatory funnels until the dithizone–chloroform

layer is blue-green. Discard the dithizone–chloroform layer. Extract the nitric acid solution twice with chloroform and discard the chloroform. Allow the second extract to stand for about 20 minutes before draining.

Transfer the nitric acid solutions to the special cells and dilute to 50 ml. Add 10 ml of buffer solution. Add, by volumetric pipet, 10 ml of 7.5 mg/liter of dithizone solution. Shake vigorously, and read the absorbance of the chloroform–dithizone solution against chloroform at 510 nm within 30 minutes.

RESULTS AND DISCUSSION

Table 2 shows the lead content of the milk solids as obtained in this laboratory and in the two other laboratories which requested the materials. Our data from different sample sizes of lot 10J25E suggests that the result reflects the smallest amount of lead which may be determined by our procedure. In these analyses, as in all others reported here, lead was determined by dithizone. Laboratory E made the measurement by atomic absorption spectrophotometry (AAS) while Laboratory F used both AAS and dithizone (Dz).

Table 3 gives data obtained on the reconstituted dry milk and the same milk treated with 5.9 and 59 μg of lead/liter. The discrepancy between results obtained on the solids (1–4 μg/liter) and those obtained on the reconstituted samples (7 μg/liter) cannot be accounted for by the lead content of the diluent. Analysis by anodic stripping voltametry of the distilled, deionized water used to reconstitute the milk showed less than 0.4 μg of lead/liter.

Table 4 cites data obtained by the cooperating laboratories. Our data in Table 4 are the average values taken from Table 3.

TABLE 2

LEAD IN DRIED MILK

	Laboratory	Sample wt (g)	Pb (μg)	Equivalence (μg of Pb/liter)
Lot 10J25E	Ethyl	10 (equiv. to 100 ml)	0.40	4.0
			0.30	3.0
		50 (equiv. to 500 ml)	0.84	1.7
			0.46	0.9
	Laboratory E	24 [a]		11.6
Lot 2A15E	Ethyl	50	1.80	3.6
			2.22	4.3
	Laboratory F	5		AAS [b] 38
		5		Dz [c] 26

[a] Pb determined on 2/5 of original sample.

[b] Atomic absorption spectrophotometry.

[c] Dithizone.

Laboratories C, E, and G analyses of the untreated milk are in general agreement with our data on the dry and liquid materials. Laboratory E result is consistent with its analysis of the dry material (Table 2). In the analysis of the treated samples, Laboratories C, D, E, F (Dz), and G were in general agreement with our data. Laboratory C reported conducting thorough review of its procedure after failure to recover more than 50% of the lead on the initial attempt. However, no faults were located. Full recovery of the added lead was found on the second attempt. Except for this instance, results are essentially the same or higher than our data.

The range of values for the untreated sample reported by the seven laboratories is 7 to 150 μg of lead/liter. Inasmuch as a quantity of lead is apparently present which can be determined with reasonable accuracy and the measurement equipment used is capable of making the measurements, it appears that equipment was used improperly, that lead was being added inadvertently during the course of the analysis or that errors made in analyzing relatively small samples are greatly magnified in computing the final results.

Murthy *et al.* (*4*), in their extensive survey of market whole milks available in the United States, used a procedure essentially the same as Willis' (*8*) method for determining lead in urine. Murthy *et al.* (*4*) dry ashed a 100-g sample in a Vycor evaporating dish, dissolved the ash in

TABLE 3

Pb IN RECONSTITUTED MILK (200-ml samples)

	Pb (μg/liter)		
		Treated	
	Untreated	59	5.9
Lot 10J25E	6.7 [a]	63.9 [a]	
	6.2 [a]	59.8 [a]	
	9.2 [b]	66.4 [b]	
	7.7 [b]	62.9 [b]	
	6.9		14.7
	7.0		13.2
	6.8		
	7.0		
Lot 2A15E	6.1 [c]	58.8 [c]	
	7.2 [c]	62.1 [c]	

[a] Portions of batches sent to Laboratories B and C. Analyzed about 2 weeks after shipment.

[b] Same samples as (*a*); analyzed about 4 weeks after shipment.

[c] Inadvertently ashed at 580°C for 2 hours.

TABLE 4

LEAD IN MILK

Laboratory	Sample, ml	Pb (μg/liter)			
		Untreated		Treated (59 μg/liter)	
A [a,b]	50	110		110	
	?	150 [c]		192 [c]	
	?	56 [d]		100 [d]	
B	?	37		89	
C	1000	9		30	
		7 [c]		65 [c]	
D	50	24 [e]		79 [e]	
		29.6		54.8	
E	250 [f]	12		65	
		AAS	Dz	AAS	Dz
F	100	27	18	—	—
	50	—	—	85	62
G	50 [g]	7.2		61	
Ethyl	200	7		62	

[a] Labs, A, B, C, D, E from Lot 10J25E; B and C received same batch of reconstituted milk.

[b] A (second sample), F and G from Lot 2A15E.

[c] Second analysis on original samples.

[d] Second samples.

[e] Tentative data obtained on initial attempt.

[f] Used ⅖ aliquot for Pb measurement.

[g] Used undisclosed aliquot for Pb measurement by ASV.

3 *N* hydrochloric acid, extracted the diethyl dithiocarbamate–Pb complex into methyl isobutylketone and determined the lead in this solution by atomic absorption spectrophotometry. The accuracy of this procedure was established by obtaining 98% recovery of 10 μg of lead added to 25 ml of milk. This is equivalent to 400 μg/liter or almost 10 times greater than the average value reported for the market milks. It does not follow that the accuracy at 400 μg/liter is the same as at 50 μg/liter. Further, the difficulties in preparing a 100-g sample are not the same as preparing a 25-g sample.

Similar unjustifiable confidence in analytical data is illustrated in the results of cooperative analyses of a variety of dried vegetables (Table 5).

The data obtained by Laboratory X indicated that the procedure used for analyzing vegetables is such that the low limit of discrimination is about 5 μg of lead/g. Subsequent analyses yielded data consistent with those obtained by others (*1, 3, 5, 6*).

TABLE 5

ANALYSES OF DRIED VEGETABLES

Sample	Pb (μg/g; dry wt)	
	This Laboratory	Laboratory X
Aqueous Standard	1.0	1.0
	5.8	6.0
Lettuce	1.0, 1.2	9, 10
Cabbage	3.0, 3.4	4.9, 5.4
Turnip	4.5, 5.2	5.0, 5.9
Carrot	1.4, 1.2	4.5, 4.0

Laboratory C supplied two 1-quart samples of whole milk. Each sample was analyzed in duplicate by the recommended procedure. These samples were subsequently identified as duplicates from a composite of market whole milk. Data obtained by this laboratory and by Laboratory C are given in Table 6.

These data and low positive and negative values given in Table 1 suggest that the lowest level of reliability of the proposed method is about 5 μg of lead/liter and that the true value may be somewhat lower. This contention is supported by several attempts to determine lead in market whole milk by anodic stripping voltammetry without prior treatment or with minimum treatment. These efforts were not unqualified successes but in some cases no lead was detected while in others, values comparable to those cited in Table 1 were obtained.

SUMMARY

Tests have been made which suggest that previously reported data on the lead content of market whole milk are too high by an order of magnitude or more. Dried milk and reconstituted dried milk, with and without added lead, were analyzed. Seven other laboratories, either experienced in performing the analysis or known to be competent in determining trace quantities of lead, also analyzed the liquid samples. Values reported by these laboratories for the untreated sample

TABLE 6

LEAD IN MARKET WHOLE MILK (composite sample)

Pb (μg/liter)	
Ethyl	Laboratory C
5.1	≤3
4.9	≤3
4.4	
5.4	

ranged from 7 to 150 μg of lead/liter. The lower value coincides with that found in this laboratory. However, this is slightly higher than results obtained on the dried material (equivalent to 1 to 4 μg/liter). Results on the milk to which 59 μg of lead/liter had been added ranged from 30 to 192 μg/liter but five of the seven laboratories obtained results in reasonable agreement with the added amount and our value of 62 μg/liter.

Quadruplicate analyses of a composite market whole milk supplied by one of the laboratories gave values of 5.1, 4.9, 4.4, and 5.4 μg of lead/liter while the originating laboratory obtained $\leq$ 3 μg of lead/liter in duplicate determinations.

High results obtained in the tests by the cooperating laboratories, and in previously reported analyses of market milk, may be attributed to inadvertent addition of lead during sample processing and/or unjustifiable confidence in the analytical procedure.

ACKNOWLEDGMENTS

The authors thank the laboratories which participated in this work; Mr. B. V. Walczak who performed the anodic stripping analyses; and Dr. Gary Ter Haar for his assistance and continuing interest.

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MICROCHEMICAL JOURNAL **16**, 121–135 (1971)

The Preparation and Properties of the Acetate Complex of Trivalent Cobalt

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Received October 12, 1970

INTRODUCTION

Salts of trivalent cobalt belong among systems with high redox potentials and, consequently, are utilizable as analytical oxidants in those cases where weaker reagents are unsuccessful. The main obstacle to fuller analytical use of cobalt(III) salts is the reaction of Co(III) with water which inevitably occurs in aqueous solutions. The only stable Co(III) compounds are complexes; for analytical purposes the acetate complex (*1*, *2*) was predominantly studied.

The configuration of the acetate complex [hereafter denoted as Co(III)Ac] was studied by Koubek and Edwards (*3*) who prepared a complex of composition $[(Ac)_2Co(OH)_2Co(Ac)_2]$ by the oxidation of cobalt(II) acetate with peroxyacetic acid. Co(III)Ac was also prepared by the anodic oxidation of cobalt(II) acetate in glacial acetic acid (4) but the solutions obtained always contained Co(II) ions and water and thus were not stable.

The present paper deals with the electrolytical preparation of Co(III)Ac yielding a reagent of defined composition and of sufficient stability, which could be used mainly for oxidation of organic substances for their determination or preparation. Further, the coulometric generation of Co(III) in nonaqueous media in a closed system was studied, the current efficiency was calculated, and the water content was determined by measuring the dielectric constant.

EXPERIMENTAL METHODS

Reagents

Technical crystalline cobalt(II) acetate was recrystallized from 10% acetic acid and, for removing crystal water, pulverized and dried at 130°C. Cobalt was determined gravimetrically as cobalt(II) sulfate. To determine the water in glacial acetic acid, the K. Fischer method with "dead-stop" end-point indication, and the melting point measurement of

the binary mixture of water and acetic acid were used. In this way a defined acid was used for the calibration of the DK-meter and for obtaining calibration curves for the ternary mixtures: water-acetic acid–cobalt(II) acetate.

Apparatus

Potentiometric titrations were carried out using platinum indicator and saturated calomel reference electrodes and the pH meter "Acidimeter EK" (Dělba, Czechoslovakia).

For the determination of water by the K. Fischer method the automatic titrator TTT lc (Radiometer, Denmark) was used.

The dielectric constant (DK) was measured with the DK-meter "Universal Dielectrimeter" OH 301 (Radelkis, Hungary) which has four ranges for $E = 1$ to 100, and ranges 1 to 50 μS and 10 to 500 μS for conductivity measurements.

Spectrophotometric measurements were carried out with the Unicam SP-800 spectrophotometer using 1-cm quartz cuvettes.

A nitrogen–hydrogen coulometer and a coulometer for explosive gas were used and were calibrated by the E211 coulometer (Metrohm, Switzerland).

Electric current for the electrolysis of cobalt(II) acetate was drawn from the source "Tesla BM 208" which supplies voltages from 0 to 500 V and currents of 0 to 500 mA, and from the source "Tesla BM 275" which supplies currents up to 100 mA at a voltage of 0 to 800 V. The line voltage was regulated by the stabilizer "Tesla 206."

The DK cell and the mantle of the gas coulometer were thermostated with a precision of 0.1 °C by the ultrathermostat (VEB Prüfgeräte-Werk Medingen, Germany).

RESULTS

Preparation and Standardization of the Reagent

The electrolytic oxidation was carried out by the modified method of Sharp and White (*4*). The process of Co(III)Ac generation was followed titrimetrically. Titration with a standard solution of potassium bichromate in a medium of 2 *N* HCl was chosen as the most suitable method for determining the excess iron(II) after the reduction of Co(III). This method yields the same results as the direct titration of Fe^{2+} with Co(III)Ac but it is faster because potentials stabilize slowly in the system Co(III)–Fe(II), especially in the vicinity of the equivalence point. No oxidation of chloride ions by the Co(III) salt took place during the determination.

For the preparation of cobalt(III) acetate were used three methods,

differing in the composition of the catholyte and the anolyte: (A) The solutions were prepared from crystalline salts [$Co(CH_3COO)_2 \cdot 4H_2O$, $NaCH_3COO \cdot 3H_2O$]; (B) anhydrous salts were used for preparing the solutions; (C) the electrolysis was carried out in the absence of sodium acetate.

A. One liter of 0.15 *N* Co(II)Ac in glacial acetic acid served both as the anolyte and the catholyte. 10 g of crystalline sodium acetate was added to increase the solution conductivity. A cylindrical platinum gauze with a surface of about 150 cm^2 (the bigger electrode of the Fischer pair) served as an anode; in the center of the anode was a cylindrical copper cathode, 30 mm long and 30 mm in diameter. Cathodic and anodic compartments were separated with a cellophane membrane. The process of electrolysis is summarized in Table 1. During first 14 days, the normality of the Co(III)Ac solution varied considerably, in fact more than that of the reagent prepared according to Minczewski (*2*) (Table 2).

Since the stability of the reagent decreases with increasing water content, anhydrous Co(II)Ac and sodium acetate were subsequently used. The crystal water added to the solution of 0.15 *N* Co(II)Ac + 10 g of NaAc amounts to 16.8 g/liter, i.e., about 2%.

B. The catholyte and the anolyte each contained 39 g of anhydrous cobalt(II) acetate and 30 g of anhydrous sodium acetate in 2 liter of the solution in glacial acetic acid.

Polythene rings were fitted to the copper cathode to prevent it from approaching the anode which would cause a discharge through the cellophane (Fig. 1).

After completion of the electrolysis, the Co(III)Ac solution was filtered through a G-4 glass filter. The progress of the electrolysis is shown in Table 3. It is evident from Fig. 2 that the Co(III)Ac solution prepared from anhydrous salts in glacial acetic acid is more stable than

TABLE 1

ELECTROLYSIS ACCORDING TO A

Time (hours)	Current (mA)	Voltage (V)	Resistance (kOhms)	Co(III)Ac (*N*)
10	20	180	9.0	0.0041
25	20	98	4.9	0.0083
40	30	96	3.2	0.0231
72	30	42	1.4	0.0503
98	30	24	0.8	0.0745
120	32	16	0.5	0.0928
135	35	12	0.34	0.1110

TABLE 2

STABILITY OF THE Co(III)Ac SOLUTION IN GLACIAL ACETIC ACID PREPARED BY ELECTROLYSIS ACCORDING TO A

Day:	0	3	7	13	18	41	120
Decrease in normality (% per day)	1.0	0.50	0.27	0.203	0.185	0.039	—
Decrease acc. to (2)	0.90	0.31	0.13	0.027	—	—	—
Normality	0.111	0.108	0.1069	0.105	0.104	0.102	0.101

that used in the previous method; the titer of the solution is practically unchanged after 1 week. The solution purity apparently also has a substantial effect on its stability.

C. 0.1 or 0.01*N* Co(II)Ac were used as both the catholyte and the anolyte. The electric resistance of the cellophane was decreased by wetting it on the inner side with 0.2 ml of distilled water; after it swelled,

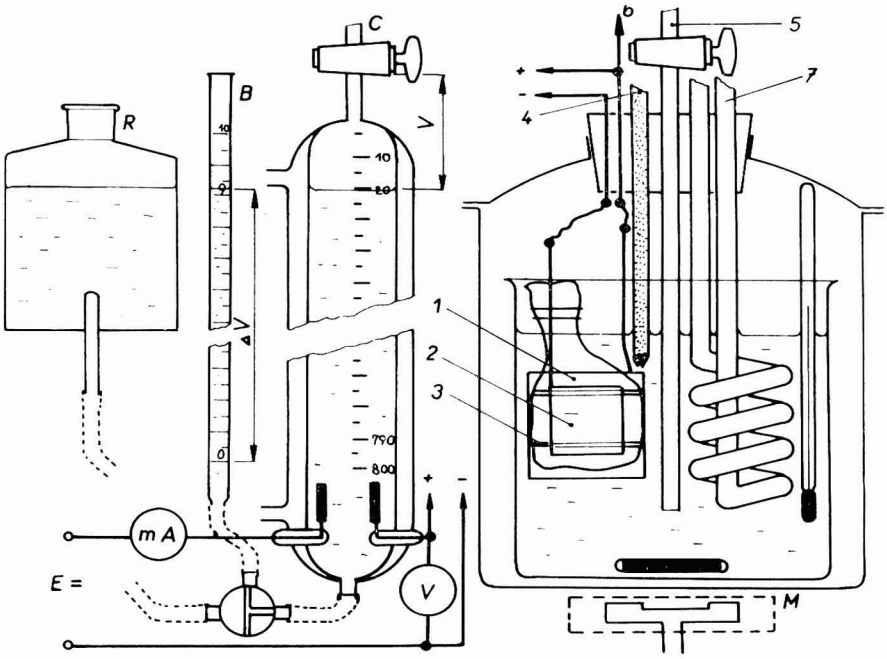

FIG. 1. The apparatus for anodic oxidation and the coulometer for explosive gas: (1) Pt anode; (2) Pt cathode; (3) insulating ring; (4) the SCE bridge; (5) the tube for taking samples; (6) potentiometer; (7) thermostatting; (M) magnetic stirrer; (C) coulometer; (B) burette; (R) the electrolyte reservoir.

TABLE 3

ELECTROLYSIS ACCORDING TO B

Time (hours)	Current (mA)	Voltage (V)	Resistance (kOhms)	Co(III)Ac (*N*)
—	40	110	2.7	—
3	40	100	2.5	0.0015
35	40	52	1.3	0.0182
106	40	12	0.30	0.0562
123	40	11	0.27	0.0650

the cell was filled with catholyte. In the case of the 0.1 *N* solution of Co(II)Ac, a current of 40 mA at 54 V was reached and after 197 hours of electrolysis a solution 0.1012 *N* in Co(III)Ac was obtained. The electrolysis of 0.01 *N* Co(II)Ac proceeded at 15 to 30 mA for 144 hours giving 0.01075 *N* Co(III)Ac.

From these results (Fig. 3) it follows that it is possible to prepare pure Co(III)Ac without a base electrolyte. The solution stability is, however, substantially lower; the reagent is not stabilized even after 2 months when the decrease in normality still amounts to 0.1 to 0.3% a day.

The Determination of Water in the System of Cobalt(II) Acetate–Cobalt(III) Acetate–Acetic Acid

It follows from Tables 1 and 3 that the initially very low conductivity of the solution increases during the electrolysis and the resistance of the system solution–membrane decreases. An increasing concentration of water which further increases the instability of the Co(III)Ac solutions could be one of the causes of this and for this reason water in this system was determined using the DK and the DS analysis.

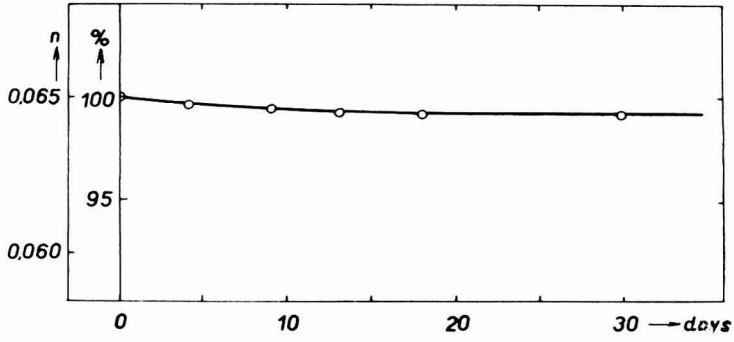

FIG. 2. The stability of Co(III)Ac prepared by electrolysis according to B.

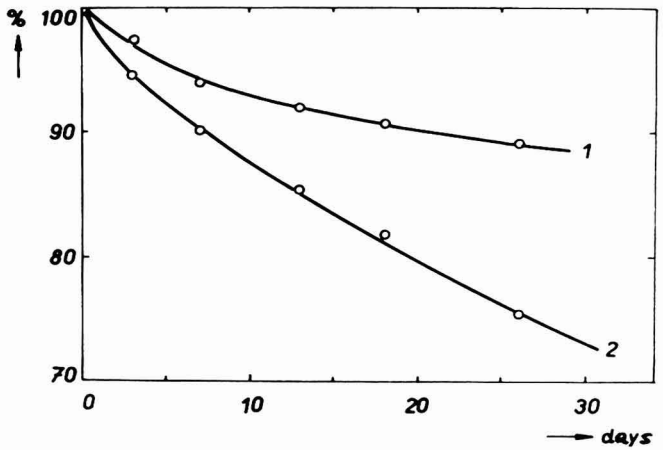

FIG. 3. The stability of Co(III)Ac prepared according to C: (1) 0.1 *N* Co(II)Ac; (2) 0.01 *N* Co(II)Ac.

The dependence of DK of the ternary mixture Co(II)Ac–HAc–H_2O on the concentration of Co(II)Ac and of H_2O was determined in the ranges 0 to 0.1 *N* Co(II)Ac and 0 to 18% H_2O in acetic acid (Fig. 4). The curve in Fig. 4 can be divided into three regions. From 0 to 2.5% H_2O, the DK value is determined by acetic acid; Co(II)Ac causes only an incremental increase in this value. In the region of 2.5 to 6% H_2O, the DK value depends linearly on the concentrations of Co(II)Ac and water. The dependence is not linear at more than 6% of water. The apparent increase in DK in the second and third regions is caused by conductivity effects of the electrolyte and the apparent capacity measured, C_{eff}, is represented by the relation

$$C_{eff} = K(1 + \mathrm{tg}^2\delta),$$

where K is the real cell capacity and tgδ is a loss coefficient (the dielectric loss — DS).

For determination of the concentration of water in Co(II)Ac and Co(III)Ac, the region up to 3% water was measured (Fig. 5). The DK of the solution varies linearly with changing water concentration and, therefore, the latter can be determined by assessing the DK value. The course of this dependence is not affected by changes in the Co(III):Co(II) ratio.

Measurement of the Current Efficiency of the Anodic Oxidation of Co(II)Ac

The high resistances of the solution and of the cellophane membrane are an obstacle to obtaining 100% current efficiency. It is necessary to

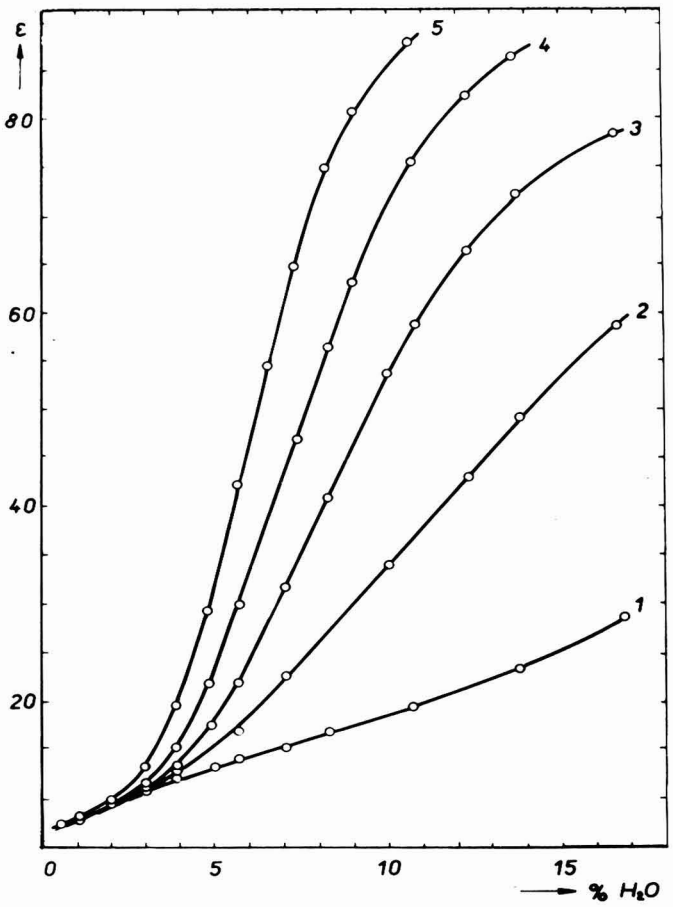

FIG. 4. The dependence of the dielectric constant on the composition of the mixture: Co(II)Ac–HAc–H_2O: (1) acetic acid; (2) 0.01 *N*; (3) 0.025 *N*; (4) 0.05 *N*; (5) 0.1 *N* Co(II)Ac.

apply voltages of the order of 10^2 V; the determination of the limiting current is difficult. The total current efficiency of the reaction

$$Co^{2+} \rightarrow Co^{3+} + e^-,$$

was determined by the explosive gas coulometer (Fig. 1), containing 0.25 *M* $K_2Cr_2O_7$. The platinum electrodes had an area of 0.9 cm^2, the electric resistance was 7 ohms. The measurement of electric charge passed with the explosive gas coulometer is accompanied by a considerable error at low current densities (up to 5%) and, for this reason, a coulometer containing 2 *N* hydrazine sulfate was used in this case. The water mantle of the coulometer was kept at 26°C. Selim and Lingane's empirical factor (*5*) was used for the calculation of charge 1 C—0.1739

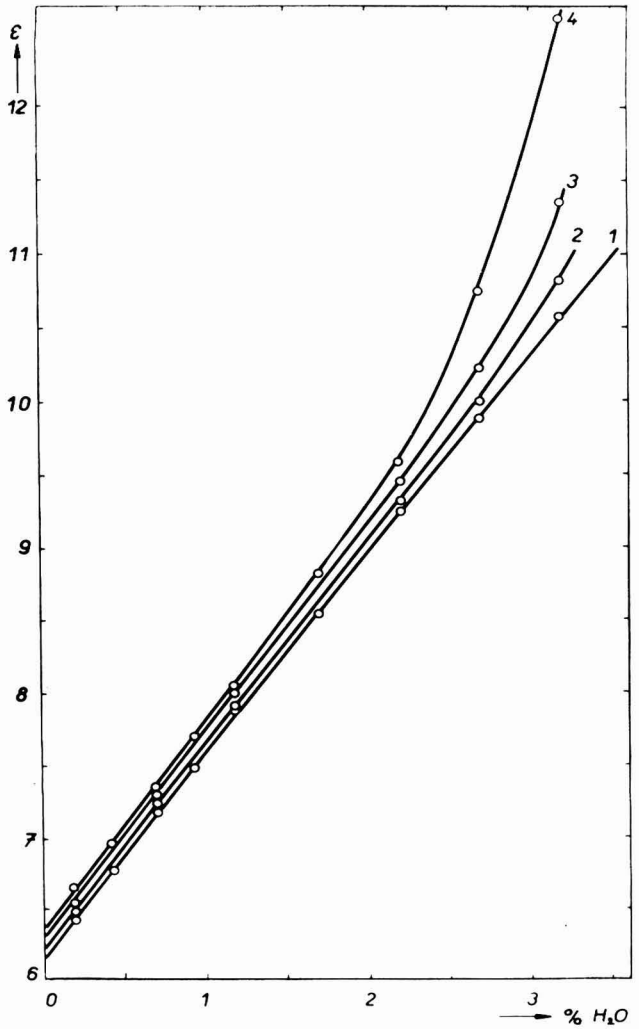

FIG. 5. The dependence of the dielectric constant of the Co(III)Ac solution in acetic acid on the mixture composition: (1) acetic acid; (2) 0.01 *N*; (3) 0.025 *N*; (4) 0.05 *N* Co(III)Ac.

ml of the explosive gas or of a mixture of nitrogen with hydrogen (*6*). The average error of the coulometer was found by calibrating it with the Metrohm coulometer: 0.9% for currents of 1 to 10 mA and charges of 6 to 50 C, and 0.6% for 20 mA and 10 to 50 C.

Spectrophotometric Determination of Co(III)Ac in Glacial Acetic Acid

Absorption curves of solutions containing Co(II)Ac and Co(III)Ac in glacial acetic acid were measured using glacial acetic acid as a blank.

The spectra conformed with the data given by Koubek and Edwards (*3*). It follows that the region of the spectrum between 500 to 700 nm, with a maximum at $\lambda = 610$ nm, is convenient for measuring the Co(III)Ac concentration within 10^{-3} to 10^{-2} *N* [absorbance is directly proportional to the Co(III)Ac concentration]. The region between 275 to 400 nm, with a maximum at $\lambda = 360$ nm, is suitable for determination of Co(III)Ac concentration within 10^{-4} to 10^{-3} *N*. Measurement in the UV region is more convenient in the presence of Co(II)Ac as there is no Co(II) maximum here, while in the visible region the main maximum of Co(II) is at $\lambda = 528$ nm and Co(III) determination must be carried out using, as a blank, the same concentration of Co(II)Ac as that in the sample.

Generation of Co(III) in a Closed System

The water concentration was followed by DK and tgδ measurements. The total current efficiency was then found by the charge measurement and the titration and spectrophotometric determination of [Co(III)Ac]. The results, given in Tables 4 and 5, show that DK and DS, as well as the water concentration (0.18%) are constant during the electrolysis. This differs from previous electrolyses where moisture was absorbed from the air and, consequently, DK and DS increased.

With decreasing Co(III)Ac concentration, DS increases due to an increase in water content (Fig. 6). The current efficiency decreases during the electrolysis down to 68 to 69% and then remains constant.

The vapors of acetic acid condensed on the outer walls of the electrolyzer at higher temperatures and caused troubles during the measurement; according to our experience, this can be prevented by using a sealed glass apparatus (Fig. 7). In this apparatus we studied: (A) the use of a glass membrane; (B) and (C) electrolysis in a nonaqueous medium and the effect of the base electrolyte on the total efficiency.

A. The arrangement of electrodes is shown in Fig. 7:the cathode—a platinum tube, 9 mm in diameter, with a surface area of 23 cm^2; the anode—two smaller Fischer platinum electrodes with a total surface area of about 110 cm^2. A glass membrane was made from a tube of a porous glass (Corning Glass Works, New York), 12 mm in diameter and 130 mm long, with the following parameters: specific weight, 1.5 g/cm^3; inner surface area, 250 m^2/g; pore diameter, 4 mm; $tg\delta/100$ Hz, 0.007. The catholyte was 0.1 *N* Co(II)Ac, the anolyte was 550 ml of 0.01 *N* Co(II)Ac. The current density was varied within 0.01 to 0.2 mA/cm^2.

B. The electrodes: a pair of platinum Fischer electrodes; a cellophane membrane; the catholyte, a saturated NaAc solution in glacial

TABLE 4

ELECTROLYSIS, 0.05 *N* Co(II)Ac

Voltage (V)	Current (mA)	Meas. DK, DS			Coulometry		Spectr.	Titr.	Current effic. (%)
		ϵ	G (μS)	tgδ	Q (coul)	$N \times 10^{-3}$	$N \times 10^{-3}$	$N \times 10^{-3}$	
300	6	7.15	125	0.101	—	—	—	—	—
	6	7.15	127	0.098	377	1.99	1.49	1.40	72.1
	6	7.15	135	0.104	478	2.80	1.98	1.95	70.1
	6	7.13	127	0.098	715	4.34	3.00	3.01	69.6
	6	7.15	135	0.104	964	5.28	3.56	3.63	68.5
280	6	7.16	130	0.101	1483	8.25	5.58	5.62	68.0
300	6.5	7.17	137	0.106	1982	11.35	—	7.75	68.2
290	8	7.16	130	0.106	2200	12.0	—	8.10	67.9
300	10	7.15	137	0.106	2856	17.32	—	11.75	68.0

TABLE 5

ELECTROLYSIS, 0.01 *N* Co(II)Ac

Voltage of 300 V.

Current (mA)	Meas. DK, DS		Coulometry		Spectr.	Current
	ϵ	G (μS)	Q (coul)	$N \times 10^{3}$	$N \times 10^{-3}$	effic. (%)
6.01	6.53	73	17.4	0.102	(0.10)	(98)
6.05	6.54	78	55.3	0.319	0.2875	90.0
6.1	6.53	77	76.8	0.445	0.3876	87.1
6.08	6.53	74	103.1	0.597	0.500	83.6
6.1	6.55	75	132.2	0.800	0.640	80.0
6.1	6.54	74	157.9	0.987	0.900	82.0
7.0	6.53	74	182.2	1.160	1.020	82.5
6.9	6.54	75	213.0	1.350	1.155	85.0

acetic acid; the anolyte, 0.01 Co(II)Ac. Current densities of 0.5 to 1.0 mA/cm^2.

C. Electrodes as in (B), the catholyte, a 5% solution of anhydrous NaAc in glacial acetic acid; the anolyte, 0,06 N Co(II) Ac in anhydrous acetic acid (with a water content of less than 0,02%).

In all these cases the amount of Co(III) generated was determined spectrophotometrically; the electric charge passed was measured in a coulometer for nitrogen and hydrogen containing 2 *N* hydrazine sulfate. The results are summarized in Table 6.

DISCUSSION

The most stable reagent obtained, which contained no Co(II), was prepared electrolytically from solutions of 0.005 to 0.1 *N* Co(II)Ac in glacial acetic acid, with 10 g of anhydrous sodium acetate dissolved in

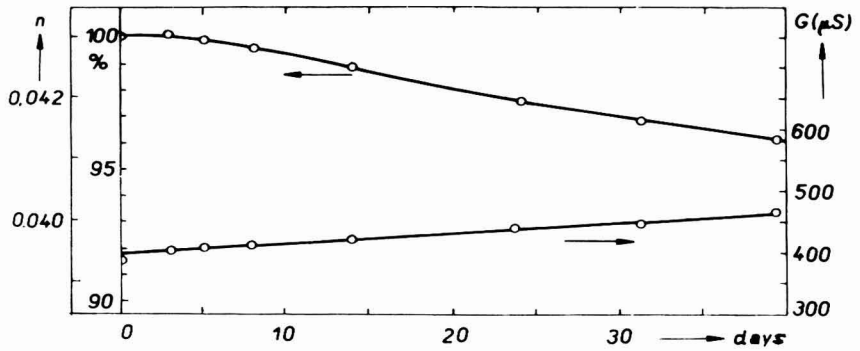

FIG. 6. The stability and the dielectric loss G of the solution of 0.05 *N* Co(II)Ac.

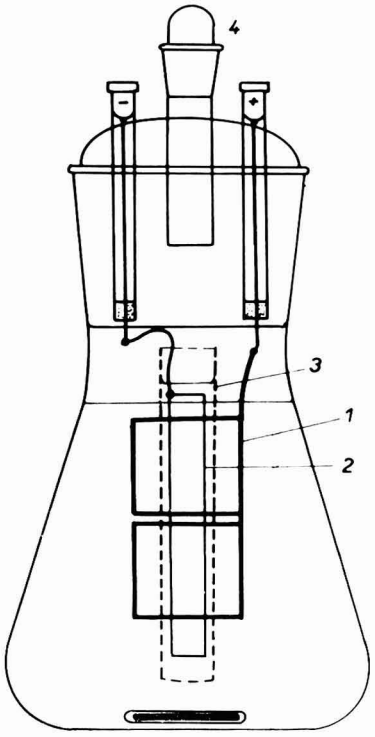

FIG. 7. The sealed electrolyzer for the volume of 250 to 600 ml of solution: (1) Pt anode; (2) Pt cathode; (3) the porous glass membrane; (4) seal for the SCE and for taking samples.

1 liter of the solution. It is necessary to use anhydrous salts and to work in a sealed electrolyzer to prevent absorption of moisture from the air. It is necessary to use salts of maximum purity (especially not containing traces of reductants). Solutions should be stored in the dark and protected by filters and desiccants against dust and moisture. The choice of a suitable material for the membrane remains an open question.

Cellophane, which has been used to date, has the following disadvantages: low mechanical resistance so that it is frequently damaged, causing the catholyte to mix with the anolyte; too high and poorly defined electric resistance, which decreases quickly during the electrolysis in the presence of sodium acetate or water.

Materials tested as membranes were: nonsintered porcelain, sintered glass, porous glass, and cellophane. Porcelain and porous glass have the disadvantage of high resistance; sintered glass permits mixing of the anolyte with the catholyte. If cellophane is used, it is convenient to im-

TABLE 6

ELECTROLYSIS AND CURRENT EFFICIENCY ACCORDING TO (A), (B), and (C)

A					B					C				
		$N \times 10^{-2}$					$N \times 10^{-2}$					$N \times 10^{-2}$		
Volt. (V)	Curr. (mA)	Coul.	Spectr.	Effic. (%)	Volt. (V)	Curr. (mA)	Coul.	Spectr.	Effic. (%)	Volt. (V)	Curr. (mA)	Coul.	Spectr.	Effic. (%)
400	7.0	0.113	0.067	58.9	545	20	0.143	0.095	66.5	400	8	0.105	0.054	56.7
400	7.5	0.294	0.175	59.5	545	22	0.259	0.200	67.8	400	9	0.263	0.150	57.0
400	9.5	0.410	0.250	60.2	545	26	0.427	0.290	67.8	300	15	0.330	0.192	57.9
500	9.5	0.539	0.320	59.5	340	20	0.562	0.393	69.7	245	20	0.585	0.346	59.0
500	11.5	0.668	0.395	59.3	300	20	0.995	0.680	68.2	240	35	0.885	0.526	59.4
600	12	0.843	0.500	59.2	290	28	1.65	1.13	68.7	160	35	0.925	0.562	60.6
600	14	1.019	0.605	59.4	280	60	2.45	1.68	69.0	140	45	1.132	0.694	61.2
700	15.5	1.196	0.717	59.8	40	50	3.10	2.14	68.9	157	50	1.345	0.826	61.3
700	19.5	1.375	0.825	59.9										
800	20	1.556	0.935	60.0										
800	20.1	1.739	1.030	59.4										
mean value				59.6					68.2					59.0
mean relative deviation				0.57					1.09					2.9

pregnate it before the electrolysis with a saturated solution of sodium acetate in glacial acetic acid or with a small amount of water (0.1 to 0.5 g), to lower its resistance. The best reproducibility was obtained with the porous glass membrane (η = 59.6% with a mean deviation of 0.32%).

The measurement of the dielectric constant, which was found useful for the determination of water, has indicated that, within the range of 0 to 2% water, the stability of Co(III)Ac is not substantially affected by the water concentration. The acetate complex obtained from the anodic oxidation has apparently the same configuration as the compound prepared by Koubek and Edwards (*3*) since the position of the absorption maxima of Co(III)Ac and the molar absorptivities (ε_{610} = 244, ε_{630} = 1660) determined here are in good agreement with the data in their paper (*3*).

The current efficiency varies from 60 to 70%, depending on the conditions. This value is an overall one, including the charge consumed for a decomposition of the base electrolyte or of acetic acid itself which is 1 to 5% according to the applied voltage. The main obstacle to increased efficiency is the high resistance of the electrolyte and the membrane, and the poor reproducibility of the working electrode potential.

It can be concluded that in a closed electrolyzer with a cellophane or a porous glass membrane it is possible to prepare anhydrous cobalt(III) acetate which is stable for practical purposes and which can be further used for the analysis and preparation of organic compounds.

SUMMARY

A reasonably stable solution of the cobalt(III) acetate complex in glacial acetic acid, containing no water or cobalt(II), has been prepared by the anodic oxidation of the corresponding cobalt(II) acetate solutions at a platinum electrode in a closed system. Cobalt(III) acetate has also been electrolytically prepared without the addition of sodium acetate. Various materials have been tested as membranes because cellophane, which is usually used, has too high electric resistance and too low mechanical strength. The concentration of water in cobalt(III) acetate solutions has been determined by dielectric constant measurements and it has been found that the cobalt(III) acetate stability is almost independent of the water concentration within the range of 0 to 2% of water. In the closed system, the current efficiency was in the range of 60 to 70%; the best reproducibility was obtained using a porous glass membrane.

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MICROCHEMICAL JOURNAL 16, 136–144 (1971)

Research in the Olfactory Detection of Chemical Agents

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Received October 14, 1970

INTRODUCTION

In an earlier publication (*2*), theoretical considerations on the rapid detection of chemical agents at concentrations less than 1 μg/liter of air led to conclusions that catalytic and chain-type mechanisms are required. Along these lines we have reported research on cyclic catalytic reactions as amplifiers in chemical detection (*10, 12*) and on various aspects of polymer degradation for use in detection (*7, 9, 13*). The present paper summarizes multidisciplinary research on achieving better sensitivity in detection through the use of olfactory sensing. Though the description is addressed toward the indirect detection of organophosphorus compounds it will be useful to anyone interested in olfactory detection of chemicals or facing sensitivity problems in microchemical detection.

Man's ability to detect small quantities of chemicals by means of olfactory senses is well documented (*6*). In some cases only a few molecules are needed to induce a response at the olfactory mucosa.

The use of odors to alert or warn man represents a method of measurement that can be rapid, inexpensive, and often reasonably precise (*16*). A specific example is the use of mercaptans and sulfides by gas companies for warning. Air pollution is a general instance in which odors serve to alert or warn. Olfactory sensing is also an integral part of the "sixth sense" needed under various situations.

CONCEPTS OF USING OLFACTORY SENSING IN CHEMICAL DEFENSE

Detection of chemical agents by using the sense of smell falls under *subjective detection methods* which also include visual sensing and irritation or injury effects. Subjective detection is usually used only as a

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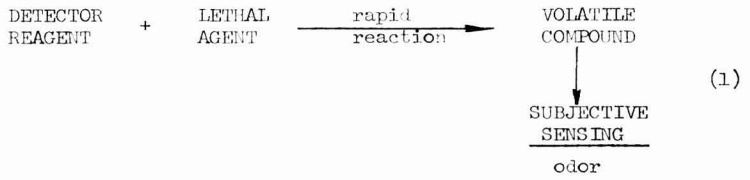
warning of the presence of chemical agents. This is followed by immediate masking and *objective methods of detection* (detector tubes, indicator papers, automatic alarms, etc.). Actually, the objective methods are used at any time when it is believed that a chemical agent might be present whether or not the senses can detect its presence. The chief value of using a subjective method such as olfactory sensing is in a surprise situation.

A number of chemical agents possess distinguishing odors (Table 1). However, there are a number of lethal chemical agents, e.g., GB (isopropyl methylphosphonofluoridate), and GA (ethyl *N,N*-dimethylphosphoramidocyanidate) which are virtually impossible to detect with the ordinary senses of smell, taste, and sight. The first indication that the agents are present would probably be the appearance of casualties.

We undertook research to find detection methods suitable for use by individuals in that an odoriferous compound would be produced in a rapid reaction of the lethal agent with a detector chemical [Eq. (1)].



(1)

DEHYDRATION OF ALKYL FORMAMIDES TO GIVE ISOCYANIDES

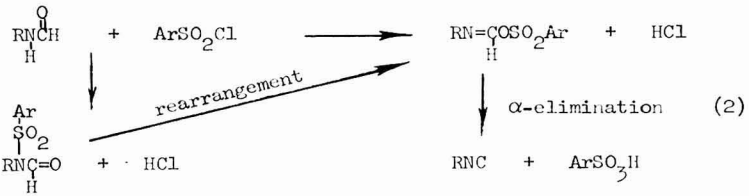
Of the number of reactions considered for use in the olfactory detection of lethal agents, the dehydration of *N*-alkylformamides is the most promising. Ugi *et al. (17, 18)* have described experiments in which *N*-alkylformamides are dehydrated easily by various electrophiles, including arylsulfonyl chlorides, to produce isocyanides. The probable mechanism of the reaction involves electrophilic attack at formamide oxygen. However, initial attack at formamide nitrogen followed by rapid rear-

TABLE 1

ODOR CHARACTERISTICS OF CHEMICAL AGENTS

Agent class	Chemical agent	Odor(6)
Chocking	Phosgene	Musty hay
Blood	Hydrogen cyanide	Bitter almonds
Blister	Mustard (dichlorodiethyl sulfide)	Garlic
	Lewisite (chlorovinyldichloroarsine)	Geraniums
Tear	Chloroacetophenone	Furniture polish

rangement has not been eliminated [Eq. (2)]. In fact, it was reported that reaction of various alkyl formamides with acetyl chloride leads to stable *N*-acetylated derivatives (*3*).



Isocyanides are especially suitable for use in warning applications. The odor is intermediate between pungent and putrid and has been described as "horrible, extremely distressing, and highly specific, almost overpowering" (*18*). It was also cited that with a few exceptions, isocyanides exhibit no appreciable toxicity for mammals. Oral and subcutaneous doses of 500 to 5000 mg/kg can be tolerated by mice (*18*). Alkyl isocyanides can be detected by odor at very low concentrations. Methyl isocyanide has a detection threshold of 6.99×10^{-9} g/liter and a recognition threshold of 6.99×10^{-8} g/liter (*14*).

A study using gas chromatographic techniques led to the finding that *N*-alkylformamides react rapidly with sulfonyl chlorides, phosphorothionochloridates and phosphorochloridates to give alkyl isocyanides (Table 2). The chemical agent GB did not react with any of the formamides. GA reacted with *N*-(*tert*-butyl)thioformamide but not with the other derivatives. In a typical experiment, 30 μl (ca. 0.18 mmole) of GA were added to a solution containing 0.20 ml of dimethyl sulfoxide,

TABLE 2

DEHYDRATION OF ALKYL FORMAMIDES

$$RNHCHO + EX \rightarrow RNC + EOH + HX$$

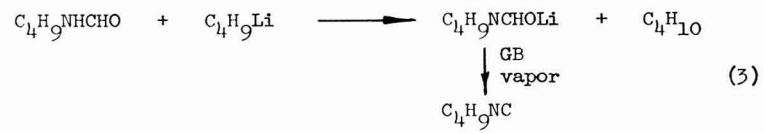
RNHCHO	EX	RNC
CH_3NHCHO	CH_3SO_2Cl	+
	$(CH_3O)_2PSCl$	+
	$(C_2H_5O)_2POCl$	+
	GA, GB	−
C_4H_9NHCHO [a]	CH_3SO_2Cl	+
	GA, GB	−
C_4H_9NHCHS [b]	$C_6H_5SO_2Cl$	+
	$(C_2H_5O)_2POCl$	+
	GA	+
	GB	−

[a] *n*-, *sec*-, and *tert*-isomers.

[b] *tert*-isomer.

33 μl (ca. 0.4 mmole) of pyridine, and 21.3 mg (0.18) mmole) of *N*-(*tert*-butyl)thioformamide. After 5 minutes, 25 μl of the solution was injected into a gas chromatograph (column: 30% SE 30, 45/60 Chromosorb W, ⅜ inch × 20 feet; column temperature 100–160°C; collector 195°C; detector: 230°C; injector: 200°C; carrier gas: He; flow rate: 200 ml/min). Seven peaks were observed. The third peak, with a relative area of 105, was attributed to *tert*-butyl isocyanide on the basis of comparing retention time (6.8 min) with that of an authentic sample of the isocyanide. The peak area of GA was 208 in comparison to the expected area of 512 if no reaction had taken place.

Whether or not the *N*-alkylformamide dehydration occurs, appears to depend on the nucleophilic strength of the formyl oxygen and the electrophilic strength of the phosphorus compound. Increasing the nucleophilicity of the formyl oxygen can be accomplished by converting the formamide to a salt form. Indeed, it was found that the lithium salts of *N*-butylformamide (prepared from the formamide and butyllithium) react rapidly in the solid state with GB vapor to give butyl isocyanide [Eq. (3)]. Unfortunately, exploitation of this discovery for detection



purposes is limited since the lithium formamide salts hydrolyze on exposure to atmospheric moisture [Eq. (4)]. Actually, high vacuum tech-

niques were required to prepare pure lithium *N*-(*n*-butyl)formamide which is a colorless solid [ir, (C=N) 6.2 μ].

Since metal ion complexation (as opposed to salt formation) also offered the possibility of increasing the nucleophilicity of the formamide oxygen without the problem described above, a program was undertaken to investigate the reaction of chemical agents with metal complexes of *N*-methylformamide (NMF). A series of these complexes were prepared and characterized (Table 3). Complete details on methods of synthesis, carbonyl stretching frequencies, electronic absorption properties, magnetic moments, and molar conductances are available elsewhere *(4, 5)*. The complexes given in Table 3 are generally soluble in highly polar solvents (e.g., CH_3NO_2, alcohols, NMF) and insoluble in less polar solvents (e.g., $CHCl_3$, CH_2Cl_2, ether, ethyl acetate. All the complexes hydrolyze in water but are generally not hydroscopic.

TABLE 3

METAL SALT COMPLEXES OF CH_3NHCHO (NMF)

Octahedral	Square planar
$[Mg(NMF)_6](NO_3)_2\,2H_2O$	$[Cu(NMF)_4](ClO_4)_2$
$[Mg(NMF)_6](ClO_4)_2$	
$[Mn(NMF)_6](ClO_4)_2$	
$[Co(NMF)_6](NO_3)_2\,2H_2O$	Square planar or tetragonal
$[Co(NMF)_6](ClO_4)_2$	$[Cu(NMF)_2Cl_2]$
$[Ni(NMF)_6](NO_3)_2$	
$[Ni(NMF)_6](ClO_4)_2$	
$[Zn(NMF)_6](ClO_4)_2$	Tetrahedral
$[Mg(NMF)_4Cl_2]\,2H_2O$	$[Zn(NMF)_2Cl_2]$
$[Ni(NMF)_4Cl_2]$	$[Zn(NMF)_2Br_2]$
	$[Zn(NMF)_2I_2]$
	$[Cd(NMF)_2Cl_2]$
	Octahedral polymeric
	$[MnCl_2(NMF)_2]$

The C=O stretching band in each case shifted to lower frequency 10–20 cm^{-1} relative to NMF. This indicates that the formamide is coordinated to the metal through the oxygen atom, although the shifts are less than those usually found for metal–amide complexes (ca. 30–70 cm^{-1}). Since coordination through oxygen will increase the importance of resonance form II relative to I, the oxygen will become more negative and the C–O band will be weakened.

I II

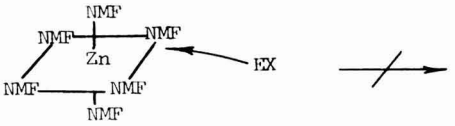
Of the compounds listed in Table 3 only the tetrahedral zinc complexes reacted with sulfonyl chlorides and chemical agents. Isocyanide production from *p*-toluenesulfonyl chloride, and GA was rapid but from GB was slow. There was no detectable reaction of any of the complexes in the solid state with GB vapor.

It was surprising to find that *p*-toluenesulfonyl chloride, which is usually very reactive with NMF, did not react with most of the NMF metal complexes. However, methyl isocyanide was formed if quinoline was added to the metal complex and *p*-toluenesulfonyl chloride mixture. A control experiment indicated that this was due to reaction with uncoordinated NMF since addition of quinoline to the complex alone gave decomposition. A weak test for isocyanide using the nitrate complexes

in nitromethane is thought to be due to the reaction of uncoordinated NMF with *p*-toluenesulfonyl chloride. The NMF arises from the nitrate interaction [Eq. (5)].

$$M(NMF)_6(NO_3)_2 \xrightarrow{CH_3NO_2} M(NMF)_4(NO_3)_2 + 2\ NMF \qquad (5)$$

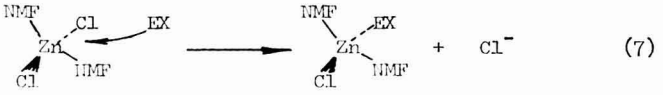
Inspection of molecular models showed that in octahedral complexes attack at the oxygen is hindered sterically. In fact, it is impossible for the sulfur atom in *p*-toluenesulfonyl chloride (or the phosphorus atom in the chemical agents) to approach closely the formamide oxygen atom. This would easily explain the lack of isocyanide formation from the octahedral complexes if attack by the electrophilic chemical (EX) on oxygen is required [Eq. (6)].



(6)

It was very evident after examining molecular models that the electrophilic agent fitted very well into the ligand sphere of the tetrahedral complex (a position that would allow it to act as a dehydrating agent).

The order of effectiveness in isocyanide production with the zinc tetrahedral complexes was $ZnI_2 > ZnBr_2 > ZnCl_2$. This indicates that the ligand (other than NMF) in the complex should be a good leaving group. It also implies that the mechanism is initially attacked by the chemical agent on the metal rather than on the formamide [Eq. (7)].



(7)

The complex $ZnCl_2 \cdot 3NMF \cdot H_2O$ was also reactive; it is probable (but not proven) that the structure is tetrahedral $[Zn(NMF)_2Cl_2] \cdot NMF \cdot H_2O$ or $[Zn(NMF](H_2O)Cl_2] \cdot 2NMF$.

The $CdCl_2$ tetrahedral complex was not reactive but this may be due to the strength of the Cd–Cl bond. The copper complexes are both approximately planar, but for unknown reasons were completely unreactive.

The minimum initial rate constants for the formation of methyl isocyanide from GA and GB (with $ZnCl_2$ and excess NMF) are 1.1 min^{-1} and 5.3 $\times$ 10^{-3} min^{-1}, respectively. It was established by glc that 20 μl of GA, when added to a solution of 98 mg of $ZnCl_2$ in 0.20 of NMF, gave after 1 minute at least 10% of the theoretically possible methyl isocyanide.

SENSORY ASPECTS

The main portion of this paper has dealt with research on the reaction of electrophilic chemicals with *N*-alkylformamides to produce odoriferous alkyl isocyanides. This research comprised part of a multidisciplinary program on the use of olfactory sensing as a means of detecting very low concentrations of chemical agents. The sensory portion of the investigation revealed that odor sensitivity (with a group of 11 odorants) was not significantly altered under a variety of physiological, psychological, and environmental conditions, as measured by detection and recognition thresholds (*14, 15*). Physical exercise, darkness, the use of a blindfold, and an auditory stress had no significant effect on odor sensitivity. A 12-hour diurnal variation produced a 10-fold reduction in odor sensitivity.

Studies of adaptation (using allyl isothiocyanate, β-ionone, and methyl isocyanide) indicate that it occurs at a fairly rapid rate on continuous exposure to an odorant. At a concentration of 10 times that of the detection threshold, adaptation is complete after 3–4 minutes of continuous exposure to stimulus. Increasing the concentration results in an increased rate of adaptation, but the magnitude of this increase is not linear. Recovery from adaptation appears to be equally rapid.

In the use of odoriferous mixtures (methyl isocyanide and one interference), it was discovered that as the concentration of the interfering stimulus increases beyond five times its detection threshold, recognition of the primary stimulus (methyl isocyanide) is decreased between two- and fourfold on the average. The interferences checked were guaiacol and ammonia.

Experiments performed in the field indicate that the range for recognition of methyl isocyanide is between 6.0×10^{-10} and 6×10^{-9} g/liter. This is in good agreement with the thresholds established in the laboratory.

Certainly, olfactory sensing can be used as an alerting or warning system for man under a variety of conditions.

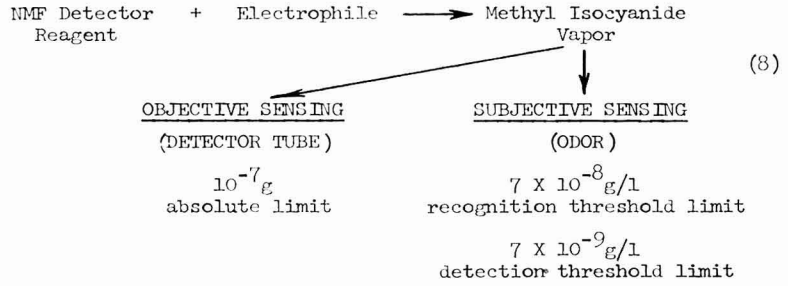
UTILITY

The factors which most severely limit broad application of isocyanide formation for subjective detection of GB (and other phosporus–fluorine compounds) are slow reaction rate and the requirement of solvent for the dehydration reaction to proceed. The ideal case would involve rapid alkyl isocyanide production from a solid detector chemical as a result of chemical vapor adsorption and reaction. However, there is a possibility of exploiting the concept for the detection of chemicals other than that of the GB type. As an illustration of a potential appli-

cation it was found that as little as 5 mg of *p*-toluenesulfonyl chloride/m^2 of asphalt surface could be detected in 30 seconds after the surface was sprayed with a NMF–$ZnCl_2$ solution. The subjects were 6 feet downwind from the contaminated area. However, 50 mg of *p*-toluenesulfonylchloride/m^2 of lawn was not detected up to 5 minutes at any distance from the contaminated area which had been sprayed with the detector solution.

The most obvious advantage of olfactory detection methods is that they can be used at night when visual monitoring of detection devices may not be possible.

A detector tube colorimetric method for isocyanide vapor was developed as an objective sensing procedure to supplement the subjective olfactory method (*1*). It is based on the oxidation of aromatic amines by cupric ion. The lowest amount of isocyanide detectable is approximately 0.1 μg. The limits of the combination objective–subjective procedures under conditions in which the electrophile gives a quantitative yield of methyl isocyanide are summarized in Eq. (8).



A convenient source of information on the design of detection reactions based on isocyanide formation was published recently (*8*). It tabulates reagent combinations which will produce isocyanides.

Details concerning the use of the combination of *N*-alkylformamide dehydration and the objective sensing method to detect down to 0.2 μg of tosyl chloride were given in a preliminary communication (*11*).

SUMMARY

This paper describes in a review fashion research on the olfactory detection of various electrophiles including toxic organophosphorus compounds. The method involves rapid formation of an odoriferous alkyl isocyanide from reaction of the electrophile with *N*-alkylformamides. Concepts of using olfactory sensing and a colorimetric procedure as subjective and objective methods, respectively, for isocyanides are discussed.

ACKNOWLEDGMENT

Helpful discussions with Dr. Arnold J. Gordon and Dr. Fred W. Oberst are gratefully acknowledged.

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MICROCHEMICAL JOURNAL **16**, 145–164 (1971)

Physicochemical Measuring Methods for End-Point Determination in Organic Elemental Microanalysis

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Received November 13, 1970

A. MEASURING METHODS FOR DETERMINING CARBON AND HYDROGEN, OR CARBON, HYDROGEN, AND NITROGEN

During the last decade, the elemental microanalytical determination of carbon and hydrogen has often been improved. Pregl's principle, the absorption of combustion products in weighing tubes and determining their weight using the microanalytical balance, was used in almost all previous papers. In 1960, results of studies were published, in which other physical measuring principles were substituted for the balance formerly used for determining the end point. In these, several measuring methods were reported and, in the last few years, others have been introduced. Table 1 shows briefly the measuring principles introduced in the last 10 years.

The aim of all this research was to find measuring methods permitting carbon and hydrogen, or carbon and hydrogen in combination with nitrogen, to be determined with greater or at least the same degree of accuracy, rapidly, with less work, and less subject to human error. The final aim is complete automation permitting the test sample to be weighed on a balance with an electrical output, automatically introduced into the combustion apparatus, combusted, the combustion products to be measured and the result printed out in percentages. How far Pregl set standards for this work is recognized in the accuracy of the measured values. In the majority of the methods introduced, one is satisfied to obtain the $\pm 0.3\%$ accuracy required by Pregl. Can Fritz Pregl's work have a better testimony?

Based on selected papers, the various measuring principles developed are discussed below.

[1] Paper presented: VI International Symposium on Microtechniques, Graz, Austria, Sept. 7–11, 1970.

TABLE 1

MEASURING METHODS FOR THE DETERMINATION OF C, H, AND N

1. Heat conductivity
   a. gas chromatographic separation of the combustion produc
   b. separation of the combustion products by absorbents, adsorbents, and by freezing out.
2. Conductimetry
3. Coulometry
4. Combination of conductimetry and coulometry
5. Colorimetric titration
6. Generation of current in a galvanic cell
7. Manometric measuring
8. Automatic weighing of the absorption tubes

1. The Thermal Conductivity Cell as Detector

Two routes have been taken:

a. the gas-chromatographic separation of the combustion products, carbon dioxide, water, and nitrogen with subsequent measuring.

b. the separation of individual combustion products using adsorbents, absorbents or by freezing out with subsequent measuring in one or several detectors.

1a. Gas-chromatographic separation was proposed by Duswalt and Brandt *(1)*, Sundberg and Maresh *(2)*, as well as Vogel and Quattrone *(3)* for determining carbon and hydrogen. Nightingale and Walker *(4)* determined carbon and hydrogen in combination with nitrogen. This same joint determination was also proposed by Hinsvark and collaborators *(5, 6)*. It forms the basis of the salable Model 185 by Hewlett and Packard *(7)* (Fig. 1).

Using helium as carrier gas, the combustion products are separated in a gas-chromatographic column and measured in a thermal conductivity detector equipped with a recorder; helium being the reference gas. The peak sequence recorded is nitrogen, carbon dioxide, and water. An electronic balance with electrical output is coupled to the recorder to compensate the sample weight differences. The peak height is measured. The percentage content of the constituents can be calculated taking into account the blank values and the test substance analyses.

The sample weight can only be varied within the narrow limits of 0.5–0.8 mg. Apart from this test substance selection is dependent on the approximate carbon content of the test sample. A series of slow-combusting substances can only be incompletely determined, if at all.

Graham *(8)* proposed a Polypak column and the use of an integrator

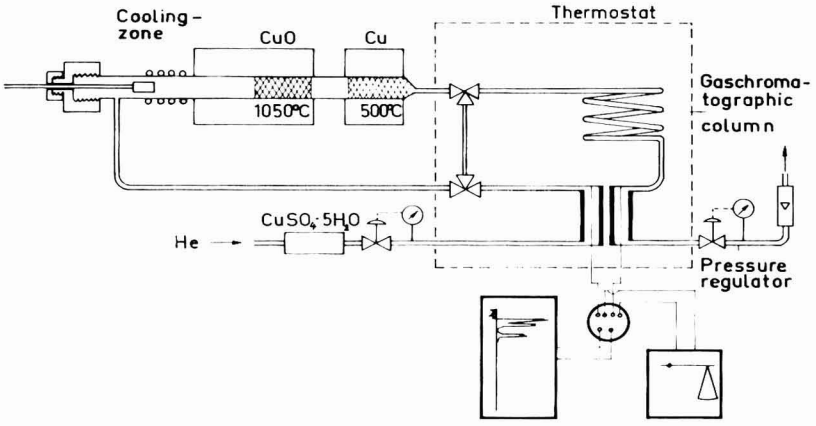

FIG. 1. C, H, N by gaschromatographic separation of the combustion products and heat conductivity measuring (Hewlett Packard Model 185).

for the Model 185 to increase the accuracy as no blank value corrections are necessary.

The apparatus developed by Carlo Erba *(9) is* based on the same principle. In this, oxygen is introduced over a limited period to increase the combustion capacity. Either a compensation recorder or an electronic integrator with a digital printer is used for data evaluation. Twenty-three samples can be introduced into the apparatus, one after the other, using an automatic sampler. Automatic weight introduction is not provided. The sample weight can be varied from 0.5 to 1.5 mg.

1b. In a series of further methods, the thermal conductivity detector is also used. However, the combustion products are not separated gas chromatographically but are extracted from the gas flow successively by adsorbents, absorbents, and freezing out. Walisch *(10)* first proposed this method. His conception was used by Technikon *(11)* (Fig. 2).

The substance is combusted in a helium stream with 3.5% oxygen over cupric oxide and the excess oxygen is removed using copper. The water formed is then adsorbed on silica gel. The gas stream now passes through the first half cell of a katharometer, then through a time delay coil and an absorption tube and finally through the second half cell of the katharometer. While the carrier gas conveying carbon dioxide and nitrogen passes through the first half cell, pure helium flows through the second half cell. The reference signal, digitally indicated, is a measurement for $CO_2 + N_2$. As the gas, free of CO_2, enters the second half cell, pure helium is again flowing through the first half cell. The second signal is a measurement of the N content. After carbon dioxide and nitrogen

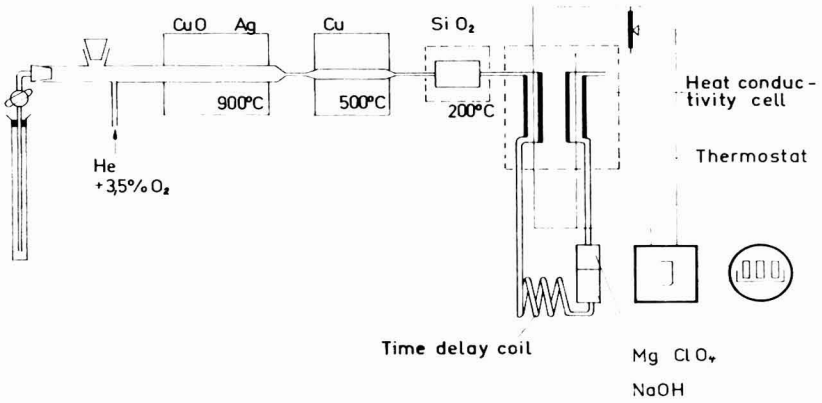

FIG. 2. C, H, N—Separation by absorption and adsorption. Measuring by heat conductivity (Walisch-Technicon).

are determined, the water is finally evaporated from the silica gel by heating up to 200°C and determined in the katharometer.

The sample weight can only be maintained in narrow limits (0.2–0.5 mg). A great deal of attention is required to operate the apparatus.

Weitkamp and Korte *(12)* primarily tried to reduce the amount of work by installing time control for all combustion cycle functions. The katharometer signal is fed to a corrector giving a linear reference between the gas concentration and the katharometer signal. Thus, the sample weight range could be extended to 0.1–0.9 mg. The printer coupled to the integrator indicates the percentage content for C, H, and N direct.

Ehrenberger *et al. (13)* have, in cooperation with Heraeus *(14)* and based on Walisch *(10)* substituted adsorption of water on silica gel by freezing out in a silver capillary at −78°C. After determining carbon dioxide and nitrogen, the silver capillary is heated electrically. In this, water is determined without a blank value. The sample weight determined by an electronic balance and the readouts for $CO_2 + N_2$, N_2, CO_2, and H_2O are printed out.

Monar *(15)* additionally adsorbed the carbon dioxide on a silica gel packing in a second silver tube at −78°. The three constituents, N_2, CO_2, and H_2O are individually determined one after the other. This apparatus is shown in Fig. 3.

Kainz and Wachberger *(16)* have taken a similar route. They combust the substance in an empty tube using helium as carrier gas. During the combustion process itself a certain amount of oxygen is supplied for oxidizing the sample in the tube, and the helium stream is cut off for this period. The combustion products, water and carbon dioxide are

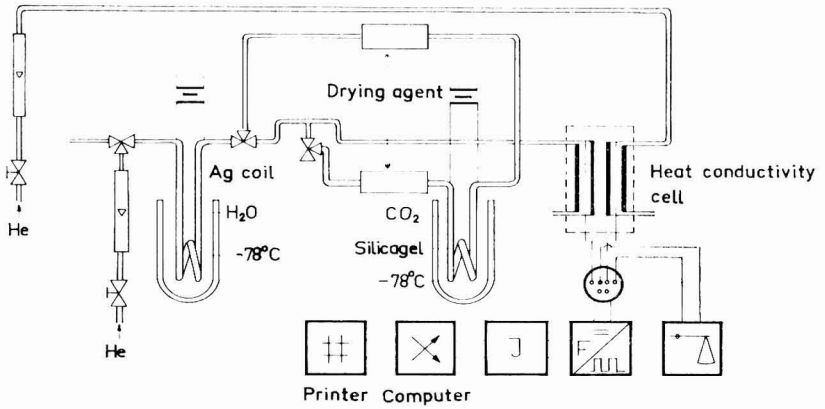

FIG. 3. C, H, N—Separation by cooling and heat conductivity measuring. (Monar-Heraeus).

retained with calcium chloride on quartz sand and molecular sieve 5A. Nitrogen passes direct to the thermal conductivity detector fitted with an integrator. Finally, carbon dioxide and water are desorbed one after the other and determined in the same detector. The sample weight can be 2–3 mg.

The Miller and Winefordner *(17)* studies form the basis of the Aminco apparatus *(18)*. In one of their methods, they determine carbon and hydrogen only and, therefore, oxygen can be used as combustion and carrier gas. The water is first retained on silica gel. Carbon dioxide is measured, using oxygen as reference gas, in the thermal conductivity detector. The water is then evaporated, converted with calcium hydride to hydrogen and this is then determined in the detector.

In the methods just discussed, one detector only is used and the individual combustion products are determined in it one after the other. In general, the sample weights are usually less than 1 mg.

Clerc *(19)* and collaborators took another route, commercially used by Perkin-Elmer *(20, 21)* (Fig. 4).

The substance is combusted over silver vanadate and silver tungstate on magnesium oxide with helium as carrier gas and oxygen fed over a limited period. The excess oxygen is removed using copper and the combustion gas collected in a mixing vessel until the pressure in it has risen to 2 atm. From this mixing vessel a portion of the gas is expanded through a 4-m glass spiral until the pressure has become almost normal. The combustion gas, uniformly distributed in the long glass spiral is then swept with helium through three detector pairs. After having passed through the first half cell in the first detector, the water is retained on magnesium perchlorate. The gas, free of water, flows through the

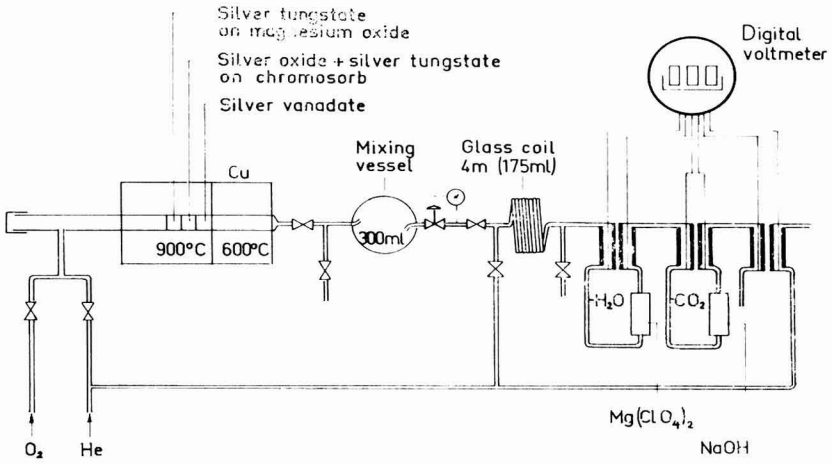

FIG. 4. C, H, N—Separation by absorption and heat conductivity measuring with three detectors (Simon-Perkin-Elmer, Model 240).

second half cell in the first detector and then on to the first half cell in the second detector. The carbon dioxide is absorbed between the two half cells in the second detector. The gas, now only containing nitrogen, then flows through the first half cell in the third detector out into the open. Pure carrier gas flows through the second half cell.

Thus, only an aliquot portion of the combustion gas is measured. Therefore, it is possible to work in a range where the conductivity functions are still sufficiently linear despite the high sample weight of up to 3 mg.

The uniform distribution of the combustion gases over a long spiral has the advantage that a stationary state occurs for a short period, at least, during measuring. The difference signals read off at the time—less the blank value—are thus a direct measurement covering the CO_2, H_2O, and N_2 content of the carrier gas. Therefore, time-based integration can be dispensed with. The difference signals can be read off from a recorder. More accurate values may be obtained using an attached digital voltmeter.

The apparatus proposed by Hozumi *(22)* and constructed by Yanagimoto *(23)* complies in principle with the Simon method.

2. Conductimetric Measuring Method for Determining Carbon and Hydrogen

In the meantime, electrolytic conductivity achieved an important position, primarily in determining carbon dioxide. Although Stuck *(24)* limited himself to determining carbon only, Malissa and collaborators

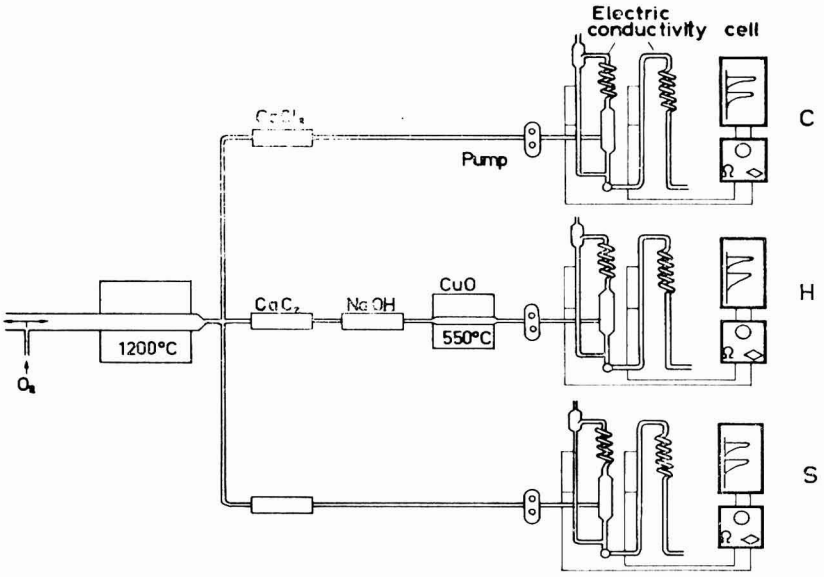

FIG. 5. C, H, S—by electric conductivity (Malissa, Pell u. Machherndl).

(25) (Fig. 5) utilize electrolytic conductivity also for determining hydrogen. To do this, the substance is combusted in the oxygen stream. The gas stream with the combustion products is divided into two equal parts using a pump system. In one of the parts, carbon dioxide is determined directly by absorption in caustic solution in a conductivity measuring cell and the conductivity modification is measured with a recorder.

In determining water, the water is converted to acetylene with calcium carbide in the second split stream. The carbon dioxide contained in the split stream is removed with sodium asbestos, the acetylene is oxidized with cupric oxide to carbon dioxide and this is determined, likewise, conductimetrically.

The carbide quality occasioned difficulties. Even on high-percentage carbides being used very frequent replacement is necessary.

Gelman and Van *(26)* also use the conductimetric method to determine carbon and hydrogen. In doing this, the water is first frozen out. After determining the carbon dioxide, the water is evaporated, converted to carbon monoxide using coal at 1120°C and, finally, oxidized with cupric oxide to carbon dioxide which is then measured conductimetrically.

Greenfield and Smith *(27)* determine carbon dioxide as well as water by the conductimetric method direct. In the first measuring cell water is absorbed in a cell filled with concentrated sulfuric acid and in the

second cell, fitted downstream, the carbon dioxide is absorbed in caustic solution. In determining water, the sulfuric acid concentration can only be varied within very narrow limits of 99.83–99.75%. The linearity of the conductivity function is no longer sufficient outside this concentration range.

According to Kainz and collaborators *(28)* this range can be extended if a solution of anhydrous acetic acid with 2% sulfuric acid is used as the absorbent for water instead of concentrated sulfuric acid.

Liebetrau and collaborators *(29)* pass the combustion gases through calcium hydride. The hydrogen formed from water is converted to HCl using palladium chloride at 200°C. The gas now containing carbon dioxide and hydrogen chloride is first passed through a conductivity cell filled with diluted hydrochloric acid and then through a second cell with diluted caustic soda solution.

3. Coulometric Measuring Methods

Haber and Gardiner *(30)* (Fig. 6) determine carbon dioxide and water coulometrically using the Keidel cell *(31)*. In doing this, the carbon dioxide must be converted to water.

Two platinum wires are wound bifilarly close to one another in a U-shaped capillary. The platinum coils are thinly coated with phosphoric pentoxide. If a voltage is applied to the electrolytic cell, and when the phosphoric pentoxide is dry, scarcely any current flows through the cell. However, if water is introduced into the cell along with a gas stream, it becomes electrolyzed. The current-time yield is a measurement for the water content. In order also to measure carbon dioxide, this is converted to water using lithium hydroxide.

Martin and collaborators *(32)* have described a further coulometric method (Fig. 7). The water contained in the combustion gases is first

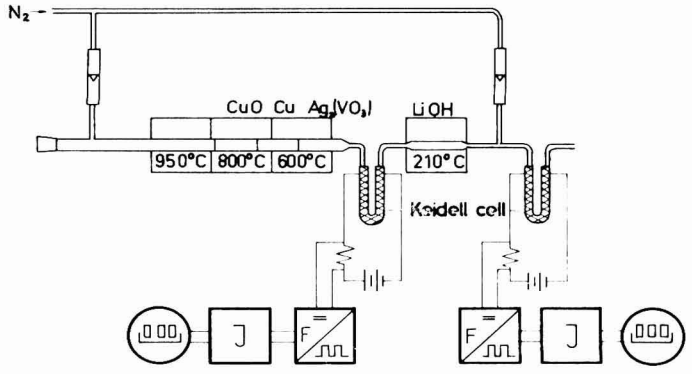

FIG. 6. C, H by coulometric measuring (Haber and Gardiner).

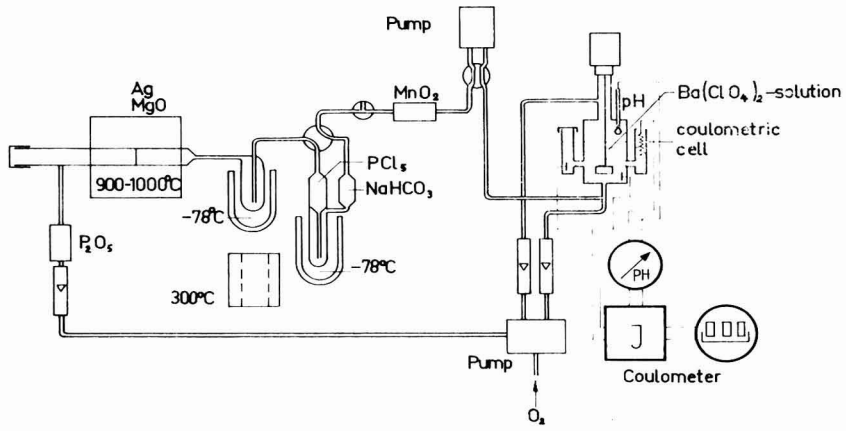

FIG. 7. C, H by coulumetric measuring (Martin, Floret u. Lemaitre).

frozen out. The carbon dioxide is passed direct into an electrolytic cell filled with barium perchlorate in which it is quantitatively neutralized with electrolytically produced barium hydroxide. The electrolysis is controlled by a sensitive pH meter. The current-time yield is indicated digitally.

After carbon dioxide is determined, the water is evaporated and converted to hydrogen chloride with phosphoric pentachloride. This is converted to carbon dioxide with sodium bicarbonate and determined.

4. Combination of the Conductimetric and Coulometric Methods

In general, the conductimetric and coulometric methods require one of the combustion components be converted into the other. Carbon dioxide can be well determined conductimetrically and water coulometrically. We *(33)* make use of this fact in our own method (Fig. 8). In this

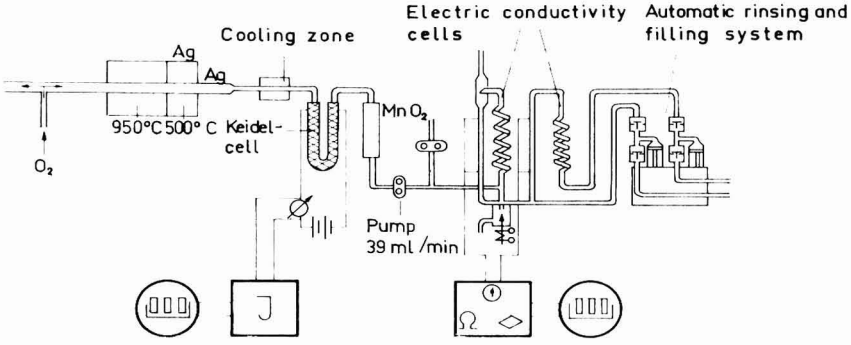

FIG. 8. C, H by electric conductivity and coulumetry (Salzer-Labormatik/ Wösthoff).

apparatus [jointly manufactured by the companies, Labormatik *(34)* and Wösthoff *(35)*], combustion is carried out in an empty tube with pure oxygen. As oxygen is sucked through the combustion tube, combustion can take place in an open-end tube. Using a pump system, the carrier gas along with the combustion products is sucked through the Keidel cell and, after removing the nitric oxide with manganese dioxide, forced into the conductivity cell filled with caustic soda solution. The measured values for water and carbon dioxide are indicated digitally. There are no blank values. The procedure is even suitable for determining ppm quantities of carbon and hydrogen. As almost all operations are fully automatic, only a few manipulations are required for the determination. Determination in series can be effected in only about 6–7 minutes.

5. *Automatic Colorimetric Titration*

Merz *(36)* determines carbon and hydrogen using the colorimetric titration of carbon dioxide (Fig. 9). The substance is combusted with a quantity of oxygen, introduced for a period, over cupric oxide, and the excess oxygen is removed with copper at 550°C. Nitrogen is used as carrier gas. First, the combustion water is frozen out, while the carbon dioxide is passed into a colorimetric titrator containing dimethyl formamide with a small quantity of monoethanolamine as absorbent. Tributyl ammonium hydroxide serves as standard solution and thymolphtalein as indicator. The titration control is effected using four light-sensitive photo

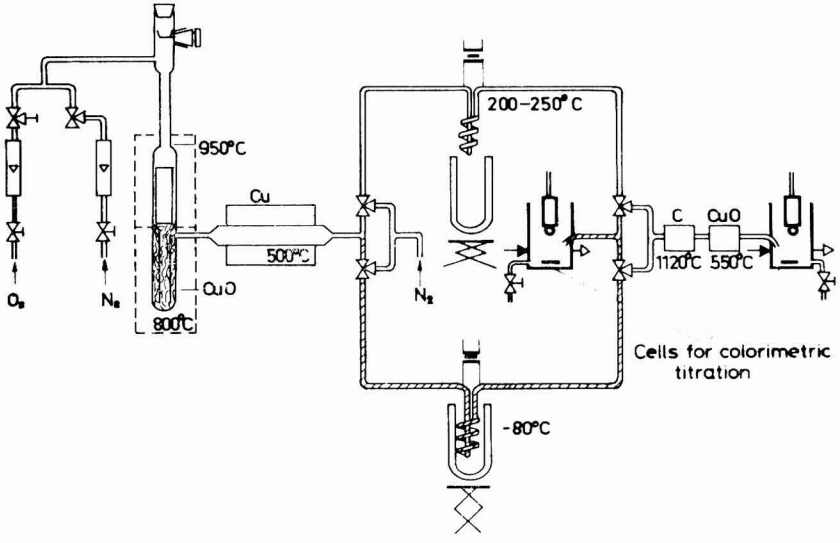

FIG. 9. C, H by automatic colorimetric titration (Merz-Heraeus).

resistances in bridge circuit with a filter arranged in each so that, on color changing, a change in voltage occurs at the bridge end points. Thus, a specific color change is measured. The entire analysis cycle, inclusive of final determination, is automatic. The titration volume is printed out digitally. It can also be fed direct to a computer.

In determining water, the water is evaporated after determination of carbon dioxide or during determination of CO_2 the next time and converted with coal to carbon oxide at 1120°C. The carbon monoxide formed is oxidized to carbon dioxide with cupric oxide and determined as such.

In calculating, blank values are used.

6. The Galvanic Cell as Measuring Instrument

Hersch *(37)* of Beckman Instruments *(38)* patented a measuring method for determining sample quantities of a few nanograms. This method is suitable for determining the C/H ratio of fractions separated gas chromatographically in a combination of gas-chromatographic and elemental analysis instruments (Fig. 10), to take an example.

The substance is combusted with cupric oxide in an inert gas stream. Carbon dioxide and water are separated and converted with coal to carbon monoxide at 1120°C. Using iodine pentoxide, the carbon monoxide is converted to carbon dioxide and iodine. The iodine formed is fed with the inert gas stream into a galvanic cell. This cell has a platinum cathode and an anode of carbon, silver, or mercury. A buffered potassium bromide solution is used as electrolyte. If the iodine comes in contact with the cathode in the galvanic cell, the carbon anode oxidizes and the iodine on the cathode is reduced to iodide. The current thus generated and integrated during the period is a measurement for the iodine having entered the cell or for the C and H content.

A microampère-minute—which can be measured with a 10 mV recorder without additional amplification—is equivalent to 9.3×10^{-9} g of carbon or 3.1×10^{-9} g of hydrogen.

7. Manometric Methods

Kirsten and collaborators *(39)* already used a method for manometrically determining carbon, hydrogen, and nitrogen with success in 1959. In doing this, combustion was effected in melting tubes outside the measuring apparatus.

Frazer and Stamp *(40)* also combust the substance in an evacuated melting tube with cupric oxide to determine C, H, and N. After cooling, the tube is opened in a vacuum apparatus. The combustion products are separated and manometrically measured one after the other.

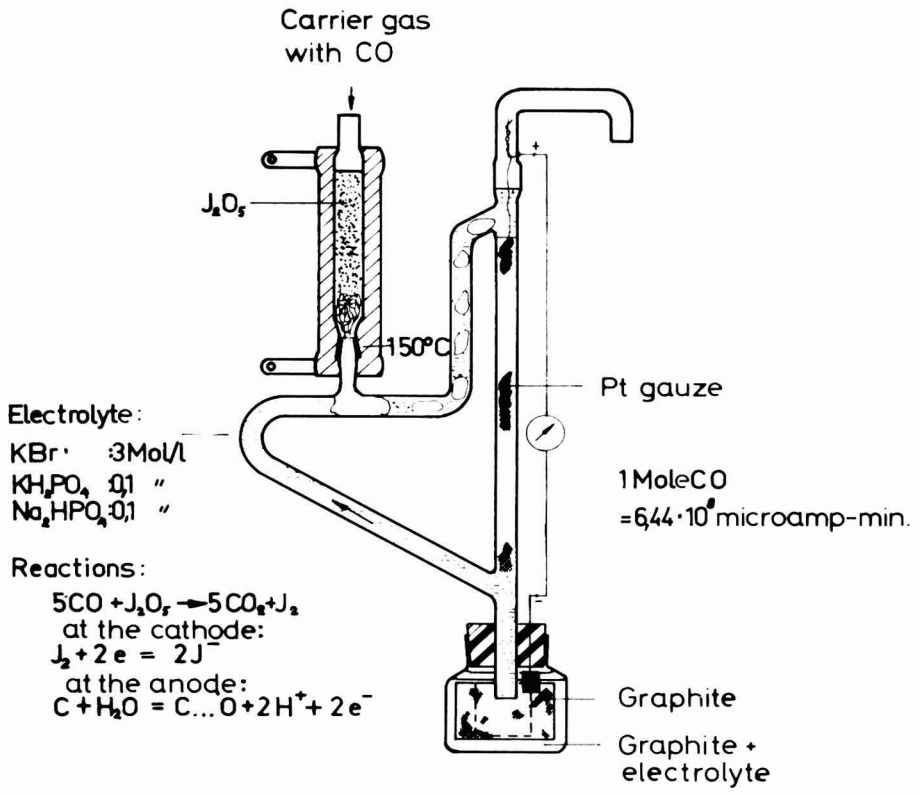

FIG. 10. Measuring cell for carbon monoxide (P. A. Hersch-Beckman Instruments).

Before measuring, the water is reduced to hydrogen. The pressure of the gases is converted into electric signals which are scanned by a computer. The operation is automatic to a great degree.

Gouverneur and Van Leuven *(41)* freeze the combustion products, carbon dioxide and water, out and pump the oxygen off. The manometric measuring then takes place. A precision mercury pressure gauge is used, in which the pressure is converted to an appropriate mercury volume and this measured with a piston burette. Level indication is given by a photoelectric cell.

8. Automatic Weighing of the Absorption Tubes

Up to this point, methods have been described which avoid weighing the absorption tubes, and other physical methods of determining carbon dioxide, water, and nitrogen were applied. Trutnovsky *(42)*, however, returned to the balance, to the measuring instrument with the greatest linear measuring range.

After combustion water and carbon dioxide are absorbed in two absorption tubes filled with ordinary material. These glass tubes are equipped with sealing heads having flat faces. A device was developed which, on the one hand, automatically establishes a tight connection between the absorption vessels and/or between absorption vessel and gas feed pipe and, on the other hand, detaches the connections and alternately places the vessels on the balance. This device is accommodated in the weighing chamber of a Mettler M5 microbalance.

Data covering a few commercial apparatuses are given in Table 2. These data were extracted from the literature.

B. MEASURING METHODS FOR DETERMINING OXYGEN

Even if one cannot include determining oxygen in the Pregl methods, it would appear to be advisable to also mention it at this point. This can be done quickly in that the measuring methods discussed for determining carbon and hydrogen can, in general, be applied for determining oxygen. In the final analysis, determining oxygen is indeed also determining carbon dioxide. Furthermore, measuring methods used for determining carbon monoxide and iodine can also be applied for determining oxygen; this increases the number of possible measuring methods even further in comparison with determining C and H.

Hinsvark and Muldoon *(43)* determine carbon dioxide gas chromatographically; while Poy *(9)* measures carbon monoxide using the Carlo Erba apparatus.

Walisch and Marks *(44)*, Kainz and Wachberger *(45)*, and Culmo *(46)* apply their methods described for C, H, N determination also for determining oxygen. Ehrenberger and Weber *(47)* prefer measuring carbon monoxide in their method.

The conductimetric determining of carbon dioxide has been used by the author in routine work for many years. Fraisse and Levy *(49)* apply automatized coulometric titration.

Colorimetric titration with automatic control of a motor burette is described by Gouverneur and Brujn *(50)*. Colorimetric titration developed by Merz *(51)* is also used for determining oxygen.

Finally, the galvanic cell by Hersch *(37)* is also suitable for determining oxygen. As already mentioned, Trutnovsky *(52)* uses his method for determining oxygen.

Apart from these methods, some other measuring methods have been described in the last few years. Calmé and Keyser *(53)* absorb the iodine formed by the Unterzaucher Method in a titration cell filled with potassium iodide and titrate this iodine potentiometrically with sodium thiosulfate directly without prior oxidation to iodate. Titration is effected automatically.

TABLE 2

SPECIFICATION OF COMMERCIAL INSTRUMENTS

Instrument type	Measuring method	Sample wt (mg)	Analysis time (minutes)	Standard deviation (% abs)		
				C	H	N
Hewlett & Packard Mod. 185	Gas chromatography + heat conductivity	0.5–0.8	15	±0.28 [a]	±0.16	±0.13
Carlo Erba	Gas chromatography + heat conductivity	0.5–1.8	10	±0.30 (*9*) [b]	±0.10	<0.20
Technicon	Adsorption + absorption + heat conductivity	0.2–0.5	16	±0.3 [ac]	±0.15	±0.4
Heraeus	Adsorption + freezing out + heat conductivity	3–6	20	±0.18 [d]	±0.08	±0.18
Aminco	Adsorption + heat conductivity	0.4–1.0	8	±0.15 (*17*)	±0.12	±0.18
Perkin-Elmer Model 240	Absorption + heat conductivity	1–3	13	±0.21 [a]	±0.15	±0.17
Yanagimoto	Absorption + heat conductivity	2–2.5	18	±0.20 (*22*)	±0.20	±0.20
Labormatik-Wösthoff	Electric conductivity + coulometry	1–2	6–7	±0.07 (*33*)	±0.03	—
Heraeus	Freezing out + colorimetric titration	3–5	8	<0.25 (*36*)	<0.25	—

[a] Detailed Information taken from Companies' Technical Papers.
[b] Maximum deviation for 90% of the values.
[c] Maximum deviation.
[d] Private communication.

Hozumi *(54)* determines iodine using light absorption measuring. The iodine vapor, along with the carrier gas, is sucked through a 38-cm glass cell heated to 120°C. Light absorption is measured at 530 mμ by a photoelectric cell and registered on a recorder. The cell is so highly dimensioned that all the iodine is distributed in the glass cell at the time of measuring.

Thürauf and Assenmacher *(55)* determine carbon monoxide with a nondispersive IR gas analyzer. The CO content of the gas mixture stream is measured without separating any other gas constituents. At the exit of the IR analyzer the direct current voltage proportional to the quantity of carbon monoxide is registered on an integrator.

C. MEASURING METHODS FOR DETERMINING NITROGEN

Although a whole series of basically new measuring methods were developed for determining carbon, hydrogen, and oxygen, this does not apply for determining nitrogen. Here, only thermal conductivity measuring in conjunction with C, H analysis has been introduced as a new method. The volumetric methods in accordance with Pregl–Dumas have been improved by Gustin *(56)* with the purpose of automating the combustion procedure. This resulted in the Coleman *(57)* apparatus, which has proved itself well. Here, the measuring of the N_2 volume is done manually using a piston burette but Monar *(58)* has improved this measuring with photoelectric cell level sensing and digital indication of the measuring volume (Azotomat of Heraeus). Finally, using the same basic principle Merz *(59)* automated the combustion and measuring still further. The complete cycle is preset and controlled by an electronic programmer. The nitrogen volume is read off from a digital counter or printed out. Heraeus *(14)* has put this equipment on the market. The analysis time is said to be about 4 minutes.

D. CRITICAL COMMENTS

Some of the measuring methods introduced in the last 10 years have been used in the construction of commercial apparatuses. It is certainly not yet possible to pass an opinion as to which apparatuses will qualify themselves. To do this, it is necessary that they be employed for routine work in many laboratories and that the results of this employment be made known. When this is done, details should always be given, e.g., which type of balance was used and what the sensitivity of these balances actually is in practice.

The susceptibility to damage of these apparatuses and the daily preparation for the routine are also of interest. As, in general, the purchase costs are very high, the apparatuses should be able to be

operated by semiskilled persons. The daily preparation should not take more than half an hour, the duration of analysis should be short. A higher purchase price is worthwhile if the time personnel, principally highly trained personnel, can be saved.

The general applicability of such apparatuses appears of especial importance. It is not sufficient if 80 or 90% of the analysis material occurring can be investigated on the apparatus selected, in each instance. The analyst often does not know the composition of the sample to be investigated by him. He is not even able to refer to this lack of knowledge. Therefore, the method should be utilizable for almost *all* substances occurring. In my opinion, it is questionable on considering this requirement whether one should continue determining as many elements as possible simultaneously. These methods demand compromises making the general applicability of the apparatuses questionable. A compromise of this type is the use of helium instead of oxygen as carrier gas.

Furthermore, data on standard deviations attainable should be given with more care. It is not fully to be understood if, for example, uniformly large standard deviations are given in measuring methods for C and H. Only the relative mistake can be somewhat the same. This certainly is much more evident in C determination than, for example, in H determination. In this context, one should also ask oneself whether the Pregl tolerance is a postulate. In my opinion, a better analysis accuracy should be achieved today so that the microelemental analytic methods can continue to compete with other physical measuring methods. Microelemental analysis only then still justifies its existence when it produces results not obtainable with clarity otherwise.

Apart from data on the standard deviations, it should also be stated in how far the mean values found deviate from the theoretical values. The standard deviation can be small and the established value can be so far from the theoretical value that these analyses are useless.

In regard to the new measuring methods it is complained that the sample weights selected are too small. The microanalyst also tends to consider the Pregl sample weight as being gospel truth. This is erroneous. More important is the fact that the substances to be analyzed are pure and uniform. Only then has an analysis a purpose; and then it is immaterial whether 1 mg or 4 mg are taken. Quite the contrary, smaller amounts of substance can be fully combusted with greater reliability.

Even if the opinion was formerly held that the chemist engaged in preparatory work must educate the analyst, one has to rethink today in terms of educating the chemist engaged in preparatory work to working with more cleanliness. One can attain this very quickly in that one

clearly proves to him the silica gel from the thin plate, beads, and filter fibers.

SUMMARY

In the last 10 years a series of physicochemical methods of measuring have been developed which could replace the classic weighing of combustion products in microelemental analysis. Microelemental analysis can be automated using these methods. The measuring principle and the commercial apparatuses for determining carbon, hydrogen, nitrogen, and oxygen are discussed extensively.

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Book Reviews

Gas Chromatography. By L. SZEPESY. English Translation (from the Hungarian). Edited by E. D. Morgan. Chemical Rubber Co. Press, Cleveland, Ohio, 1970. 7 + 384 pp. $23.50.

The aim of the book under review is to teach the student the elementary principles of gas–liquid chromatography (GLC) and then to apply them to the qualitative and quantitative analyses of organic mixtures. The author has attempted to do this by a reasonably comprehensive and well designed teaching program. The reader who wishes to be instructed in the techniques of GLC is able to proceed at his own pace discovering not only useful practical information but also sufficient of the theoretical background of the technique to be able to comprehend the reasons for the choice of stationary phase and other experimental parameters necessary for a good separation.

This book is a timely one, and is intended to serve as an up-to-date guide for the beginner. Emphasis is on the practical aspects of the subject, but enough theory is included to serve as groundwork for further reading; selected references are given at the end of each chapter covering the more important aspects of the subject and major current developments. There are 11 chapters, which include liberal use of tables and diagrams. After a general introduction including a guide to the literature, a chapter is devoted to the definition of subject matter terms and to fundamentals on which the theory of GLC is based. A comprehensive section deals with the practical aspects of carrier gas, thermostats, injectors, columns, detectors, and other ancillary equipment; another large section provides a guide to the choice of columns and stationary phases, methods of preparing columns, and factors that affect their efficiency. Other topics covered include qualitative and quantitative analyses of substances separated by GLC, preparative GLC, the role of GLC in industrial processes, and the linking of automatic analysis by GLC to computer controlled continuous processes. Throughout the book attention is given to special techniques such as pyrolysis and reaction gas chromatography, elementary analysis, and use of multiple columns. A subject index but no author index is provided.

Typographically the book is excellent, the translation is clear and lucid, and the book reads easily. Paper, printing, and cloth binding are of good quality. The book will be useful to teachers of analytical chemistry and to those who wish to use GLC as an analytical technique applicable to a wide variety of problems. However, in view of its relatively high price the volume appears rather expensive to permit its widespread private ownership.

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Enzymatic Methods of Analysis. By GEORGE G. GUILBRAULT. Pergamon, New York, 1970. xv + 347 pp. $10.00.

In the last 25 years, there have been many novel developments in industrial chemistry. Biological catalysts, reacting in vats of 10,000-gallon capacity, produce

tremendous amounts of organics and biologicals hitherto obtained only in laboratory quantities. Enzymes have been used in clinical chemistry for at least 50 years and their use in analytical chemistry has not been neglected, but acceptance has been slow. This book is an indication of the growing importance of the use of Nature's catalysts and Dr. Guilbault has done the analyst a service by gathering procedures from scattered sources and presenting them in one place. His approach to the subject has taken into consideration both the novice and the experienced investigator—it is sufficiently explicit for the former without being too elementary for the latter.

The first chapter reviews the properties of enzymes and the principles of enzymatic analysis, and the second covers both chemical and instrumental methods of analysis. The next four chapters report in detail the types of determinations for which enzymes can be employed—the determination of enzymes themselves; substrates; activators and coenzymes; and inhibitors. In the last two chapters, Dr. Guilbault discusses the two developments which point the future of enzymatic analysis—immobilization and automation. Immobilization elimirates the high expense and makes continuous or semicontinuous routine analyses possible and practical. Automation, saving time and costs, is the "wave of the future."

Enzymatic methods of analysis will undoubtedly be used more and more as time goes on. The unique substrate selectivity of these compounds together with their specific requirements for activity or for inhibition of that activity make possible the determination of extremely small amounts of material. For example, submicrogram quantities of fluoride can be determined by its inhibition of liver esterase, even in the presence of large amounts of phosphate. The presence of Al^{3+} in concentrations of 3.3×10^{-4} can be detected by the inhibition of isocitric dehydrogenase by that ion. These are but two of many applications.

The book is well written and each chapter is amply documented. Separate author and subject indexes make reference easy. The book itself has remarkably few typographical errors; is sturdily bound and printed in clear, readable type; and the photographs, tables, drawings, and charts, of which there are many, are all well reproduced. A particularly useful section is an appendix listing all commercially available enzymes and their producers, with addresses of the latter.

This books is highly recommended. The analyst will find it most useful and will be well advised to add it to his library.

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Pulse Radiolysis. By MAX S. MATHESON AND LEON DORFMAN. M.I.T. Press, Cambridge, Mass. 1969. x + 202 pp. $11.75.

Pulse radiolysis is a relatively new technique of particular interest in radiation chemistry and chemical kinetics. The authors present an authorative overview of the subject which is quite understandable to those not experts in the field (such as this reviewer). The first portion of the book deals with the instrumental details and the experimental techniques used in pulse radiolysis. The authors then devote four chapters (~130 pp.) to a summary of the reactions of the short-lived intermediates. This section should be very useful to kineticists who are interested in the reactivity of these intermediates. Of particular note are the two fairly extensive chapters on organic systems and reactions of the hydrated electron. The

rather extensive bibliography provides a useful index to the literature although the authors state that the literature coverage is fragmentary after 1968. This book will be of value to those interested in pulse radiolysis and the very useful information which this technique provides.

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Applied Spectroscopy Reviews. Volume 3. Edited by EDWARD G. BRAME, JR., Dekker, New York, 1970. x 11 + 345 pp. $17.50.

The rapidly growing output of papers dealing with spectroscopy has caused scientists seeking information in particular fields to resort increasingly to authoritative reviews such as these where the main trends of development are summarized by experts. Volume 3 contains seven reviews covering the literature thoroughly through 1967, with only but a handful of recent references cited—a regrettable deficiency in an otherwise flawless book. Being an international publication, the volume under review lists contributors from the U.S., India, and Japan.

The scope of subjects and techniques covered is considerable, and as was the case in previous volumes, infrared spectroscopy is seen again to receive major attention in the current volume. In addition to IR spectroscopy there are reviews dealing with emission and X-ray spectroscopy along with mass spectrometry. There are three reviews dealing with IR spectroscopy, two of which cover its use in biological applications: "The Application of IR Spectroscopy to Structure Studies of Nucleic Acids" being a comprehensive review of the subject covering studies that can be performed from 4,000 to 400 cm^{-1}, and "IR Studies of Hydrogen–Deuterium Exchange in Biological Molecules" discussing the various methods that can be used in studying the exchange mechanism to determine primary, secondary, and tertiary structure in biological systems. The third one "Quantitative Analysis by IR Spectrometry" is devoted to the general subject of quantitative analysis; it covers some of the more practical and down-to-earth aspects of the subject. Two other brief but excellent reviews, one on "The Application of X-ray Spectroscopy to Clinical Analyses in Biological Research" dealing with recent developments in X-ray spectrometry and electron-probe microanalysis as applied to biological systems; and the other devoted to "Recent Advances in Analytical Emission Spectrometry" covering latest developments including the use of computers, constitute fascinating reading. The review on "Electronic Spectra of Radical Ions" taking up about one fifth of the book is a most comprehensive article on the subject. Methods used in producing the various radical ions and related species as well as peak absorptions in the UV and visible regions of the spectrum are presented. The final review titled "The Combination of Gas Chromatography with Mass Spectrometry" deals primarily with the coupling of gas chromatographic and mass spectrometric techniques, and the parameters of each that are pertinent to the successful operation of the combined system. However, this review does not deal completely with all aspects of gas chromatograph–mass spectrometer coupling, but an attempt is made to correlate the salient features of gas chromatography and of mass spectrometry that are necessary for an operational gas chromatograph–mass spetrometer system.

To sum up, Volume 3 is a worthy follow-up to its predecessors. The book is printed on strong paper in easily readable type. The drawings, tables, and graphs

of which the book abounds are clear and well produced. It constitutes excellent value, and is certainly a must for all up-to-date technical libraries as well as for all scientists engaged in spectrographic research.

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Cellulose Acetate Electrophoresis: Techniques and Applications. By H. P. CHIN, Ann Arbor Sci. Pub., Ann Arbor, Mich. 1970. vii + 139 pp. $15.75.

As is evident from the title. this book deals primarily with the practical application of cellulose acetate electrophoresis to macromolecular analysis. The book is designed to be used by persons with some basic knowledge of biochemistry, although it will provide a good basic introduction to the subject of electrophoresis for most laboratory scientists.

Over three-quarters of the book is devoted to electrophoretic analysis of blood proteins. A minor portion of the book deals with application of the technique to other large molecules, such as insulin.

The book is roughly divided into three general areas: theory, practical equipment, and various procedures for blood protein separations.

The first few chapters contain an interesting historical description of electrophoresis and a discussion of the basic principles of electrophoresis. The latter chapter contains a good balance between the theoretical and practical factors which govern electrophoretic separations. On the whole, certain important physcochemical properties of solutions, such as ionic strength, are not covered in depth. On the other hand, some not too well appreciated practical factors which can influence electrophoresis (e.g., "self slowing") are pointed out.

The following chapter describes the physical and chemical properties of cellulose acetate membranes. Accessory electrophoretic equipment is described. A list of manufacturers of equipment is provided.

The fourth and fifth chapters offer a plethora of detailed practical procedures for the preparation of the cellulose acetate membrane, for conditions for electrophoretic, for preparation of important buffers, for preparation of various stains and, finally, for preparation of the completed ionophoretogram for subsequent quantitative evaluation. However, quantitative techniques such as planimetry and densitometry are not covered in depth.

The sixth chapter describes general guidelines for protein electrophoresis as well as exact procedures for the preparation of protein stains. Also included is a brief overview of the physiology and pathology of plasma proteins.

The heart of the book deals with the practical applications of cellulose acetate electrophoresis to the separation of various types of blood proteins. Major classes of blood proteins such as lipoproteins, hemoglobins, glycoproteins, and isoenzymes are covered. Each of these chapters is excellent.

The chapter on lipoproteins provides a procedure for electrophoretic separation, for staining, and for quantitative analysis. Also included is a brief discussion of the chemistry of lipoproteins. Various types of genetically and nongenetically associated lipoprotein disorders are also described. The widely used phenotyping system for classification of various types of lipoproteinemias is only briefly discussed. This is unfortunate, since it represents the most systematic approach yet developed for aiding in the detection of lipoprotein disorders.

The remainder of the chapters on blood protein analysis (hemoglobins, hapto-

globins, glycoproteins, and isoenzymes) were brief. Each contains specific procedures for cellulose acetate electrophoresis.

The last two chapters are devoted to immunodiffusion, immonelectrophoresis, and miscellaneous applications. The former contains a brief discussion of the fundamentals of immunoelectrophoresis, as applied to cellulose acetate electrophoresis. Although practical approaches are discussed, no exact procedural details such as those provided in the preceeding chapters are given. The last chapter briefly describes various applications of this electrophoretic technique to the separation of other small and large molecules such as amino acids, nucleotides, mucopolysaccharides, and insulin. However, little is said about the utilization of this technique for the analysis of structural or cellular proteins.

The book deserves the attention of all who are interested in obtaining a firm practical foundation in the use of cellulose acetate electrophoresis. It is refreshing to read a book which accomplishes its aims. The high cost of the book may limit its accessibility to many individuals, but it should prove to be a noteworthy addition to all libraries.

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Micromethods for the Clinical and Biochemical Laboratory. By HERMANN MATTENHEIMER. Ann Arbor Sci. Pubs., Ann Arbor, Mich. 1970. xii + 232 pp. $18.75.

As defined by the late Dr. Benedetti-Pichler, "Microchemistry is . . . the technique involved in the performance of chemical experiments on an essentially smaller scale than is usually employed in laboratory practice" (1). The development of microtechniques in the biochemical laboratory has permitted the tremendous advances of recent years in our knowledge of life functions.

Nearly 10 years ago, Dr. Mattenheimer published, in German, a limited number of micromethods which he had developed for the biochemical laboratory. The wide acceptance of his book prompted a revised and enlarged edition 5 years later. Now, the commercial availability of specialized equipment has made practical the use of these methods in routine clinical chemistry, so Dr. Mattenheimer has translated this second edition into English with the normal revisions and updating. A significant addition is a chapter on ultramicrotechniques, mainly developed by Dr. H. O. Lowry, applicable to the quantitive determination of enzyme activity in cells from freeze–dried tissue sections.

The first chapter defines terms and the next two are concerned with equipment and general working conditions. Following these is a chapter on chemical methods which is most comprehensive. The remaining four chapters cover enzymatic determination of metabolites; determination of enzyme activity; enzyme assays in tissue homogenates and extracts; and the ultramicromethods for the determination of enzymes in cells from freeze-dried tissue mentioned above.

Procedures are given for practically all of those items of clinical importance which are usually available only in small quantities or in low concentrations. There is no bibliography—only one or two references for each method—but the directions are so explicit that such references need serve only as credit lines. The book is definitely usable by the technician but is not limited to him. Four appendixes give, respectively, a table for the conversion of absorbances into millimi-

cromoles of HADH (NADPH); preparation of buffer solutions; sources of reagents and reagent kits; and the use of test papers and tablets.

The book is sturdily bound for hard laboratory use; printed in clear, readable type, with a profusion of tables, drawings, and photographs which are well reproduced; and there is a good index. It is highly recommended as a useful tool in every clinical and biochemical laboratory whether commercial, hospital, clinical, or academic.

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Guide to Fluorescence Literature. Vol. 2. By RICHARD A. PASSWATER. Plenum, New York, 1970. 369 pp. $22.50.

Workers in any field that encompasses a vast literature are always indebted to the compiler of a bibliography in that field.

The author of the present volume has gone beyond mere compilation; he has indeed created a "Guide to Fluorescence Literature." This Volume and Volume 1 are the first resource for workers in Fluorescence.

References are grouped by year (1964 to mid–1968) under five major categories and are arranged alphabetically by first author. A cross-reference subject index and a complete author index will save many hours of library time.

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Microanalysis by the Ring-Oven Technique. 2nd ed. By HERBERT WEISZ. Pergamon Press, Ltd., Oxford—New York—Toronto, 1970. ix + 170 pp. $8.00.

Years ago the ingenuity of the Ring-Oven Technique created an interesting situation. At a time, when the general trend pointed towards increase in sophistication, size, and price of analytical machinery, the simple little Ring Oven aroused an astonishing amount of interest. Very soon, enough material and publications were at hand to compose a little monograph, which appeared in 1961 and was widely acclaimed. Again, at a time when many methods became obsolete before even fully invented, the Ring Oven Technique not only stood against such fate, but after nine years, a second edition of the monograph was necessary. The overal increase in volume is from 112 to 170 pages. The original number of 26 special "Ring-Oven Publications" grew to 136, and the number of general references expanded from 106 to 299 in both cases give and take a little, since in an appendix are found a dozen or so more references which were added during a late stage in the printing process to bring the book up to date as much as possible.

This remarkable increase in relevant publications shows clearly to what extent the method has been accepted in chemical circles and what immense interest still

exists for a simple wet analytical method, when it is capable of doing so much as the Ring-Oven Technique can do.

The expansion of the field required rewriting of several sections of the book, and a few chapters had to be added. Of those, one on application in organic analysis and one about radiochemistry will be particularly welcomed by the relevant workers.

The revision and additions have been made in the meticulous, exact manner characteristic of the author. Several figures have been added and some old ones redrawn, resulting in an improvement over the already fine previous edition.

There is no doubt that this excellent little book in its second edition will not only please the old friends of the first edition, but will acquire many new admirers and will create increased interest in the method. The book should be read with much profit even (or possibly especially!) by those who do not work in the field.

H. FLASCHKA, *School of Chemistry, Georgia Institute of Technology, Atlanta, Ga. 30332*

Encyclopedia of Industrial Chemical Analysis, Vol. 9, Casein to Chromium. Edited by F. D. SNELL AND L. S. ETTRE. Wiley (Interscience), New York, 1970. xii + 709 pp. $45.00 ($35.00 by subscription).

Earlier volumes of this significant compendium have been reviewed by this Journal ((see, *Microchemical Journal,* **15,** 378 (1970)). This encyclopedia can again be recommended to any library having substantial holdings in the fields of chemistry and chemical technology, or a clientele involved actively in applied chemical analysis.

With this volume, the trek through the chemical alphabet for products and groups of products extends from casein and reaches chromium (and its alloys and compounds). Intermediate stops include such diverse topics as caulking compounds; cellulose; cellulose derivatives; cements, mortars, and concrete; ceramics; cereals; cerium (and its alloys and compounds); cesium (and its compounds); chlorine; chlorine compounds, inorganic; chlorocarbons; chorophenols; chlorosulfonic acid; chocolate and cocoa products; and choline and its derivatives.

According to the plan of the work, general techniques not treated in Volumes 1 through 3 are considered in the main body of the work. Volume 9, as a result of this organization, includes a well-conceived, 33-page article on centrifugal separations that may arise in either preparational or analytical procedures. The editors have not adopted a narrow view of "industrial chemical analysis;" consequently, in the present volume the physical testing of caulking compounds, cements, and concrete is treated, and for chocolate and cocoa products some 10 pages are devoted to methods for assuring microbiological purity.

For the practice of organic microchemistry, it is noteworthy that the article "Chlorocarbons" includes an 11-page presentation of procedures for the determination of chlorine in such compounds (combustion via Carius sealed tube, peroxide bomb, and oxygen-filled flask techniques; hydrolysis via the action of sodium in liquid ammonia and ethanolamine, and via the use of the sodium-biphenyl reagent).

A. J. BARNARD, JR., *J. T. Baker Chemical Co., Phillipsburg, N. J. 08865*

Assay of Proteins and Polypeptide Hormones. Volume 33. By H. VAN CAUWENBERGE AND P. FRANCHIMONT. Pergamon Press Ltd., Oxford, 1970. vi + 243 pp. $13.50.

The book sets out to survey the assay of proteins and polypeptide hormones as applied in medical practice. The range of assays described includes the early biological tests, bioassay techniques which have provided valuable information but were often lacking in specificity, sensitivity; and precision; thereafter, the introduction of immunological methods created a new field of investigation for biologists and clinicians alike; however, even though these methods had the advantage of being based on excellent specificity of the antigen–antibody reaction, the techniques used to demonstrate this reaction were often insensitive and imprecise. This deficiency was remedied by the introduction of radioimmunological techniques which provided a system that was far more sensitive and at the same time more resistant to the various factors capable of altering visualization of the immunological reaction.

The book is divided into seven sections constituting sixteen chapters. The opening two chapters are devoted to a review of the general principles which must be applied to arrive at assay techniques of good quality. In each succeeding chapter a particular hormone is considered. Hormones covered include growth hormones, adrenocorticotropin, thyrotropin, gonadotropins, prolactin, vasopressin, and oxytocin, insulin, glucagon, erythropoietin, renin, parathormone, thyrocalcitonin, human chorionic gonadotropin, and human placental lactogen. The problems involved in assaying the hormone are taken up; the principal regulatory mechanisms involved in the normal and pathological secretion of the hormones are discussed. The current assay methods for each hormone as well as those used in the past stressing problems of technique and interpretation are presented in detail.

The presentation is adequate with only a few errors in the text being noted. The index is surprisingly short and incomplete; and there is no author index. However a good and adequate bibliography is provided at the end of each chapter. The text is clearly printed and well illustrated and is written in a readable style that will capture and hold the interest of the reader.

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

Topics in Mass Spectrometry. Edited by A. L. BURLINGAME. **Vol. 8 of Advances in Analytical Chemistry and Instrumentation.** Wiley, New York, 1970. xi + 471 pp. $22.50.

This volume comprises nine chapters, each by an active contributor in his field: Techniques of Molecular Ionization, Mass Discrimination Caused by Electron-Multiplier Detectors, Correlation of Fragment Ion Structure with Energetics of Formation, Gas–Liquid Chromatography—Mass Spectrometry Combination, High Resolution Mass Spectrometry, Mechanisms of Ion Decomposition Reactions, Mass Spectrometry of Complex Natural Compounds, and Applications of Mass Spectrometry to Organic Geochemistry.

The editor has compiled an author index and a subject index, and he has included a Cumulative Index for Volumes 1–8 of the "Advances" series.

As would be expected from the authors, the articles are authoritative and well

written. The editor apparently coped reasonably well with the problem of the time lag between solicitation of the papers and publication of the volume. Most of the chapters include 1968 references.

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Analytical Chemistry of Zirconium and Hafnium. By ANIL K. MUKHERJI, Pergamon Press, Oxford/ New York/Toronto, 1970. xiii + 281 pp. $12.00.

With this work the *International Series of Monographs on Analytical Chemistry* reaches Volume 40. Professor Ronald Belcher is to be congratulated on his services as Senior General Editor of this series from its initial conception. On balance, the quality of the series has been excellent. Many volumes stand as major contributions to the literature of analytical chemistry and some have now advanced to a second edition.

Dr. Mukherji in the monograph under review in 280 pages has attempted to survey for zirconium and its natural associate, hafnium, the aqueous chemistry; the dissolution of alloys and ores; the detection of these elements and their determination (by gravimetry, titrimetry, electroanalytical methods, and solution photometry); their separation from other elements by ion exchange and solvent extraction; the analysis of diverse materials by arc and spark spectrography, by flame emission and absorption photometry; and by X-ray, neutron activation, and radiotracer techniques. The coverage of literature is selective and extends into 1967.

The separation of hafnium from zirconium is the subject of a 41-page chapter with some 146 references cited. Here the author has gone beyond procedures of the preparational or analytical laboratory to provide an understanding of large-scale and industrial processes by which this now-important separation can be effected.

The reviewer has examined work from his special interests in organic reagents for inorganic analysis. All common selective reagents for zirconium and hafnium were found to be either mentioned or considered in some detail. The author (or his copy editor) has often failed, however, to achieve unity and consistency in the selection of trivial names or systematic chemical names for some reagents. For example, "Pyrocatechol Violet" and "Catechol Violet" are treated at separate points and are indexed separately although both are names for a single reagent. Again "*N*-phenylbenzohydroxamic acid" and "*N*-benzoyl-*N*-phenylhydroxylamine" are entries at various points in the text and have separate entries in the subject index!

Dr. Mukerji has fulfilled his aims. The work can be recommended to libraries having significant holdings in applied chemical analysis and metallurgical analysis. The volume should be shelved adjacent to the Elinson and Petrov monograph *"Analytical Chemistry of Zirconium and Hafnium"* now available in English as a translation from the Russian (Israel Program for Scientific Translations, Jerusalem).

A. J. BARNARD, JR.,
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Man's Impact on the Global Environment: Assessment and Recommendations for Action. Edited by WILLIAM H. MATTHEWS, The M. I. T. Press, Cambridge, Mass., 1970. xvi + 319 pp. (paperback) $2.95.

This is the report of the Studies of Critical Environmental Problems sponsored by the Massachusetts Institute of Technology and is of interest to microchemists as members of society. Carefully thought out group studies and concrete recommendations are presented with the hope that "a greatly extended and deepened search for knowledge concerning critical environmental problems" will be stimulated in both governmental and private areas. The technology is available. The difficulty is in motivation.

Everybody is urged to read this book and act upon it to the extent of his individual capacity.

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Fluorescence Analysis: A Practical Approach. By CHARLES E. WHITE AND ROBERT J. ARGAUER, Marcel Dekker, Inc., New York, 1970. x + 389 pp. $18.75.

It is always a pleasure to review a book that one can highly recommend and that will be essential reading for a wide range of chemists. The basic theory and practice of fluorescence are described, and the various disciplines where fluorescence trace analysis is used in the fields of chemistry, biology, and agriculture are illustrated. Sufficient theory is given to provide a background for practical experiments and the use of the apparatus involved.

The text of this book constitutes 18 chapters. The first three opening chapters are devoted to the nature of fluorescence, correction of excitation and emission spectra, and instrumentation. Subsequent chapters deal with fluorescent metal chelates, quantitative fluorescence methods for metals and nonmetals, fluorescent indicators, fluorescence in qualitative inorganic analysis, spectrofluorometry with column, gas, paper, and thin layer chromatography, as well as application of fluorescence in agriculture and clinical chemistry. Substances covered in detail include proteins and amino acids, aldehydes, amines, alcohols and acids, drugs, carbohydrates, vitamins, and steroids. Considerable length is devoted to metal chelates and to a discussion on the effect of substituent groups on fluorescent intensity. About eighty specific procedures for the quantitative determination of elements and organic compounds are included.

The style is lucid; the text is pleasant to read and well illustrated with diagrams as well as tabular presentation of data. The printing is clear, but unfortunately, there is a substantial number of misprints throughout the text. Author and subject indices are provided, and the bibliography at the end of each chapter, although not completely comprehensive, is adequate.

Although all sections are equally praiseworthy in their approach to the various topics dealt with, from an analytical viewpoint, the chapters on fluorescence in chromatography for separation, characterization, and identification of materials, as well as fluorometric procedures in clinical chemistry deserve special attention. These new analytical techniques bid fair to supplement or even replace some of the currently used instrumental techniques in our armory for inorganic and organic trace analysis. There is no doubt whatsoever that even for these two sections alone, this book represents a milestone along the highway of analytical chemistry.

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

Announcement

EASTERN ANALYTICAL SYMPOSIUM

In order to broaden the scope of the Symposium in the sense of including the very latest developments in the fields of Analytical Chemistry and Spectroscopy, it has been decided that the EAS will include in its next program (November 10–12, 1971) a maximum of three half-day sessions of submitted papers. Each speaker will be allowed 30 minutes for his presentation; 25 minutes for the talk, and 5 minutes for questions and answers. All those interested in presenting papers at this meeting should send *four (4) copies of a 300-word abstract to:*

A. Z. Conner,
Hercules Inc.
Research Center
Wilmington, Delaware 19899.

In order for abstracts to be reviewed for consideration they must be received by May 1, 1971.

FUTURE MEETINGS

1971	November	10–12	Statler-Hilton	New York, N.Y.
1972	November	15–17	Traymore Hotel	Atlantic City, N.J.
1973	November	14–16	Statler-Hilton	New York, N.Y.

Erratum

Vol. 15, No. 3 (1970), in the article, "Coulometric Microdetermination of Oxygen in Organic Compounds," by Kan ichi Nakamura, Masaaki Nishimura, and Tetsuo Mitsui, pp. 461–469:

The Equation on p. 465 should read as follows:

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

An extraneous line of type was inadvertently included.