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Microchemical Journal

devoted to the application of microtechniques in all branches of science

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MOLECULAR COLLISION THEORY

M. S. Child
Department of Theoretical Chemistry
University of Oxford, England

September 1974, x+302 pp., £8.50/$22.00
0.12.172650.9

This book contains an introduction to molecular collision theory for those seeking an interpretation of experimental results. Emphasis is given to the validity and relevance of available theoretical techniques, rather than to the formal structure of the theory. The argument starts from a quantum mechanical standpoint, but the reader is quickly led to appreciate the importance currently given to semi-classical methods.
Molecular Collision Theory will serve primarily as a reference book, but one which also consolidates recent advances in the literature, and should prove of value to chemists and physicists with a grounding in bound state quantum mechanics.

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Microchemical Journal, Volume 20, Number 1, March 1975

Briefs

2,2'-Diquinoksalil as a New Reagent for Determination of Bivalent Tin in Thin-Layer Chromatography. R. BARANOWSKI, B. KOT, I. BARANOWSKA, AND ZB. GREGOROWICZ, *Department of Analytical and General Chemistry, Silesian Technical University, Gliwice, Poland.*

The new reagent gives a blue complex with bivalent tin. No reaction occurs with tetravalent tin.

Microchem. J. **20,** 1 (1975).

Spectrophotometric Determination of Copper with Uramyldiacetic Acid. F. BERMEJO-MARTINEZ AND MERCEDES MOLINA-POCH, *Department of Analytical Chemistry and Analytical Chemistry Section of High Council of Scientific Research, Faculty of Sciences, Santiago de Compostela, Spain.*

Uramyldiacetic acid is used as the chromogenic reagent. The complex is formed over a wide range of pH and has a maximum of absorption at 775 nm. The metal ligand ratio is 1:1. Beer's law is obeyed over the range of 20–420 μg Cu(II)/ml.

Microchem. J. **20,** 7 (1975).

A Simultaneous EDTA–Metric Determination of Calcium and Magnesium with Antipyrylazo III and Thymolphthalexon. B. W. BUDESINSKY, *Analytical Laboratory, Phelps Dodge Corporation, Morenci, Arizona 85540.*

The simultaneous determination of calcium and magnesium in limestone and dolomite is done by EDTA titration at pH 13.0–13.3 and 10.2–10.5 using antipyrylazo III and thymolphthalexon, respectively, employing masking agents.

Microchem. J. **20,** 17 (1975).

Spectrophotometric Study of a Direct Determination of Serum Calcium. W. L. CLARK, E. S. BAGINSKI, S. S. MARIE, AND B. ZAK, *Departments of Pathology, Holy Cross Hospital and Detroit General Hospital, Departments of Biochemistry and Pathology, Wayne State University School of Medicine, Detroit, Michigan and Department of Pathology, St. Joseph Mercy Hospital, Pontiac, Michigan.*

A modification of a direct manual procedure is described in which a one-piece color reagent is used. Rapid and stable color formation occurs enabling the procedure to serve either as a manual stat system or in robotized automatic instrumentation. A low dielectric solvent is used to repress the ionization of the blank while allowing linear and reproducible calibration characteristics.

Microchem. J. **20,** 22 (1975).

Continuous Titrations in Ultramicrodeterminations. B. GRIEPINK, F. G. RÖMER, AND W. J. VAN OORT, *Analytical Chemistry Laboratory, The University, Croesestraat 77A, Utrecht, Netherlands.*

Problems in scaling down procedures of analyses are generally stated. Data are presented for several determinations.

Microchem. J. **20,** 33 (1975).

A New Spot Test for Aliphatic Aldehydes and Ketones. JAMES W. MUNSON AND THOMAS HODGKINS, *College of Pharmacy, University of Kentucky, Lexington, Kentucky 40506.*

Microgram quantities of aliphatic aldehydes and ketones may be detected by the adaptation of a reaction utilizing free radical formation.

Microchem. J. **20,** 39 (1975).

Determination of Sulfite by Reaction with Mercury(I) Chloride and Spectrophotometric Measurement of Mercury(II) Complexes. WILLIE L. HINZE, DONALD J. KIPPENBERGER, AND RAY E. HUMPHREY, *Department of Chemistry, Sam Houston State University, Huntsville, Texas 77340.*

Sulfite ion is determined in the 0.4–12 ppm range by reaction with insoluble mercury(I) chloride to form the soluble $Hg(SO_3)_2^{2-}$ ion and elemental mercury. The uv absorption of the sulfite complex or an anion species, HgX_4^{2-}, formed on adding an excess of KBr, KCl, KI, or KSCN is measured. The mercury(II) in solution can also be determined by lowering the pH, adding KCl, and forming the crystal violet adduct of the $HgCl_3^-$ ion. This adduct is extracted into benzene and the absorbance measured at 605 nm.

Microchem. J. **20,** 43 (1975).

Methods for the Isolation and Characterization of Constituents of Natural Products. XIX. Use of a Celite–Sodium Borohydride Column for Reduction of Carbonyl Compounds at the Micro Level. DANIEL P. SCHWARTZ AND ROBERT REYNOLDS. *Dairy Products Laboratory, Eastern Regional Research Center, Agricultural Research Service, U. S. Department of Agriculture, Philadelphia, Pennsylvania 19118.*

A Celite–sodium borohydride column is used to reduce micromole quantities of simple carbonyl compounds to the corresponding alcohol. A hexane solution of the carbonyl compound is applied to the column and the effluent containing the alcohol collected. A column constructed in a melting point capillary suitable for microgram quantities is also described.

Microchem. J. **20,** 50 (1975).

Auxiliary Conversion Tables for Sensitivity Performance Tests of Atomic-Absorption Instruments. J. RAMÍREZ-MUÑOZ, *Beckman Instruments, Inc., Irvine, California 92664.*

Conversion tables have been prepared which give the signal (in absorbance units and percentage of absorption) corresponding to a given sensitivity level, expressed as concentration limit, when measuring the response of a solution of a given concentration. These tables permit the operator a rapid check of the sensitivity performance of an instrument by comparing signals obtained under his or her conditions with the signal expected for a given concentration from limits already published or previously obtained by the operator with other conditions.

Microchem. J. **20,** 56 (1975).

Potentiometric Microtitration of Cobalt. D. A. LEE, *Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830.*

Microgram quantities of cobalt were determined by potentiometric titration with potassium ferricyanide. The electrode response was improved by the use of polarized Sb reference and Pt indicating electrodes. The titration method together with γ-scintillation counting was used to determine isotopic assays of cobalt isotopes.

Microchem. J. **20,** 62 (1975).

An Improved Selective Determination of Mercury(II) by Complexometric Titration. G. S. VASILIKIOTIS AND C. D. APOSTOLOPOULOU, *Laboratory of Analytical Chemistry, University of Thessaloniki, Thessaloniki, Greece.*

The determination is done in alkaline solution, using an excess of EDTA, titration of the unreacted EDTA with a standard zinc solution, and addition of *N*-allylthiourea. The mercury complex is decomposed by heating, and the liberated EDTA is titrated with the zinc solution, using Eriochrome Black T as indicator.

Microchem. J. **20,** 66 (1975).

Ultramicro Photometric Titrations Using A Long-Path Photometer. H. A. FLASCHKA AND D. C. PASCHAL, *School of Chemistry, Georgia Institute of Technology, Atlanta, Georgia 30332.*

The long-path photometer previously described was adapted for photometric microtitrations. Turbidimetric titrations of silver with chloride and of barium with sulfate did not give good results, but iron(II) with permanganate, zinc with EDTA in the presence of excess Zincon, copper with EDTA in the presence of excess SNAZOXS, and calcium with EDTA in the presence of excess arsenazo III all gave excellent results.

Microchem. J. **20,** 70 (1975).

Data on the Determination of Calcium and Phosphate in the Presence of Citric Acid. L. SZEKERES AND J. A. JOSEPH, *Linden Laboratories, Inc. Subsidiary of Chromalloy American Corporation, Los Angeles, California 90066.*

The determination of calcium and phosphate in citrate extracts of the natural raw phosphates is accomplished by complexometric–precipitate titration after oxidation of the citric acid with hypochlorite.

Microchem. J. **20,** 78 (1975).

Rapid Potentiometric Method for the Analysis of Mica, Talc, Feldspar, Nepheline Syenite, and Clay. H. KHALIFA, I. A. ISMAIL, AND A. KH. GHONAIM, *Chemical Department, Ministry of Industry, Cairo, A. R. E.*

There is described for the analysis of a number of insoluble silicates, an application of the method of back titration of unconsumed EDTA in hexamine-buffered media using Hg(II) and Bi(III) as titrants and silver amalgam as the indicator electrode.

Microchem. J. **20,** 84 (1975).

Design and Construction of a Fluorotitrator. J. M. WHITE AND H. FLASCHKA, *School of Chemistry, Georgia Institute of Technology, Atlanta, Georgia 30332.*

An instrument is described that permits fluorometric titrations in a large, open compartment that accommodates titration vessels of a wide variety of shapes and sizes. The influence of ambient light is excluded by applying geometric exclusion in combination with electronic monitoring and compensation. In test titrations of copper and calcium with EDTA and calcein, the instrument performed satisfactorily.

Microchem. J. **20,** 89 (1975).

Sodium–Lead Alloy in the Decomposition of Organic Samples for the Detection of Heteroelements. R. C. LANCE, A. J. BARNARD, JR., AND E. F. JOY, *Research & Development Department, J. T. Baker Chemical Co., Phillipsburg, New Jersey 08865.*

The decomposition of organic samples using open-tube fusion with sodium–lead alloy was demonstrated to be safe, complete, and controlled, in most cases. The detection of heteroelements in the fusion mixture of common organic samples was successful with samples of 10–20 mg. Chlorine, nitrogen, and fluorine were included in the study. The detection limits for heteroelements were in the microgram region.

Microchem. J. **20,** 103 (1975).

MICROCHEMICAL JOURNAL 20, 1–6 (1975)

2,2′-Diquinoksalil as a New Reagent for Determination of Bivalent Tin in Thin-Layer Chromatography

R. BARANOWSKI, B. KOT, I. BARANOWSKA, AND ZB. GREGOROWICZ

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Received December 12, 1973

Determination of bivalent tin by the thin-layer chromatography method is difficult and is connated with its easy oxidation to tetravalent tin. According to literature references chromatographic systems have been used to determine bivalent tin, where supporting materials were the following: silica gel G (*10, 15, 17, 19*), Al_2O_3 (*15*), a mixture of silica gel G with cellulose in a proportion 1:1 (*20*). Chromatograms were developed in mixtures of solvents: *n*-butyl alcohol: 1 *M* dihydroxysuccinic acid:3 *N* HCl (10:1:1) [*10*], acetone (dimethylketone):HCl (99:1) [*19*], propionic acid:H_2O (1:4) [*20*], amyl acetate:HCl (50:5) [*17*], 2% acetic acid:*n*-propyl alcohol:isopropyl alcohol (6:3:1) [*20*].

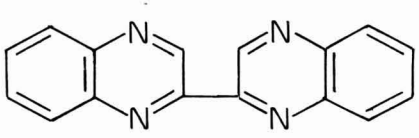
Tin is visualized on chromatograms by oxine (8-hydroxyquinoline), NH_3, H_2S in the light of uv lamp [*10*], $(NH_4)_2$ S [*17*], NH_4OH (*15*).

In the scientific literature the determination of tetravalent tin was described more often. Chromatograms from Sn^{4+} were developed in mixtures of solvents, mainly mixtures of alcohols and hydrochloric acid, e.g., methyl alcohol, ethyl alcohol, *n*-propyl alcohol, and butyl alcohol with HCl (*1*), as well as hexane with benzene (80:20) [*3*].

Tetravalent tin was developed by ditizone (*3, 9, 11, 21*), quercetin (*6*), *o*-dihydroxybenzene (*22*), diphenylcarbazone (*18, 21*).

For the determination of tin a method of paper chromatography (*3, 5, 7–9, 12, 18, 21, 22*) was elaborated where tin was developed in the form of complex compounds.

The aim of our research work was to examine the properties of



2, 2′ - Diquinoksalil

1

2,2′-diquinoksalil (*2, 4, 16*) as a new reagent for the detection of bivalent tin in the technique of thin-layer chromatography.

2,2′-Diquinoksalil gives with bivalent tin ions a blue coloration, and does not react with Sn^{4+}, which has the unquestionable advantage when determining Sn^{2+} and Sn^{4+} at the same time.

EXPERIMENTAL PART

Reagents and apparatus: 2,2′-Diquinoksalil (*2,4,16*) (available from Polish Chemical Reagents 8 Sowinskiego Street, Gliwice, Poland), benzene, dioxane, methanol CH_3OH, absolute ethanol C_2H_5OH, *n*-propyl alcohol, izopropanol, hexane, cyclohexane, acetonyl acetone, diphenylcarbazide, L-ascorbic acid, hydrochloric acid, formic acid, glacial acetic acid, *N*-butanol, silica gel G for the thin-layer chromatography—Merck $SnCl_2.2H_2O$ obtained as after Ref. (*13*), thin-layer chromatography made by Shandon assembly, Hamilton's microsyringe

PREPARATION OF SOLUTIONS

2,2′-Diquinoksalil (0.06%). A 30-mg weighed portion of 2,2′-diquinoksalil was transferred into a measuring flask of 50 ml volume and dissolved in a $HCl:H_2O$ (3:2) solution.

Standard solution Sn^{2+} in 99.8% ethanol (1 μg/1 μl). A 0.1901-g weighed portion of $SnCl_2 \cdot 2H_2O$ was dissolved in 99.8% ethanol in a flask of 100-ml volume and then made up with ethanol to the measuring mark.

$SnCl_2$ standard solution in water (1 μg/1 μl). $SnCl_2 \cdot 2H_2O$ solution was made up by dissolving 0.1901 g $SnCl_2 \cdot 2H_2O$ in a mixture of 25 ml distilled water and 27.5 ml concentrated HCl, and then made up with distilled water to the measuring mark in a measuring flask of 100-ml volume.

Solution 2% standard of diphenylocarbazide in 99.8% ethanol. Thin-layer chromatography. Glass plates sized 20 × 20 cm were covered with a silica gel G suspension in the standard way. The gel layer was 0.30 mm thick. On glass plates prepared in such a way, a tin solution of a concentration 1 μg/1 μl was introduced, a chromatogram was developed at a distance of 14 cm and proceeded with 0.06% solution of 2,2′-diquinoksalil in a mixture of $HCl:H_2O$ (3:2). The absence of blue spots confirmed that Sn^{2+} was completely oxidized to Sn^{4+}. The tetravalent tin was detected with diphenylcarbazide in ethanol (*14*).

Searching for means of Sn^{2+} stabilization, the plates were sprayed with 20 ml of saturated solution of L-ascorbic acid in methanol. The presence of acid on the plate has caused the diminution of the degree

of Sn^{2+} oxidization. To ensure the distribution of L-ascorbic acid at a uniform rate, the L-ascorbic acid was diluted in methanol at a rate of 1% by weight of silica gel. The prepared solution was combined with silica gel, and the plates were covered with the suspension formed, in the standard way.

Tin solutions were put on chromatographic plates by means of a micro-syringe at rates of 1 μg to 20 μg. In determining Sn^{2+} it is essential to reduce the time of introducing the standard solution to the plate to prevent Sn^{2+} oxidation to Sn^{4+}. Therefore, tin diluted in ethanol was used and spraying was carried out in a carbon dioxide atmosphere.

A series of tests were carried out in the aim of determining the conditions in which Sn^{2+} would remain on the second oxidation stage, after its introdution on the chromatographic plate and development in the solvent systems. Best effects have been achieved by introducing tin from an alcoholic solution on a plate covered with silica gel G, with a 1% addition of L-ascorbic acid. Chromatograms have been developed in chromatographic chambers filled with solvents after removing air from them by means of carbon dioxiode to prevent tin oxidation during the development of the chromatogram. The chromatograms have been developed then in a 2,2′-diquinoksalil solution to cause dyeing of divalent tin spots to a rich-blue color. Spots of sharp contours were achieved, suitable for a quantity interpretation. Tetravalent tin does not react with 2,2′-diquinoksalil. Subsequently Sn^{4+} has been developed by a diphenylcarbazide solution obtaining thereby red spots.

Some chromatographic systems have been examined to determine divalent tin. In these systems silica gel G, silica gel G after iron removal, and Al_2O_3 have been used as the stationary phase, and the following as solvents mixture:
n-butanol: 1.5 *N* HCl (50:5), *n*-butanol: 1.5 *N* HCl: acetonyl acetone (50:10:0.25), methanol: *n*-propanol: 1.5 *N* HCl: acetonyl acetone (25:25:10:0.25), izo-propanol: methanol: acetonyl acetone: 1.5 *N* HCl (40:10:10:0.25), hexane: dioxane: formic acid (15:27.5:3.5), ethyl acetate: HCl (50:5), hexane: benzene: propanoic acid (40:10:10), benzene: acetic acid (50:10), dioxane: formic acid (25:25), benzene: dioxane: formic acid (30:30:5).

Oxidation of Sn^{2+} to Sn^{4+} took place in the above systems, preventing the detection by 2,2′-diquinoksalil. Best effects were obtained when using the following systems: silica gel G as a stationary phase with a 1% addition of L-ascorbic acid. I mobile phase—hexane: benzene: acetic acid (40:10:20); II mobile phase—benzene: bioxane: formic acid (60:30:5); III mobile phase—hexane: benzene:

TABLE 1
AVERAGE VALUES R_f FOR Sn^{2+} IN CHROMATOGRAPHIC SYSTEMS I, II, III

Chromatographic system	Average value R_f
I	0.05
II	0.11
III	0.12

dioxane:formic acid (40:10:26:10). The distance of the starting point from the front of solvents was for all systems 14 cm.

Applying chromatographic system III and detection by 2,2'-diquinoksalil solution, a rectilinear dependence of the spot surface size from the amount of examined Sn^{2+} introduced on the plate was found and illustrated in Fig. 1.

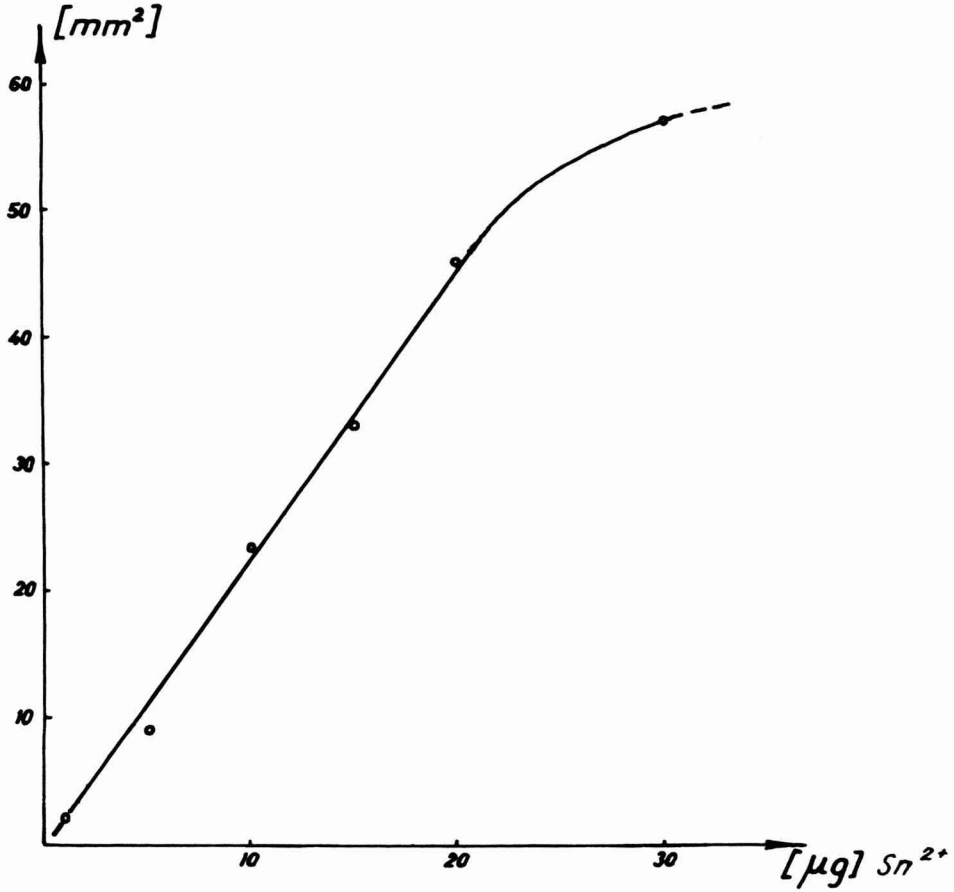


FIG. 1. Quantity curve showing the dependence of spot sizes on the Sn^{2+} rate introduced to the chromatographic plate.

On the basis of the performed examinations it was found that 2,2'-diquinoksalil is a new, sensitive reagent to Sn^{2+} ions, enabling the determination of Sn^{2+} from μg on the chromatographic plate. Absence of coloring of the given reagent with tetravalent tin ions is an additional advantage enabling the determination of Sn^{2+} and Sn^{4+} at the same time.

2,2'-Diquinoksalil asables the detection of bivalent tin beside tetravalent tin also by the method of spot analyses on a chromatographic plate, without the necessity of developing chromatograms.

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Spectrophotometric Determination of Copper with Uramyldiacetic Acid[1]

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INTRODUCTION

Several polyamino-polycarboxylic acids had been successfully employed for the photometric determination of copper. Thus, NTA forms a blue complex with copper which gives a maximum of absorption in slightly acid solutions at 619–710 nm and in alkaline medium at 640–648 nm, Beer's law being obeyed between 0.04 and 7.6 mg of copper(II)/ml (*14*).

It is also possible to determine copper in slightly acid or alkaline solutions by forming the copper(II)–EDTA chelate. The absorption is measured at 720 nm remaining unchanged in the pH interval 4.75–6.50 and little affected when the pH is about 10. By using this procedure it is possible to determine from 0.035 to 8.0 mg of copper(II)/ml (*15*). The method was applied to the determination of copper in tin, nickel alloys, and in aluminium (*13*) or in cyanide plating copper baths (*11*). When working in ammonia medium it is necessary to carry out the measurements at 630 nm (*16*).

The copper(II)–DCyTA chelate found application for the photometric determination of 10–540 μg of copper(II)/ml at 710 nm (*5*), the absorption remaining unchanged in the pH range 4–12. Copper reacts with EGTA to give a complex which can be used for its evaluation in a wide pH interval (4.90–9.77) and at 680 nm conforms to Beer's law between 20 and 500 μg of copper/ml (*4*).

Bermejo and Blas (*3*) utilized the EEDTA as chromogenic chelating agent for the copper determination. The complex formed exhibits a maximum at 720 nm in acidic medium. Beer's law is obeyed in the range 25–650 μg copper/ml with an error less than 2%.

[1] Part LXXII in the series "Analytical Applications of the Chelons." Paper presented at the International Symposium on Microchemical Techniques-1973, held at the Pennsylvania State University, University Park, Pennsylvania, August 19–25, 1973.

Frausto *et al.* (*10*) developed a procedure using the EDPA as reagent. The formed complex at pH 3–8 shows a maximum at 670 nm with a molar absorptivity of 140.

Bermejo-M. and Rodriguez-Campos have studied the reaction copper(II)–DTPA for absorptiometric and spectrophotometric measurements (*8*). The given system presents a maximum at 650 nm in alkaline medium. Beer's law is followed between 20 and 300 μg of copper(II)/ml when using the filter photometer and from 9 to 200 μg of copper(II)/ml when using the spectrophotometer.

Below is described a new copper photometric determination using uramyldiacetic acid (UDA) as a chromogenic reagent; this new method has the advantage over the oldest analogous methods with other chelons using the 775-nm wavelength for the measurements. Most wavelength maxima for copper(II)–chelon complexes reported to date are below 730 nm.

EXPERIMENTAL

Reagents

Standard copper(II) solution. A standard aqueous copper(II) solution was prepared from $CuSO_4$, X.A.R., its concentration being determined by titration with EDTA (*6*), resulting of 2.59 mg of copper(II)/ml ($0.04086F$). From this other solutions were prepared by dilution.

Standard uramyldiacetic acid (UDA) solution. A standard aqueous solution of UDA was prepared and titrated with standard zinc solution (*7*), resulting 0.0379 *F*. From this other solutions were prepared by dilution.

Uramyldiacetic acid (UDA) solution. A 5% m/v aqueous solution was prepared by dissolving the appropriate amount of reagent in distilled water with the aid of sodium hydroxide to achieve its complete solution.

Buffer solution pH=3. A citrate–hydrochloric acid buffer solution (E. Merck) was used.

Sodium hydroxide solution 0.1 F. Aqueous solution with 4 g sodium hydroxide in 1 liter.

Hydrochloric acid solution 0.1 F. Aqueous solution prepared in the usual way.

Apparatus

A spectrophotometer, Beckman model DU equipped with 10 mm quartz cells was used. (*18*).

A pH meter, Beckman model Expandomatic with glass and calomel electrodes (sensitivity $\pm$ 0.01 pH), was used.

Procedure

To a series of 25-ml volumetric flasks transfer aliquots of the standard copper(II) solutions, add 1 ml of 5% UDA solution, the necessary drops of 0.1 *F* hydrochloric acid or sodium hydroxide solution to approximate the pH to 3, and then 5 ml of buffer solution of that pH, diluting to the mark with distilled water. The absorbance measurement is carried out at 775 nm (or the scanning was made) using 10-mm quartz cells and a solution on 1 ml of 5% UDA solution diluted to 25 ml as a blank.

RESULTS AND DISCUSSION

Absorption spectra. In order to fix the optimum working wavelength, the spectral characteristics of the copper(II)–UDA system at various pH values were studied. In Fig. 1 are shown the results obtained at pH 2, 5, 7, and 10.2; from which it is concluded the identity of the spectra at the given pH values, exhibiting a maxima at 775 nm in either case, although the absorbance value is slightly higher in alkaline medium.

Effect of pH. The absorbance constance in the pH interval 2–8,4 was found. Figure 2 illustrates the results obtained when using a copper concentration of 207.69 μg/ml. To avoid the potential increase of the conversion of UDA to murexide it is convenient to work at an acidic pH value; thus, pH of about 3 was chosen for further studies.

Effect of reagent amount. Once the pH optimun had been fixed, the effect of the amount of reagent was studied. It was found that 0.5 ml of 5% UDA solution were enough to form the complex and further additions of reagent did not appreciably affect the absorbance of the system

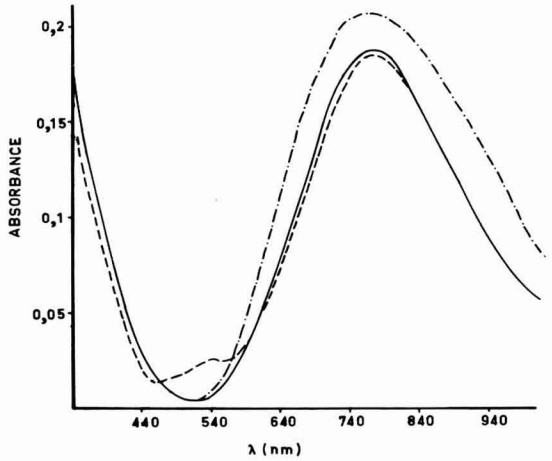

FIG. 1. Absorption spectra of copper(II)–UDA complex solutions at pH: 2.5 —, 7 ----, 10.2 ·-·-· and concentration of 207.69 μg copper(II)/ml.

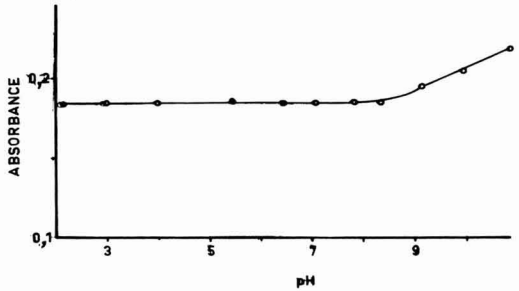

FIG. 2. Effect of pH variations on the absorbance of the copper(II)–UDA complex solutions. Copper(II) concentration was of 207.69 μg/ml.

(Fig. 3). As a blank, a solution of reagent obtained by taking in each case the same volume of 5% UDA solution diluted to 25 ml was employed.

Time and temperature effects. The effects of time and temperature on the stability of the system copper(II)–UDA were also studied; copper reacts instantly with uramyldiacetic acid, the absorbancy of the formed complex solutions remaining unchanged for up to 25 hr and up to 100° C.

Beer's law. The relationship between the absorbed radiant energy and complex concentration was studied with the previously established conditions. Several standard solutions of copper(II)–UDA complex were prepared by taking aliquots of the standard solution of copper(II) and after adding the reagent and adjusting the pH, diluting with distilled water up to the mark. The absorbance of the solution was measured at 775 nm; the results are lineal on the calibration graph over the range 20–420 μg copper(II)/ml. The molar absorptivity was calculated as $\epsilon = 57.1$.

Ringbom's plot and minimum photometric error. In order to evaluate the optimum interval of Beer's law application, the percentage of the absorbance against the log of copper concentration was plotted obtaining the graph shown in Fig. 4, from which it was found the optimum range

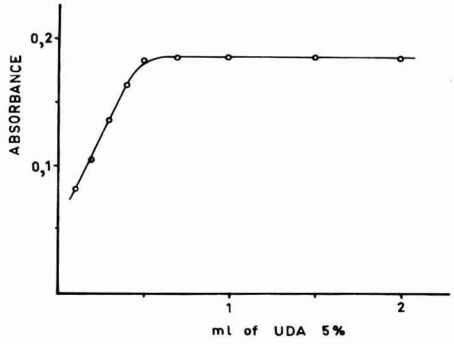

FIG. 3. Effect of reagent amount on the absorbance of the copper(II)–UDA complex solutions. Copper(II) concentration was of 207.69 μg/ml.

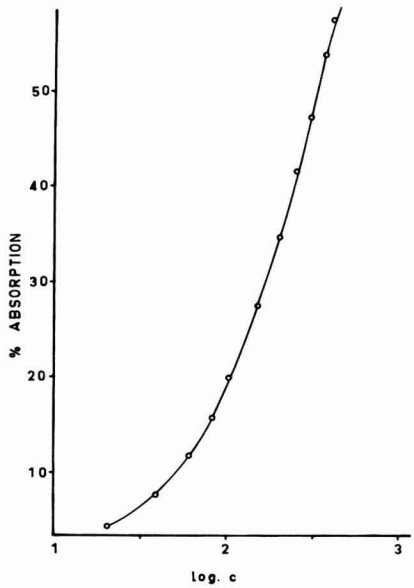

FIG. 4. Ringbom's plot for the copper(II)–UDA complex solutions.

to be from 70 to 400 μg of copper(II)/ml. In the same way and from the Ringbom's plot (*9,17*) the minimum photometric error was calculated by using Ayres' (*1,17*) method from which it was deduced that the copper(II)–UDA solutions show a minimum photometric error of 2.8.

Reproducibility and precision. To carry out these studies four series of different concentration solutions were prepared following the pre-

TABLE 1
METHOD REPRODUCTIBILITY

Series A: 20.77 μg copper/ml		Series B: 62.31 μg copper/ml		Series C: 207.69 μg copper/ml		Series D: 373.86 μg copper/ml	
A	Deviation	A	Deviation	A	Deviation	A	Deviation
0.020	0.001	0.056	0.000	0.186	0.000	0.332	0.000
0.019	0.002	0.056	0.000	0.184	0.002	0.332	0.000
0.021	0.000	0.056	0.000	0.185	0.001	0.332	0.000
0.021	0.000	0.056	0.000	0.186	0.000	0.333	0.001
0.021	0.000	0.054	0.002	0.184	0.002	0.332	0.000
0.021	0.000	0.056	0.000	0.185	0.001	0.332	0.000
0.022	0.001	0.056	0.000	0.186	0.000	0.331	0.001
0.021	0.000	0.055	0.001	0.186	0.000	0.331	0.000
0.021	0.000	0.055	0.001	0.187	0.001	0.330	0.002
0.020	0.001	0.056	0.000	0.186	0.000	0.330	0.002
$\bar{X} = 0.021$		$\bar{X} = 0.056$		$\bar{X} = 0.186$		$\bar{X} = 0.332$	

viously described procedure. The results obtained for the reproducibility of the method are presented in Table 1.

The precision data were obtained by using statistical technique (*2*), and the calculated values were the following;

Series A:

$\sigma = \pm 0.0009$ $\qquad \sigma_m = \pm 0.00028$

$\bar{X} \pm \sigma t = 0.021 \pm 0.002$ $\qquad \dfrac{\sigma_m \cdot t}{\bar{X}} \times 100 = \pm 2.99\%$

Series B:

$\sigma = \pm 0.008$ $\qquad \sigma_m = \pm 0.00026$

$\bar{X} \pm \sigma t = 0.056 \pm 0.002$ $\qquad \dfrac{\sigma_m \cdot t}{\bar{X}} \times 100 = \pm 1.05\%$

Series C:

$\sigma = \pm 0.0011$ $\qquad \sigma_m = \pm 0.00035$

$\bar{X} \pm \sigma t = 0.186 \pm 0.002$ $\qquad \dfrac{\sigma_m \cdot t}{\bar{X}} \times 100 = \pm 0.42\%$

Series D:

$\sigma = \pm 0.0011$ $\qquad \sigma_m = \pm 0.00035$

$\bar{X} \pm \sigma t = 0.332 \pm 0.002$ $\qquad \dfrac{\sigma_m \cdot t}{\bar{X}} \times 100 = \pm 0.24\%$

from which are deduced, what the confidence limits with 95% probability must be for each series in the interval 0.019–0.023, 0.054–0.058, 0.184–0.188, 0.330–0.334, respectively.

Stoichiometry of the reaction. To stablish the composition of the

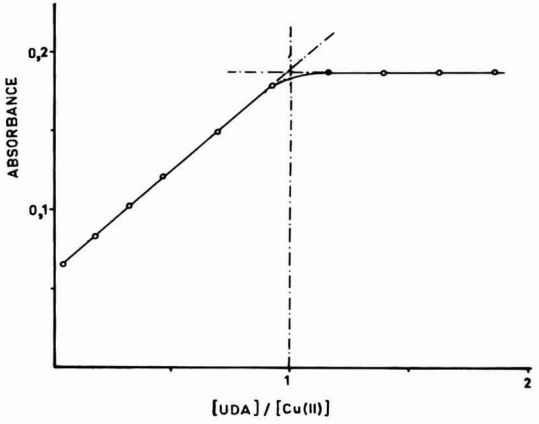

FIG. 5. Application of the molar ratio method for determination of copper(II)–UDA complex composition.

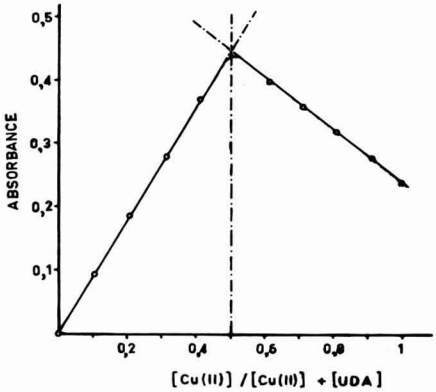

FIG. 6. Application of the continuous variations for determination of copper(II)–UDA complex composition to pH = 3.

copper(II)–UDA chelate two methods were followed: (a) "molar ratio" of Yoe and Jones (*19*) and (b) "continuous variations" of Job (*12*).

The results are illustrated in Figs. 5 and 6, from which study it is inferred that the formation of the complex takes place in the ratio copper(II) to UDA of 1:1.

Effect of diverse ions. The effect of the other species on the formation of the copper(II)–UDA complex was studied. See Tables 2 and 3. Sev-

TABLE 2
EFFECTS OF DIVERSE ANIONS[a]

Anion	Amount in 25 ml (mg)	Absorbance
Acetate	12.5	0.186
Nitrate	12.5	0.185
Chloride	12.5	0.186
Arseniate	12.5	0.185
Bromide	12.5	0.184
Thiocyanate	12.5	0.214 (turbid blue)
Thiocyanate	5.0	0.188
Citrate	12.5	0.186
Phosphate	12.5	0.185
Vanadate	4.9	0.219
Vanadate	0.9	0.188
Perchlorate	12.5	0.185
Nitrite	5.0	0.187 (pink violet)
Iodide	5.0	0.186
Iodide	12.5	0.243 (turbid green)
Oxalate	5.0	0.178
Dichromate	1.8	0.182 (violet)
Tungstate	9.2	0.179
Tungstate	1.8	0.185

[a] Copper concentration 207 μg/ml; absorbance 0.186.

TABLE 3
EFFECTS OF DIVERSE CATIONS[a]

Cation	Amount in 25 ml (mg)	Absorbance
Ni^{2+}	1.2	0.186
Tl^{3+}	10.2	0.180
Cd^{2+}	11.2	0.185
Ce^{4+}	7.0	0.184 (light brown)
Ce^{4+}	1.4	0.185
Cr^{3+}	0.5	0.185
Cr^{3+}	2.6	0.187
Ca^{2+}	1.2	0.185
Mg^{2+}	9.6	0.184
Co^{2+}	5.6	0.182 (violet)
Co^{2+}	1.1	0.185
UO_2^{2+}	2.7	0.183
Fe^{2+}	5.5	0.186 (brown)
Fe^{3+}	0.6	0.186 (light brown)
Pd^{2+}	1.1	0.201 (yellowish green)
Pd^{2+}	0.5	0.187 (yellowish green)

[a] Copper concentration 207 μg/ml; absorbance 0.186.

eral solutions containing 207.69 μg of copper(II)/ml, 1 ml of 5% UDA solution, 5 ml of the buffer solution (pH=3), and amounts of the ions investigated ranging from 0.5 to 12 mg in 25 ml, were prepared. After filling the volumetric flask up to the mark with distilled water, the absorbance measurements were made as usual. It was found that iodide, thiocyanate, vanadate, oxalate, and palladium must be present in amounts not higher than 20 μg/ml when the copper concentration is the above mentioned.

Application of the method for the determination of copper in ores with a low content of the element. The method described above was used for the determination of the copper content in an ore in which copper is present in amounts not higher than 1.5% . The sample was treated with aqua regia during 2 hr in the way copper together with other elements

TABLE 4
DETERMINATION OF THE COPPER CONTENT OF AN ORE WITH A LOW PERCENTAGE OF THE ELEMENT

Sample no.	UDA method (%)	DTPA method (%)	Average difference (%)
1	0.58	0.54	0.04
2	0.58	0.54	0.04
3	0.72	0.75	0.03
4	1.21	1.24	0.03

was solubilized. The trivalent ions, mainly iron, were eliminated by double precipitation with ammonium hydroxide solution. The filtrate obtained was made up to a volume of 100 ml, from which an aliquot was transferred to a 25-ml volumetric flask and treated as in the general procedure previously described.

The copper content was also determined by another method employed as reference method which uses DTPA as reagent (*8*). In Table 4 are shown the results obtained for various samples with both methods indicating only the mean average values of copper content. There is good agreement between the values found when using both methods.

CONCLUSIONS

The above investigation has led to the development of a method for determination of minute amounts of copper after forming the copper(II)–uramyldiacetic acid chelate at pH about 3 and measuring the absorbance of the complex at 775 nm. Although the complex is formed in a wide pH interval, 2–8.4, the neutral or alkaline interval values must be avoided owing to some decomposition of uramyldiacetic acid giving murexide which interferes in the determination.

Those ions which interfere in the procedure can readly be eliminated by using some of the methods already described (*18*). Although the method was applied to the determination of copper in an ore, it can be used with a variety of materials.

SUMMARY

A new method for the spectrophotometric determination of copper using uramyldiacetic acid as chromogenic reagent is proposed. The complex is formed in a wide pH range (2.5–9); but in order to avoid the potential conversion of UDA in murexide it is convenient to work at pH < 3; and has a maximum of absorption at 775 nm. Beer's law is obeyed in the interval 20–420 μg of copper(II)/ml. The Ringbom optimal range falls between 70 and 400 μg of copper(II)/ml, with a minimum photometric error of 2.8. The reaction between the metal and the ligand takes place in the ratio 1:1. The method has been applied to the determination of copper in ores with low content of the metal.

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A Simultaneous EDTA–Metric Determination of Calcium and Magnesium with Antipyrylazo III and Thymolphthalexon

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INTRODUCTION

The simultaneous determination of calcium and magnesium is the subject of many analytical methods. A considerable part of them is based on the application of polyaminocarboxylic acids as titrants (*9*). Recent methods of that type are mostly instrumental employing either electrometric (*4*, *7*), or photometric (*8*) indication of the end point.

The selectivity of calcium titration is based on sufficient difference between the values either of stability constants of polyaminocarboxylic complexes or of solubility products of metal hydroxides.

In the former alternative, the selective determination of calcium is given by equation $\log [c_{Ca}\beta_{Ca}/(c_{Mg}\beta_{Mg})] \geq 6$ (assuming the 0.1% maximum interference and ignorable side reactions of both elements), where c_{Ca}, β_{Ca}, and c_{Mg}, β_{Mg} are the total concentrations and over-all stability constants of calcium and magnesium, respectively. Of all polyamino carboxylic acids used as titrants only the ethyleneglycol bis(2-aminoethyl-ether)tetraacetic acid (EGTA) approximately obeys the above relationship. Therefore, EGTA has been used in several modifications with instrumental end point indication for selective titration of calcium in the presence of magnesium (*8*, *9*). A suitable calcium selective indicator for a visual titration is not known.

In the latter alternative, the corresponding pH of selective calcium titration is given by the equation $\text{pH} = 10.3 + \frac{1}{2} \log ([Y]\beta_{Mg}/c_{Mg})$[1] (assuming the 0.1% interference of magnesium, the logarithmic solubility product of magnesium hydroxide −10.4, and ignorable side reactions of both elements), where β_{Mg} and c_{Mg} have the same meaning as above, $[Y]$ is the actual concentration of the free ligand of corresponding polyaminocarboxylic acid. For most titrations it is reasonable to assume $\log (c_{Mg}/[Y]) \geq 3$. In that manner we have for the titration with EDTA $\text{pH} \geq 13.1$ ($\log \beta_{Mg} = 8.7$). Even this alternative has

[1] This is obtained by combination of equations $10^{-10.4} = [Mg][OH]^2$, $10^{-14} = [H][OH]$, $\beta_{Mg} = [MgY][Mg]^{-1}[Y]^{-1}$ and $1000[MgY] = c_{Mg}$.

been used in several modifications for selective calcium determination (*8*, *9*).

If EGTA is used for calcium, magnesium is subsequently determined usually with EDTA and an instrumental indication of the end point (*9*).

In the second alternative, magnesium is usually determined by a separate titration of the sum of calcium and magnesium and the amount corresponding to calcium is subtracted (*9*). If the murexide indicator is used for calcium, it can be destroyed by hydrolysis and magnesium determined in the same solution (*6*). In another modification (*2*) employing EDTA, the sum of calcium and magnesium is determined first at pH 10 and then after a pH increase to 13 the liberated EDTA (equivalent to magnesium) is titrated with a standard calcium solution.

The principal disadvantages and insufficiences of the discussed calcium–magnesium determination are:

(a) EGTA is approximately five times more expensive than EDTA (a fact which counts in routine analysis) and its complexation is slower than that of EDTA.

(b) Indicators exhibiting the sharpest endpoint (Calcon, Calcein, Eriochrome Black A) are adsorbed on magnesium hydroxide substantially deteriorating the sharpness of the endpoint.

(c) If the ratio (in weight percent) of magnesium/calcium is higher than 0.1, there is a substantial adsorbtion of calcium on magnesium hydroxide so that the results for calcium are lower and for magnesium higher.

(d) Most sensitive indicators (see (b)) for calcium cannot be used for analysis of natural materials (limestone and dolomite) since they are blocked (in spite of most added masking agents) by trace amounts of polyvalent elements (iron(III), aluminum, and titanium(IV)).

Recently (*3*), antipyrylazo III (or diantipyrylazo, i.e., 3,6-diantipyrinylazo-4,5-dihydroxy-2,7-naphthalenedisulfonic acid) was described as a very selective reagent for photometric determination of calcium. This reagent in combination with thymolphthalexon (thymolphthalein complexone, i.e., 3′,3″-bis[bis(carboxymethyl) amino]methylthymolphthalein) was found as most convenient indicator for a simultaneous EDTA–metric determination of calcium and magnesium. The troubles discussed under (a), (b), and (d) are removed, only (c) persists.

MATERIALS AND METHODS

Apparatus

All photometric measurements were made with a Gilford Spectrophotometer Model 2400 (Oberlin, OH). An Orion Model 801 pH-

meter (A. H. Thomas Co., Philadelphia, PA), with a glass and calomel electrode pair, was used for pH measurements.

Reagents

Standard aqueous solutions of 0.1 *M* EDTA–disodium salt, 0.1 *M* calcium chloride, and 0.1 *M* magnesium chloride. Aqueous 0.1% solution of Antipyrylazo III, the mixture of thymolphthalexon with potassium chloride (1 : 100). The solution containing 50 g of sodium cyanide, 400 g of sodium hydroxide, and 15 g of triethanolamine per liter has been used as calcium buffer. The solution containing 70 g of ammonium chloride and 570 ml of concentrated ammonia (28%) per liter has been used as magnesium buffer. The bleaching of antipyrylazo III has been accomplished by 40% aqueous solution of hydroxylamine hydrochloride. The solution of EDTA has been standardized by means of 99.999% lead nitrate using Xylenol Orange as indicator. Natural samples of limestone and dolomite were of Arizona origin. Antipyrylazo III was the product of Aldrich Chemical Co., Milwaukee, WI. All other chemicals were Bakers Analyzed Reagents.

Titration of Calcium and Magnesium

The volume and acidity (hydrochloric acid) of a solution containing 1–4 mmole of calcium and 0.02–1 mmole of magnesium has been adjusted to about 100 ml and 0.02 *N*, respectively. Two milliliters of calcium buffer and 5 drops of antipyrylazo III indicator have been added and calcium has been titrated with 0.1 *M* EDTA–DiNa. The color change is from bluish–violet to orange–red at the endpoint. Then 5 ml of magnesium buffer and 2 ml of 40% hydroxylamine hydrochloride have been added, the solution has been mixed and allowed to stand to decompose the antipyrylazo III for 10 min. About 50 mg of thymolphthalexon indicator has been added and magnesium titrated again with 0.1 *M* EDTA–DiNa. The endpoint is from blue to yellow.

The titration can also be performed with photometric indication employing the wavelength of 600 nm in both cases.

RESULTS AND DISCUSSION

The results of calcium and magnesium determination are collected in Table 1. The dolomite samples exhibit the effect of calcium coprecipitation as discussed under (c) in the Introduction.

The selectivity of antipyrylazo II complexation with calcium (*3*) is a main prerequisite of this compound as an excellent indicator for EDTA-metric determination of calcium in natural materials (limestone and dolomite). This was proved by analysis of thousands of

TABLE 1
TITRATION OF CALCIUM AND MAGNESIUM

Ca (mg)		Mg (mg)		Ca (mg)		Mg (mg)	
Taken	Found	Taken	Found	Taken	Found	Taken	Found
40.1	40.0	0.5	0.5	160.4	161.2	0.5	0.5
40.1	39.9	2.4	2.5	160.4	159.9	2.4	2.5
40.1	39.8	24.3	24.6	160.4	159.7	24.3	24.6
80.2	80.5	0.5	0.5	91.6[ac]	91.8	1.5[c]	1.5
80.2	80.0	2.4	2.5	92.2[ac]	91.8	0.5[c]	0.5
80.2	79.7	24.3	24.7	91.1[ac]	90.7	1.3[c]	1.3
120.3	121.2	0.5	0.5	54.0[bc]	53.3	32.1[c]	32.7
120.3	119.6	2.4	2.6	53.8[bc]	52.7	32.5[c]	33.0
120.3	119.4	24.3	24.8	52.5[bc]	51.8	31.8[c]	32.4

[a] Limestone sample.
[b] Dolomite sample.
[c] Results found by classical analysis (*5*).

samples during several years. There is a considerable number of natural samples where the application of murexide, calcone, calceine, and metal phthaleine as indicators fails, so that a preliminary separation of polyvalent ions by ammonia precipitation or cupferron extraction is necessary. But not the same is true about antipyrylazo III. In fact, we did not find a single case of its failure. The easy preparation of chromatographically pure product (*3*) and the stability of aqueous solutions for several months are other noteworthy features of that reagent.

To bleach the aqueous solutions of antipyrylazo III for the subsequent magnesium determination we investigated the reduction effect of ascorbic acid, sodium dithionite, hydrazine hydrochloride, and hydroxylamine hydrochloride. Only the last compound exhibits a convenient behavior, the reduction is sufficiently fast and the reagent and its decomposition products do not interefere with the magnesium determination. The reduction itself is catalyzed by some trace metals (iron, copper, titanium) so that its speed is not constant. However, the time given in the procedure is generally quite sufficient.

Thymolphthalexon is an excellent indicator for calcium and magnesium (*1*, *9*). It is not clear why so little attention has been paid to that fact. The basic advantage of thymolphthalexon is the color change between blue and colorless (corresponding to the metal complex and free indicator, respectively) at pH 10.8–11.2. That enables the application of thymolphthalexon as an indicator in a subsequent titration. The pH of magnesium titration is 10.2–10.5. Within those conditions, a weak residual blue color of the metal-free indicator still

remains in pure aqueous solution. At the determination discussed, however, that color is completely covered by the yellow color of antipyrylazo III reduction products.

In addition it should be pointed out that antipyrylazo III is also an excellent indicator for EDTA–metric determination of copper(II) (0.001–0.01 *M*) in aqueous ammonia (0.5–3.0 *M*). The color change is between blue and orange–red (or red–violet if more concentrated copper solution is used). No other known indicator matches the sharpness of the endpoint.

SUMMARY

The simultaneous determination of calcium and magnesium in natural materials (limestone and dolomite) can be made by EDTA–metric titration at pH 13.0–13.3 and 10.2–10.5 indicated by antipyrylazo III (diantipyrylaxo; 3,6-diantipyrylazo-4,5-dihydroxy-2,7-naphthalenedisulfonic acid) and thymolphthalexon (3′,3″-bis[bis(carboxymethyl)aminomethyl]-thymolphthalein), respectively. Sodium cyanide and triethanolamine are used as masking reagents, hydroxylamine hydrochloride is the bleaching (reducing) reagent to remove the antipyrylazo III color for the subsequent magnesium titration. Antipyrylazo III is also a convenient indicator for EDTA-metric determination of copper(II) in aqueous ammonia.

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Spectrophotometric Study of a Direct Determination of Serum Calcium[1]

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INTRODUCTION

Calcium is an important cation whose concentration needs to be monitored frequently under various disease conditions either on a routine or an emergency basis. Although atomic absorption is considered by some to be the system of choice (*18*), most laboratories must still rely on other means of determination, usually titrimetry (*4,5,13,21*), or spectrophotometry (*9,11,18,19,20*), both of several sorts. The use of chelating agents such as cresolpthalein complexone (*6,10,14,16,17*), alizarin sulfonate (*7*), and methylthymol blue (*8*) have become popular because they yield strongly colored complexes with calcium. The positive error caused by the reaction of magnesium has been eliminated in several procedures by means of the addition of 8-hydroxyquinoline (*6*).

Originally cresolpthalein complexone (CPC) had been used as an indicator in the titration of alkaline earth metals by another chelating agent, EDTA. However, a deeply colored blank caused by the ionized forms of the dye created problems. Earlier investigators who used these compounds in visual titrations suppressed the deepness of color of the blank by incorporating an organic solvent into the reagents (*1*). Since a strongly absorbing blank is also a serious problem in spectrophotometric measurements, an attempt has been made to at least partially suppress the ionization of one of the chelometric indicators, CPC, by incorporating into this system a selected solvent of low dielectric constant. Both manual and automated systems using a similar reaction medium have

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been described (*2,3,22*). The present report concerns the following aspects of the reaction:

1. The effect of organic solvent concentration on the apparent molar absorptivities of the Ca(II)–CPC complexes and on the reagent blank.
2. The effect of a variable reagent blank which has a similar absorption spectra to the measured complex.
3. The effect of 8-hydroxyquinoline (8-HQ) in the reaction as a masking agent for magnesium.
4. The effect of other metals normally found in biological materials on the reaction.
5. The use of a single combined reagent for a rapid serum calcium determination.

MATERIALS AND METHODS

Reagents

Stock reagent. Add 75 ml of ethylene glycol (EG) and 25 ml of 2-amino-2-methyl-1-propanol (AMP) to a 1-liter volumetric flask containing about 800 ml of H_2O. Weigh in 30 mg of CPC and dilute to the mark with metal-free distilled H_2O. This solution is stable for 3 wk.

Working reagent. Prepare this solution by dissolving 100 mg of 8-HQ per 100 ml of the stock reagent. This dissolves quite slowly and is stable for approximately 1 wk.

An alternative approach can also be used for the preparation of the color reagent. In this case, a CPC solution containing 8-HQ and a base solution containing AMP and EG are premixed before use, the pH adjusted with 10% NaOH and the reagent then used as described for the other one-piece reagent. It is necessary to adjust the pH here because some HCl is required for dissolving the CPC and this lowers the pH of the resulting mixture.

Calcium stock standard (1 g/liter). Transfer 250 mg of anhydrous $CaCO_3$ into a 100-ml volumetric flask, dissolve in a minimum amount of concentrated HCl and dilute to volume with water.

Calcium working standard (100 mg/liter). Dilute the stock standard 1:10. Alternatively, an appropriate dilution of commercially available atomic absorption standards may be used.

Ethylene diamine tetraacetic acid (1% EDTA). An equivalent amount of ethylenebis (oxyethylenenitrilo)-tetracetic acid (EGTA) will also serve the same purpose.

Procedure

1. Add 20 μl of 100 mg/liter standard to a clean test tube or cuvette (4 ml polystyrene autoanalyzer cups work well), and 20 μl serum to another.

2. Add 2 ml of color reagent to both tubes. The color reagent without the addition of 20 μl of H_2O serves as the reagent blank because such dilution of its color is too small to be significant.

3. Measure the absorbance of the standard, and unknown against the reagent blank at 575 nm.

If the serum is turbid or chylous, add 20 μl of EDTA (or EGTA) solution to the cups containing the serum and the reagent blank and determine the absorbances again. The difference in absorbance between this solution and the original sample reading is the corrected absorbance used in calculation.

RESULTS

Effect of organic solvent. The inverse effect of variation in EG concentration on the absorbance spectra of the 100 mg/liter calcium standard versus water as a blank is shown in Fig. 1, left. As the concentration of EG is increased to 25%, the peak absorbance reading of the Ca (II)–CPC complex is decreased. When the EG concentration was varied in the reagent blank the effect was similar (Fig. 1, right). The resultant plots of this depressant effect of the solvent are shown in Fig. 2 for the standard, the reagent blank, and the standard minus the reagent blank. These are the peak absorbance values versus the EG values in percent.

Effect of variable blank. The spectra of three calcium standards, 50, 100, and 150 mg/liter scanned against H_2O are shown in Fig. 3, left. Also, the spectra of two reagent blanks, the lower one treated with EDTA are shown below the standards. The latter points to the contamination of the chemicals, H_2O, or glassware with calcium. On the right

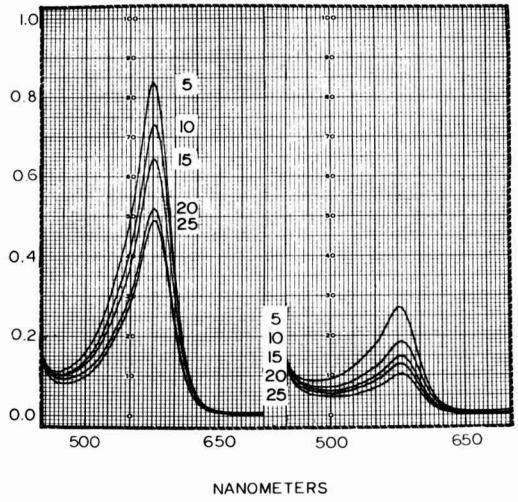

FIG. 1. Effect of varying the EG solvent concentrations from 5–25% on a 100 mg/liter standard on the left and the reagent blank on the right.

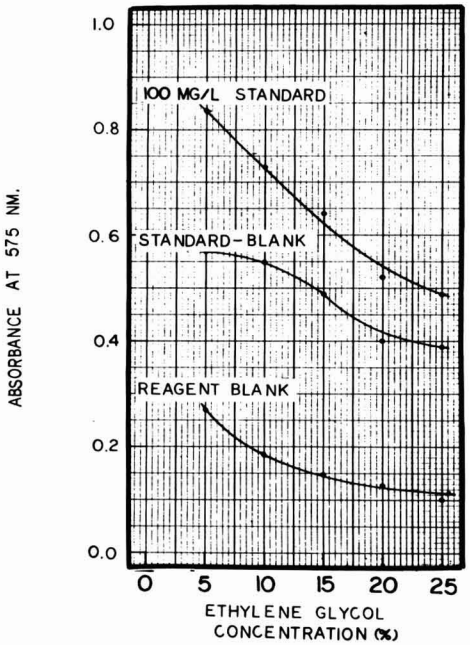

FIG. 2. Plot of peak absorbance values versus the reagent concentrations of EG for a standard, a standard blank, and reagent blank.

side of the figure are shown the spectra of reagent blanks prepared by using solutions which contained no calcium as determined by the additions of EDTA to each and which only differed from each other by their mg/liter concentrations of CPC as shown. Both standards and blank plot

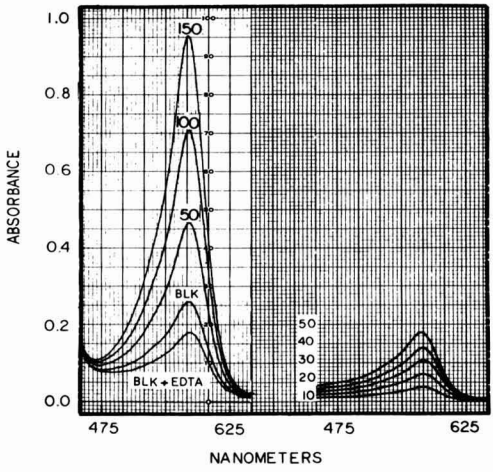

FIG. 3. Standard and reagent blank curves to the left versus water, also showing the calcium contamination of the blank. Calibration curves of the CPC reagent at the right covering 10–50 mg/liter.

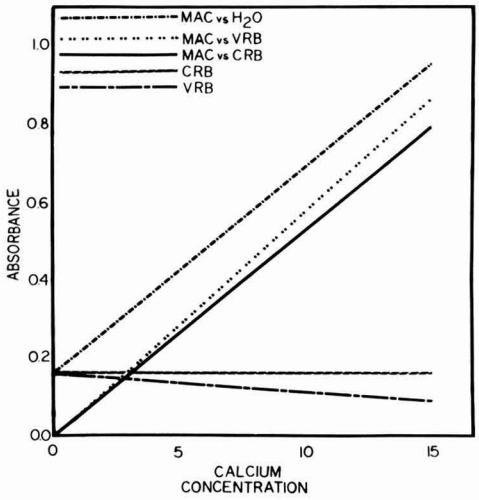

FIG. 4. Graphs of the continuous reagent blank used (CRB), the variable reagent blank (VRB), the measured absorbance curve against CRB (MAC vs CRB), the true absorbance curve (MAC vs VRB) and the measured absorbance curve versus H_2O (MAC vs H_2O).

linearly and their peak values were necessary to present the blanking discussion of Fig. 4.

Figure 4 contains five calibration curves derived from the spectra of the previous figure. Its purpose is to show in part that the total absorbance of the Ca(II)–CPC complex is never measured because the true reagent blank is variable and regressive whereas the reagent blank actually subtracted is always a constant value. MAC versus H_2O is the measured absorbance calibration curve one would obtain if the reacted standards were read against H_2O. The constant reagent blank curve (CRB) which is to be subtracted from the several standards is the curve parallel to the X-axis which meets MAC at the Y-axis. When curve CRB is subtracted from MAC (MAC vs CRB) the actual calibration curve which one uses results. But, since increasing amounts of calcium in the several standards use up increasing and measurable amounts of CPC, the true reagent blank results in a variable blank effect shown as the regressing curve, VRB. When the difference between CRB and VRB is applied to MAC, it results in the true absorbance curve and this is shown as the curve MAC vs VRB. Therefore, the slope of the true absorbance curve is greater than the slope of the measured absorbance curve inversely as the difference between the two reagent blank curves. One could conclude that the true absorbance of any standard is never obtained in a regressive blank situation such as the one described in this figure because curve VRB cannot be as easily measured as is curve CRB.

Interference. Some of the original work on the determination of cal-

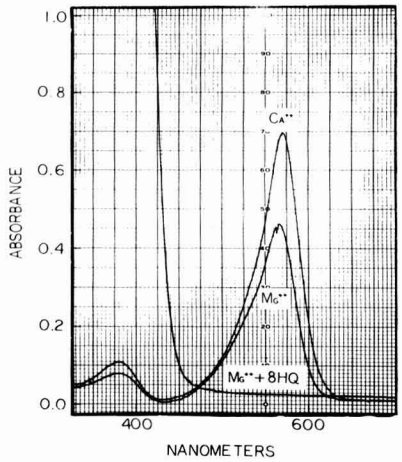

FIG. 5. Graphs of Ca(II)–CPC and Mg(II)–CPC without 8-HQ and Mg(II)–CPC with 8-HQ.

cium with CPC suggested that a constant amount of magnesium should be included in the standards to partly offset the interfering reaction of magnesium with CPC (*10*). The Mg(II)–CPC spectrum is identical in slope but less intense than that of Ca(II)–CPC as shown in Fig. 5 for 100 mg/liter of calcium and 100 mg/liter of magnesium. When 8-HQ is included in the reagent as described by Connerty and Briggs (*6*), then the total magnesium reaction is eliminated as shown in the same figure (bottom).

In order to illustrate the extent of any reaction between calcium and

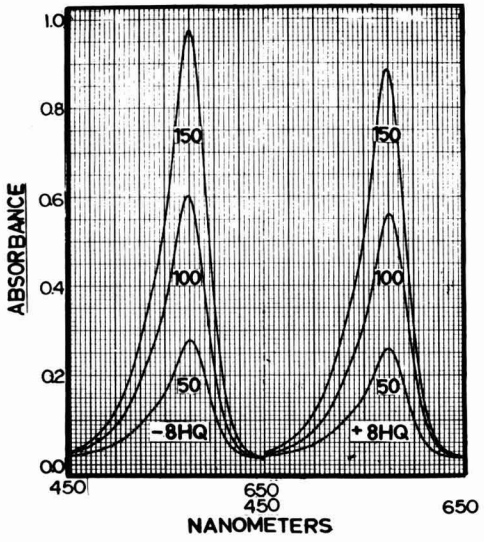

FIG. 6. Comparative effect of 8-HQ on calcium calibration standards.

8-HQ, the graphs in Fig. 6 were prepared. Calcium standards at the concentrations shown were reacted with CPC as described but in one set of standards, the 8-HQ was eliminated. The resulting differences in absorbances between the two standard sets indicated that 8-HQ has a depressant effect on the absorptivity of the reaction no doubt due to a limited but measurable binding of calcium.

It is common in chelatometry that certain interfering metals are eliminated as interferences by the addition of cyanide (*15*). It has also been suggested that cyanide may increase the stability of the diethylamine (DA) buffers used in the automated procedure for calcium (*10*). The metals present in serum in significant amounts which can form cyanide complexes are copper, iron, and zinc. When an excessive amount (10 mg/liter) of each metal was added to the 100 mg/liter of calcium standard and the spectra of the reactions obtained, the graphs of Fig. 7 resulted. It can be seen that their effect is small, considering that their normal concentrations are only one tenth of the amounts tested. Since the buffers described under procedure were also reasonably stable and this stability was not increased by cyanide, it seemed reasonable to eliminate the ion from the system altogether.

Color stability. Figure 8 illustrates the color stability over a period of 1 hr. The serial spectra indicate that the color is stable for at least the period studied. The initial spectrum scanned after 1 min shows a small elevation above the peak values of the spectra that follow. A repeat of the experiment in which a continuous scan of color formation was made showed that the fall to the plateau value was a rapid one, reaching it in about 1 min.

Reagent stability. This is an important consideration and the results of

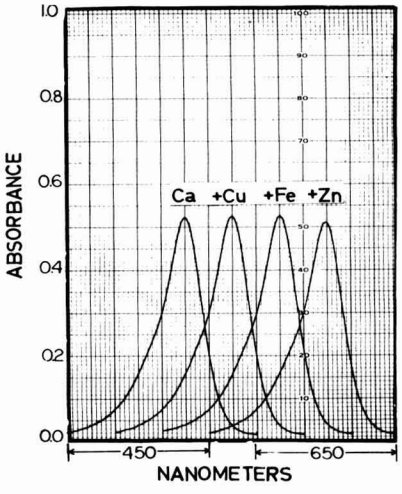

FIG. 7. Graphic effects of copper, iron, and zinc on the Ca(II)–CPC reaction.

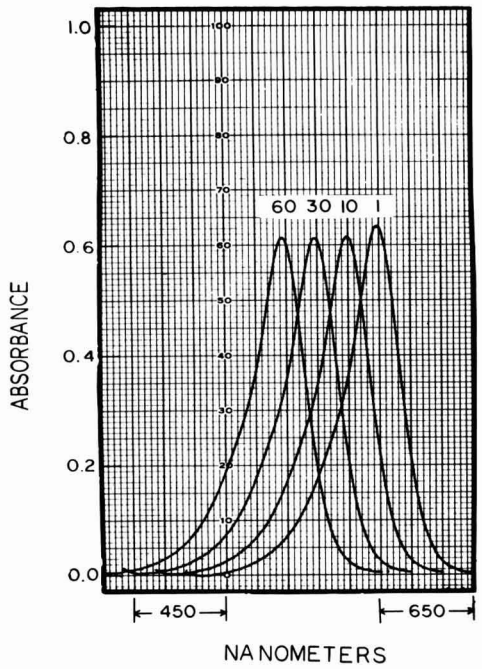

FIG. 8. Time study of the stability of the Ca(II)–CPC reaction.

such a study are shown in Fig. 9. Three concentrations of calcium standard were checked once a week for a period of 4 wk as were the pH values of the reaction solution. Each week the 8-HQ was dissolved in the buffered CPC 24 hr before using it in the study.

Recoveries. The calcium concentrations of a number of randomly selected serum specimens were measured by using EG in the solvent system in comparison to that of dimethyl sulfoxide (*19*). In a similar manner and using another batch of serums, AMP was substituted for

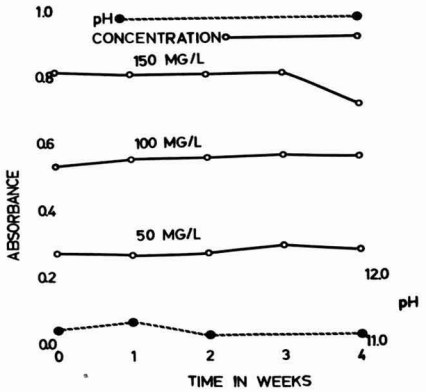

FIG. 9. Reagent stability with time is shown for the one-piece reagent system (left ordinate). The measured apparent pH with time is also shown (right ordinate).

TABLE 1
COMPARISON OF RESULTS OBTAINED WHEN EG IS SUBSTITUTED FOR DMSO AS THE SOLVENT AND WHEN AMP IS SUBSTITUTED FOR DA AS THE BASE REAGENT[a]

EG	DMSO	DA	AMP
92	91	89	90
99	96	94	88
133	139	88	86
96	99	96	97
96	98	105	109
94	95	93	90
86	88	92	93
100	93	91	93
101	97	87	84
96	95	100	99
98	98	90	89
93	93	97	90
98	98	93	92
94	94	101	98
101	102	91	89
95	96		

[a] All results are shown in milligrams per liter.

diethylamine in a dimethylsulfoxide (DMSO) procedure (*3*). The results are summarized in Table 1.

DISCUSSION

The procedure described here is similar to the previously described direct manual and automated ones (*2,3,22*). The main modifications are the single-piece reagent and the substitution of EG for DMSO, a change which eliminates a somewhat noxious odor while still maintaining the dielectric constant necessary to lower blanks, to produce linear reaction characteristics, to increase reagent solubility without the presence of an acid excess, and to maintain clarity in the presence of proteins. Studies of interferences due to lipemia, jaundice, and hemolysis were not deemed necessary since they had already been discussed previously and corrective measures for the case of turbidity due to lipemia were suggested (*3*). The amount of EG chosen was a reasonable compromise between a low blank and high sensitivity. The concentration of AMP buffer was that which resulted in an apparent pH of 11.0–11.5. Initially, a two-reagent system based on the DMSO work (*3*) was used but no difference was noted when each reagent was added separately or the two reagents were mixed in correct proportions before the sample was added. The reaction was found to be essentially complete in 1 min after mixing. It seems that there is no need to acidify the serum first in order to liberate calcium

from protein prior to the addition of the base reagent, because the formation constant of CPC with calcium is sufficiently high to allow rapid reaction even in an alkaline medium.

SUMMARY

A modification of a direct manual procedure for serum calcium has been developed in which a one-piece color reagent is used. When a micro amount of serum is added to the reagent, rapid and stable color formation occurs enabling the procedure to serve either as a manual stat system of in robotized automatic instrumentation. Total color measurement is not attained in such a procedure owing to the described regressive nature of the reagent blank. However, accurate and linear reaction characteristics are effectively achieved. The use of a low dielectric solvent, EG, served well for repressing the ionization of the blank while allowing linear and reproducible calibration characteristics.

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Continuous Titrations in Ultramicrodeterminations

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INTRODUCTION

Recently the lower horizon of quantitative elemental analysis was extended by the work of Belcher [Gr. Brit.] (*1*), Kirsten [Sweden] (*4–8*), Tolg [German F. R.] (*15*), and many others. Yet their methods are not in general use. This may be due to the required skill and experience of the analyst.

Many special problems occur in scaling down a procedure for chemical analysis. We feel that so-called ultra microanalysis will be accepted by far more analysts if a general and simple solution for all special problems can be found.

In every scaling-down procedure one has to cope with the same five problems in some form. These are: sample losses due to adsorption (minimization involves increasing surface-to-volume ratio), reagent contamination (enlarged blanks usually cause a low signal-to-noise ratio), limited reaction rates and efficiencies, caused by dilution, human failures (reproducibility in manipulations is necessary but difficult to achieve), and problems of detection of minute sample quantities.

For these five basic problems one simple solution may exist. In our laboratory we had good results with so-called continuous analysis. In a continuous system the determination of a substance is carried out by reacting to the substance's effect upon an already established situation. After appropriate sample preparation, the sample is introduced into the established system and disturbs the original situation. The action needed to restore the initial situation is a measure for the disturbance introduced and thus for the substance to be determined.

Elsewhere (*3*) we discussed the advantages of such a system for the solution of adsorption problems and the other stated problems of incom-

[1] Parts of paper presented at the International Symposium on Microchemical Techniques – 1973, The Pennsylvania State University, University Park, Pennsylvania, August 19–24, 1973.

plete or low reaction rates, purity of reagents (especially solvents), and human failures. Detection is made easier as well.

Detection in Chemical Ultramicroanalysis

In a continuous titration system the sample is detected by its effect on a preestablished stationary situation.

It is not necessary that the sample itself is detected; the detection of the changed situation is important. This makes detection easier. It is an old and well-known fact that changing phenomena are detected better (e.g., traffic lights, warning signals, etc.). Our sense organs detect very small effects better when these effects are not constant, e.g., pulsating, and so do many physical or chemical detectors. Modern theories dealing with information transport suggest that detection or perception of very small signals is possible when the signals have an alternating nature.

As stated, the use of a signal change instead of the absolute signal value itself, enables the use of very simple detectors.

Normally in micro- and ultramicro analysis, many demands are made on the detectors. The detector is expected to have a linear response over a region (e.g., obey Beer's or Nernst's law), it must be reproducible over the same broad region and its sensitivity often has to be as good as possible.

In a continuous system, however, these requirements are less rigid. As a matter of fact the only requirement for a detector is reproducible behavior in a small region. The signal of the initial situation must be within this region. Thus, the detector has to detect only whether the initial situation is restored again or not. This involves one bit of information.

In a continuous system no strict requirements are needed with respect to linearity and working range of the detector. Even no ultimate requirements are demanded of sensitivity. Using the principle of detection of signal changes we could apply relative simple detection systems with good results.

Some time ago we used a glass electrode to detect whether a chemical reaction proceeded or not (*2*). We made use of the hydronium ions involved in the reaction. At a pH of about 7 about 10^{-4} nmole of hydronium ions theoretically can be detected, or better: a chemical reaction producing or consuming about 10^{-4} nmole of hydronium ions can be followed. An example of such a reaction is:

$$H_2O + HPO_4^{2-} + La^{3+} \rightarrow LaPO_4 \downarrow + H_3O^+ \qquad (1)$$

In Fig. 1 the detection of the proceeding of the titration reaction is presented. The reaction sets hydronium ions free. The pH of the system

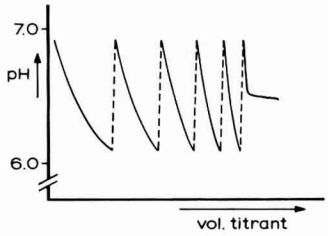

FIG. 1. Detection of the proceeding of the titration using La(III).

is lowered upon titrant (La(III)—ions) delivery. When the pH becomes too low (incomplete reaction) sodium hydroxide is added (in Fig. 1 represented by the dotted lines). After the equivalence point no hydronium ions are generated and the pH remains fairly constant.

In Fig. 2 the titration curve of this phosphate determination is given for the continuous titration system. The titration solution contains a larger extent of La(III) ions at a pH of about 5.5. When the sample of phosphate is introduced (A) the pH is decreased due to reaction 1. Immediately the delivery of the sodium hydroxide titrant starts and after a short time the initial situation is reset (B). The dotted line C represents the situation after sample introduction when the titration is not carried out. It is clear that in case C adsorption becomes important.

Using an automated system with a proportional band or by a manual titration, down to about 3 μg phosphorus in, for example, a few milligrams of organic materials can be determined (*2,14*). If better apparatus is used, the lower limits are decreased (e.g., Table 1 and lit. ref. (*16*).

A glass electrode really is a very stable detector. Recently (*10*) we were able to titrate down to about 300 ng of carbon dioxide in an aqueous titration solution having a volume of about 50–80 ml. This amount was trapped from a gas flow using the enzyme carboanhydrase. We titrated with hydroxyl ions (from a buret or coulometrically generated) to a set point pH of about 8. In this region the system's sensitivity was too large. We added a minute amount of diluted veronal buffer to "stabilize" or "damp" large pH changes. Some results are shown in Table 1.

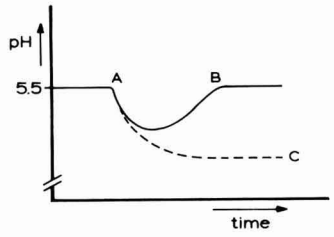

FIG. 2. Titration curve for phosphate determination in a continuous titration system.

TABLE 1

Given μg C (aq. solution of sucrose burned in oxygen)	C found (μg)	Number determinations	SD (%)
0.50	0.43	45	2.1
0.75	0.76	30	0.9
1.00	0.98	80	0.6
5.00	5.08	80	0.2
10.00	9.92	80	0.1

In a stream of carrier gas (e.g. oxygen, after the burning of an organic compound) the carbon dioxide is swept through about 80 ml of an aqueous solution of the enzyme carboanhydrase and a very dilute veronal buffer (pH = 8). The whole system is cooled to 0°C to enlarge the enzyme's lifetime. The pH disturbance caused by the carbon dioxide is eliminated by a titration with 0.01 M sodium hydroxide or coulometrically generated hydroxyl ions to the set-point pH value of 8. A determination takes 2–6 min.

In a continuous system one can use ion-selective electrodes as detectors as well. In fact, ion-selective electrodes may be used even below their lower detection limit as specified by the manufacturers. For example, we used an ORION silver sulfide electrode to determine minor sulfur quantities (*13*).

Not only are determinations of quantities below the linear detection limit possible, but also very simple, homemade electrodes may be used as well.

Some time ago Růžička and coworkers (*11,12*) described very simple electrodes, which may be manufactured in the laboratory within a few minutes. These low-cost electrodes were used in a continuous system for the chelatometric determination of a few nanograms of copper, zinc, lead, and cadmium (*9*).

The detector electrode is made by rubbing onto a graphite–Teflon surface a mixture of CuS and Ag_2S. The titration solution for the determination of zinc, or lead is a solution of the complex Cu–EDTA (0.05 *M*) buffered at pH 4.7. Upon introduction of the sample, copper ions are set free, according to reaction2.

$$\text{Cu-EDTA}^{2-} + \text{M}^{2+} \rightleftarrows \text{M-EDTA}^{2-} + \text{Cu}^{2+} \qquad (2)$$

The change in the low initial free copper concentration is detected and a titration system delivers an EDTA–solution (10^{-4} *M*). Typical results for a Zn determination are given in Table 2. A titration takes about 1–4 min.

TABLE 2

Given ng Zn	Zn found (ng)	SD (ng Zn five determinations)
16.0	17.5	1
32.0	32.5	1
64.0	64.0	1
96	96	1
128	128	1
320	317	2
512	509	2

Again it is clear from this example that linear detection behavior is unnecessary. The only requirement for the detector is good reproducibility in a small region.

SUMMARY

Problems in scaling-down procedures of chemical analysis are generally stated. Titrations are to be preferred over single measurements. Continuous titration in a titration system may help in solving the stated problems. Detection is easy and may be done by simple electrodes. Illustrations from the authors' practice are given.

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MICROCHEMICAL JOURNAL 20, 39–42 (1975)

A New Spot Test for Aliphatic Aldehydes and Ketones

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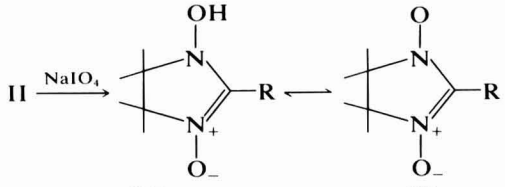
INTRODUCTION

Several aldehydes have been found to react with 2, 3-dimethyl-2, 3-bis-(hydroxylamino)-butane (**I**) to form an anhydro product (**II**) which can be converted to a stable free radical (**III**) by the addition of sodium periodate or lead dioxide (1).

I **II**



III **IV**

The stable free radicals (nitronyl nitroxides) are intensely colored; usually red or blue, depending on the solvent. This report utilizes this reaction as a spot test for some aldehydes and ketones—an adaptation to the microgram scale. The limits of sensitivity and the scope of the reaction are discussed.

MATERIALS AND METHODS

2, 3-Bis-(hydroxylamino)-2, 3-dimethyl butane sulfate[1] (V) was recrystallized from a 2-propanol–water mixture. Sodium meta-periodate[2]

[1] Eastman.
[2] J. T. Baker.

($NaIO_4$) was reagent grade and was used without further purification. All aldehydes and ketones were obtained from commercial sources and used without further purification. An aqueous solution of formaldehyde was analyzed by oxidation to formic acid and titration with standard sodium hydroxide.

In all tests, 100 μl of a solution of V (0.01 *M* in water) and 10–100 μl of an appropriately diluted stock solution (in methanol or ethanol) of the aldehyde or ketone were mixed in a spot plate depression. After 5 min at room temperature, 100 μl of $NaIO_4$ solution (0.05 *M* in water) was added to this mixture. The appearance of a pink color within 2 min which persisted for at least 1 min was taken to be a positive test. An appropriate blank was run with each test. All solvents were pretested for the presence of interfering substances by subjecting 100 μl of the solvent to the above procedure.

RESULTS AND DISCUSSION

The results of the test with a series of aldehydes is presented in Table 1. All aliphatic aldehydes tested gave a positive test while among the aromatic aldehydes, only benzaldehyde gave a positive test. The sample of benzaldehyde used was redistilled to rule out impurities. It has been reported that benzaldehyde does undergo this reaction (*1*) but the reac-

TABLE 1
SPOT TEST RESULTS WITH ALDEHYDES

Compound	Test results	Minimum detectable quantity (μg)
Formaldehyde	+	0.2
Acetaldehyde	+	0.2
n-Propionaldehyde	+	2
n-Butyraldehyde	+	1
Isobutyraldehyde	+	1
2-Ethyl-butyraldehyde	+	10
n-Valeraldehyde	+	2
3,7-Dimethyl-2,6-octadienal[a]	+	200
3,7-Dimethyl-6-octenal[b]	+	20
Phenylacetaldehyde	+	50
Benzaldehyde	+	250
p-Hydroxybenzaldehyde	−	—
p-(Dimethyl amino) benzaldehyde	−	—
5-Nitrosalicylaldehyde	−	—
trans-Cinnamaldehyde	−	—

[a] Citral.
[b] Citronellal.

TABLE 2
SPOT TEST RESULTS WITH KETONES

Compound	Test results	Minimum detectable quantity (μg)
Acetone	+	80
2-Butanone	+	2000
3-Methylbutanone	+	1000
2,6-Dimethyl-4-heptanone	−	—
Cyclopentanone	+	400
Cyclohexanone	+	400
2-Cyclohexen-1-one	+	100
Acetophenone	−	—
Benzophenone	−	—
2,4-Dibromoacetophenone	−	—
p-Nitrobenzophenone	−	—
1,2-Diphenylethandione[a]	−	—
Ninhydrin	−	—

[a] Benzil.

tion time is much greater than those for aliphatic aldehydes. This method is quite sensitive for the detection of aliphatic aldehydes.

Table 2 gives the results of this test when applied to ketones. Only the aliphatic ketones gave a positive test but the minimum detectable quantities are much larger than for the aliphatic aldehydes. 2-6-Dimethyl-4-heptanone did not give a positive test but this may possibly be due to

TABLE 3
SPOT TEST RESULT WITH MISCELLANEOUS COMPOUNDS

Compound	Test results
Acetic acid	—
Acetyl chloride[a]	—
Acetic anhydride	—
Acetonitrile	—
Acetoxime	—
Acethydrazide	—
Formamide	—
Ethyl acetate	—
Semicarbazide	—
Dimethylsulfoxide	—
Coumarin	—
Benzohydroxamic acid	—
2-Nitropropane	—
N-Methyl-*N'*-nitro-*N*-nitrosguanidine	—

[a] Gave a pink color that faded rapidly.

steric hindrance by the isobutyl groups. It is not clear if the ketones undergo the same reaction as the aldehydes. In the case of the aldehydes the nitronyl nitroxide is formed by the elimination of a proton (**II→III**) while with the ketones it would require the elimination of alkyl group.

Table 3 gives the results of applying this test to a variety of compounds with various functional groups. In these cases, 100 μl of the pure liquid or 10–20 mg of the pure solids were subjected to the test. Only acetyl chloride gave what appeared to be a positive test but the color did not persist for more than 30 sec. Several compounds gave precipitates or gas evolution but this was easily distinguishable from a true positive test.

The blank gives a yellow color that gradually fades. While this is clearly different from the positive test, it is important to run a blank with each sample in order to rule out misinterpretation of the color formation. This was found to be particularly important when analyzing very small quantities.

SUMMARY

A new spot test for aliphatic aldehydes and ketones has been developed utilizing free-radical formation. Aliphatic aldehydes are detected in the range of 0.2–200 μg while aliphatic ketones are detected from 100–2000 μg. Benzaldehyde was the only other compound tested that gave a positive result.

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Determination of Sulfite by Reaction with Mercury(I) Chloride and Spectrophotometric Measurement of Mercury(II) Complexes

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INTRODUCTION

Relatively insoluble mercury (I) compounds undergo reaction with anions which form very stable or extremely insoluble mercury(II) compounds or complexes to yield the compound or complex of mercury(II) and elemental mercury. Cyanide, hydroxide, and sulfide are examples of ions which cause this disproportionation of mercury(I) ion (*2*). Recently this type of reaction with sulfite ion was made the basis for the determination of sulfur dioxide in stack gases (*9*). The very stable mercury(II) sulfite complex, $Hg(SO_3)_2^{2-}$, is formed which is soluble in the solution. The reaction occurs as shown in the following equation.

$$Hg_2Cl_2 + 2SO_3^{2-} \rightarrow Hg(SO_3)_2^{2-} + Hg^0 + 2Cl^-$$

The mercury in solution is determined by absorption of low energy X-radiation. An instrument built for detection of sulfur dioxide based on this reaction is reported to be useful for measuring SO_2 in the range of 100–4000 ppm after 15-min reaction time (*9*).

Several methods for the spectrophotometric determination of sulfite based on the reaction of that ion with mercury(II) compounds have been reported. The decrease in absorption of the diphenylcarbazone complex of a mercury(II) nitrate solution on reaction of the mercury(II) ion with sulfite was made the basis for a colorimetric method for sulfite(*7*). An ultraviolet method was reported based on the reaction of a solution of $Hg(NO_3)_2$ in sulfuric acid to form the sulfite complex, $Hg(SO_3)_2^{2-}$, and measurement of the absorption of this species at 237 nm (*8*). The molar absorptivity, based on mercury, is approximately 25,000. Beer's law is reported to be obeyed over the range of $3.5–6.0 \times 10^{-5}$ *M* sulfite. This appears to be the only direct method for sulfite based on the measurement of mercury. Other indirect methods involving the use of mercury(II) compounds include the reaction of the iodate salt with sulfite followed by colorimetric

measurement of the starch-iodine blue species formed by reduction of the iodate with iodide (*3*) and formation of the $FeSCN^{2+}$ complex by adding iron(III) ion to a reaction solution of mercury(II) thiocyanate and sulfite (*4*).

Spectrophotometric methods for chloride have been based on the absorption of mercury complexes. In one procedure the absorption of the dithiocarbamate complex of phenyl-mercuric chloride was measured (*1*). Another method involved the reaction of chloride ion with solid mercury(II) iodate to yield $HgCl_2$ in solution followed by addition of a large excess of Br^-, Cl^-, I^-, or SCN^- to form the corresponding complex ion, HgX_4^{2-}, which absorbs strongly in the uv region (*5*). Very small amounts of mercury(II) have been determined by forming the chloride complex, $HgCl_3^-$, combining this anion with the crystal violet cation, extracting the association complex into toluene, and measuring the absorbance at 605 nm (*6*). The molar absorptivity, using benzene as the solvent, was reported to be 82,000 (*11*). Apparently this crystal violet-extraction procedure has not been applied to the determination of anions.

We have found that the $Hg(SO_3)_2^{2-}$ species produced in solution by reaction of mercury(I) chloride with sulfite will yield the corresponding complex ion on adding an excess of Br^-, Cl^-, I^-, or SCN^- to the solution. The uv absorption of the $Hg(SO_3)_2^{2-}$ ion or the appropriate HgX_4^{2-} complex can be related to the concentration of sulfite in the reaction solution. For measurement in the visible region, the $Hg(SO_3)_2^{2-}$ complex can be converted to the chloro-anion, $HgCl_3^-$, and extracted as the crystal violet adduct into benzene. The sensitivity is considerably higher using this procedure.

MATERIALS AND METHODS

Equipment

Absorption measurements were made with Beckman ACTA III and Kintrac spectrophotometers. A Lab-Line Junior Orbit shaker was used to agitate some of the solutions.

Chemicals and Solutions

Mercury(I) chloride was a J. T. Baker "Baker Analyzed" chemical. The halide ions and thiocyanate ion used were all Baker and Adamson reagents. The crystal violet was a Matheson, Coleman, and Bell product and was used as an 0.2% solution. The buffer employed was 0.025 *M* KH_2PO_4 and Na_2HPO_4 with a pH of 6.86. Sodium sulfite solutions were about 0.001 *M* and were made up in distilled water containing about 5% glycerol. Reaction solutions were prepared by dilution.

Procedure

Reaction solutions were made to a volume of 10 or 20 ml by dilution of the stock solution of sodium sulfite with either a 0.001 or 0.002 *M* KCl solution or the pH 6.86 buffer. The buffer was not used for the crystal violet work. Approximately 20–60 mg of Hg_2Cl_2 was then added and the solution shaken for 20 min. The excess solid was then removed by filtration.

For the uv work, the mercury(II) complexes were then formed by adding 0.5–1.0 g of the respective potassium salt of the halide or thiocyanate to a 5.0-ml volume of the reaction solution. The absorbance was then measured at the wavelength maximum of the HgX_4^{2-} present. In some of the work the absorption of the $Hg(SO_3)_2^{2-}$ species was measured.

For the crystal violet procedure, 8.0 ml of the sulfite reaction solution in dilute KCl were used. The pH was lowered to about 1.4 by adding 0.20 ml of 1.5 *M* HCl. The chloro-anion, $HgCl_3^-$, was then formed by addition of 0.20 ml of 2.5 *M* KCl. One milliliter of an 0.2% crystal violet solution was added and the crystal violet-$HgCl_3$ species extracted twice with 4.0-ml portions of benzene. The absorbance of the combined benzene extracts was then measured at 605 nm after 20–30 min.

RESULTS AND DISCUSSION

Absorption of Mercury Species.

The mercury-anion complexes formed, HgX_4^{2-}, where X is Br^-, Cl^-, I^-, or SCN^-, all absorb intensely in the uv region, each complex having a maximum absorption at a different wavelength. Molar absorptivity values, which range from 17,000 to 32,000, were summarized previously (*5*). These species are readily formed on adding an excess of the appropriate anion and are stable. The sulfite complex, $Hg(SO_3)_2^{2-}$, which is present in the solution after reaction of sulfite ion with Hg_2Cl_2, evidently reacts completely with the anion added. The sulfite complex also absorbs strongly in the uv and has been used for measuring sulfite by a different reaction (*8*). Since two sulfite ions are required to yield one mercury(II) complex in the reaction solution, the effective molar absorptivity values for sulfite would be lower than those for mercury. The effective molar absorptivity values are listed in Table 1. The reaction of sulfite with Hg_2Cl_2 is apparently almost complete and, in fact, the molar absorptivity values based on sulfite are higher than would be expected based on the stoichiometry of the reaction.

Although the crystal violet-trichloromercurate adduct has been used to determine low levels of mercury (*6*), apparently this species

TABLE 1
SENSITIVITY OF MERCURY COMPLEXES FOR MEASUREMENT OF SULFITE

Complex	λ (nm)	ϵ^a	SO_2 range (ppm)[b]
$HgBr_4^{2-}$	248	27,000[c]	1.0–6.0
$HgCl_4^{2-}$	230	17,000[d]	1.0–8.0
HgI_4^{2-}	322	15,000[c]	1.0–8.0
$Hg(SCN)_4^{2-}$	280	12,000[d]	1.0–10
$Hg(SO_3)_2^{2-}$	220	11,000[c]	1.0–12
CV · $HgCl_3$[e]	605	48,000[d]	0.4–3.0

[a] Corrected for blank value obtained by extrapolation. Values are based on sulfite concentration.
[b] Approximate range assuming a maximum absorbance of 2.0, 1-cm cell.
[c] Solution was a pH 6.86 buffer.
[d] The reaction solution was 0.001–0.002 *M* in KCl.
[e] Crystal violet adduct.

has not been applied to anion measurement. The effective molar absorptivity for sulfite is rather high so that the applicable concentration range is about half that of the anion complexes. The procedure is more involved since the pH must be adjusted somewhat accurately and two extractions conducted. Some gain in sensitivity could be realized by using a smaller volume of solvent for the extraction.

Sensitivity for Determination of Sulfite.

Beer's law is obeyed by all of these mercury complexes and by the crystal violet adduct. The useful concentration range for each species in terms of ppm of SO_2 is shown in Table 1. Absorbance-concentration data for the bromide and crystal violet species are presented in Table 2, and some recovery data to indicate precision is shown in Table 3. Reproducibility of the procedures seems to be acceptable

TABLE 2
BEER'S LAW DATA FOR BROMIDE AND CRYSTAL VIOLET COMPLEXES

$HgBr_4^{2-}$			CV · $HgCl_3$		
SO_2 (ppm)[a]	$A_{,248\ nm}$[b]	ϵ	SO_2 (ppm)[c]	$A_{,605\ nm}$[d]	ϵ
1.0	0.41	25,500	0.64	0.50	50,000
2.0	0.85	26,500	0.96	0.67	45,000
3.1	1.32	27,500	1.28	1.01	50,500
4.1	1.80	28,000	1.60	1.29	51,500
5.1	2.42	30,000	2.40	1.82	48,500

[a] Reaction in 6.86 buffer.
[b] Blank correction 0.05.
[c] Reaction in 0.002 *M* KCl.
[d] Blank correction 0.30.

TABLE 3
RECOVERY DATA FOR SULFITE EMPLOYING BROMIDE AND IODIDE COMPLEXES

$HgBr_4^{2-}$			HgI_4^{2-}		
Present (ppm)[a]	Found (ppm)[b]	Error (%)	Present (ppm)	Found (ppm)[c]	Error (%)
1.0	1.1	+10	2.1	1.9	−9.4
2.0	2.1	+5.0	4.2	4.3	+2.4
3.1	3.2	+3.2	6.3	6.3	0
4.1	4.0	−2.5	8.3	8.2	−1.2

[a] Calculated as parts per million SO_2.
[b] Reaction in pH 6.86 buffer, absorbance measured at 248 nm.
[c] Reaction in pH 6.86 buffer, absorbance measured at 322 nm.

above about 1.0 ppm as SO_2. Some difficulty might arise at lower levels because of the possibility of air oxidation of the SO_2 during the time allowed for reaction with Hg_2Cl_2. The highest effective molar absorptivity values for these species, $\epsilon = 48{,}000$ for the crystal violet adduct, is comparable to the values in the range of 37,000–48,000 for sulfite using the West-Gaeke method (*10*). Absorption of the $Hg(SO_3)_2^{2-}$ complex present in the reaction solution can also be used to measure sulfite although the sensitivity is not as high as that of the other species.

General Considerations of the Method.

The reaction of sulfite ion with solid Hg_2Cl_2 was conducted in distilled water, dilute KCl solutions which were 0.001–0.002 *M*, and in a pH 6.86 phosphate buffer. Results in water showed lower sensitivity and poor precision. Reproducibility and linearity were considerably better in the buffer and KCl solutions. In work involving this reaction followed by measurement of X-ray absorption, an acetate buffer was used (*9*). This would not be suitable for uv work because of the absorption of the acetate ion. There appeared to be little difference between the buffer and KCl solutions as far as reproducibility and adherence to Beer's law are concerned. There did seem to be a small, but real, improvement in sensitivity for bromide and iodide complexes when using the buffer as compared to the KCl solutions. Although the reactions of the sulfite solutions with Hg_2Cl_2 were allowed 20 min, time studies indicated that these were about 90% complete after 5 min in the buffer solutions. The solid potassium salts added were not weighed out in most instances. An alternative to adding the solid compounds would be to use a small volume of a concentrated solution of the salt as was done for determining chloride (*5*). The decrease in sensitivity due to dilution would not be significant.

The crystal violet procedure could be modified by using a different solvent for extraction and possibly smaller volumes. Other organic dye molecules such as methyl green (*12*) also could possibly be employed as these have been used for determination of mercury after forming a halide complex.

The mercury-anion complexes and the crystal violet adduct seem to be stable for several hours. The absorbance values of these solutions did not change significantly after standing for periods of 4–6 hr.

Possible Interferences

One of the possible advantages of these procedures should be selectivity as far as the reaction of anions with mercury(I) chloride is concerned. The only anions which would likely interfere would be those which can cause the disproportionation of the mercury(I) and produce a soluble mercury(II) species in solution. Bromide, chloride, and iodide ions were found to cause no interference at the millimolar level when measuring the uv absorption of the mercury-anion species, but the presence of iodide gave high results in the crystal violet work. It is evident that iodide does react as insoluble, yellow mercury(I) iodide apparently forms. Cyanide ion will interfere seriously at the millimolar level in both methods. The possibility of determining cyanide by a similar procedure is being investigated. Sulfide should not cause significant interference since the mercury(I) sulfide formed is not soluble. The presence of sulfide did lead to slightly lower results in the crystal violet procedure but was not investigated in the uv method. High results were obtained in the presence of carbonate and phosphate ions using the crystal violet method while acetate, borate, nitrate, and nitrite showed no appreciable interference.

SUMMARY

Sulfite ion was determined in the 0.4 to 12-ppm range by reaction with insoluble mercury(I) chloride to form the soluble $Hg(SO_3)_2^{2-}$ ion and elemental mercury. The uv absorption of the sulfite complex or an anion species, HgX_4^{2-}, formed on adding an excess of KBr, KCl, KI, or KSCN is measured. The mercury(II) in solution can also be determined by lowering the pH, adding KCl, and forming the crystal violet adduct of the $HgCl_3^-$ ion. This adduct is extracted into benzene and the absorbance measured at 605 nm.

ACKNOWLEDGMENT

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Methods for the Isolation and Characterization of Constituents of Natural Products XIX. Use of a Celite–Sodium Borohydride Column for Reduction of Carbonyl Compounds at the Micro Level

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INTRODUCTION

This paper describes a column technique for carrying out sodium borohydride ($NaBH_4$) reductions of some simple carbonyl compounds at the microgram and micromole level. The procedure is rapid and easy involving only the application of a solution of the carbonyl compound to a column of Celite–$NaBH_4$ and collection of the effluent which contains the corresponding alcohol. For reductions on the microscale, at least, the technique is considered superior to the classical methods of performing $NaBH_4$ reactions (*1,3*) in that fewer steps are involved, the need for finding a suitable solvent system for the reactants is circumvented, and commercially pure or easily purified nonpolar solvents are employed.

MATERIALS AND METHODS

Celite 545 (Johns Manville Co., Baltimore, MD)[2] is dried overnight at 500–600°C. The dried Celite (4 g) and 1 g of $NaBH_4$ (Matheson Scientific, East Rutherford, NJ) are ground in a 4-inch mortar until homogeneous. The powder is stored in a tightly closed receptacle and kept in a desiccator over phosphorous pentoxide when not in use. It is active for at least 1 yr under these conditions.

n-Hexane (J. T. Baker, Phillipsburg, NJ) is rendered alcohol- and car-

[1] Research conducted while Dairy Products Laboratory was located in Washington, DC.

[2] Mention of brand or firm names does not constitute an endorsement by the Department of Agriculture over others of a similar nature not mentioned.

bonyl-free by passage over a chromic acid column (*4*) and then over a sulfuric acid column (*2*). It is finally distilled from KOH pellets. Carbon tetrachloride (Baker) and dichloromethane (Baker) were sufficiently free of carbonyl and alcoholic contaminants and were used without further treatment. All solvents were kept over calcium hydride.

Disposable Pasteur pipets (145 × 7 mm o.d.) were used for columns. They were cut just below the crimp to facilitate insertion of column materials and were plugged with a small wad of glass wool. For experiments at the microgram level, columns were made from melting point capillaries, 100 mm long, 1.5–2.0 mm o.d., which were cut approximately in half.

PROCEDURES AT THE MICROMOLE LEVEL

Procedure 1. Celite-$NaBH_4$ powder (300 mg) is transferred to a Pasteur pipet and tamped into a compact column 2.5–3.0 cm in length. Tamping should be firm enough to eliminate air pockets and to prevent the column from separating in the event that sufficient hydrogen is evolved to cause separation. On the other hand, the column should not be tamped excessively tight so that inordinately slow flow rates are obtained. A flow rate of 15–20 min/0.5 ml (after the column has been completely wetted with solvent) is adequate to reduce all but one of the carbonyl compounds studied to the corresponding alcohol in fair to excellent yield. Several practice runs are recommended to gain sufficient experience for preparing columns with the desired characteristics.

The carbonyl compound dissolved in hexane is pipetted onto the column and collection of the effluent is begun. When all of the solution has drained, the wall of the column is washed with a little hexane and when this has entered the bed, one column volume of dichloromethane[3] is added.

Procedure 2. The column is prepared as in procedure 1 except that 400 mg of the Celite–$NaBH_4$ powder is used. The hexane solution of the carbonyl compound (0.5 ml) is pipetted onto the dry column and when drained the wall is washed with a minimum of hexane. After these operations there should remain some unwetted portion of the column. After 10 min the alcohols are eluted as in procedure 1.

Quantitative aspects of the reduction were followed by assaying the effluent directly for the alcohol using the procedure of Schwartz (*4*). At least a 2.5 molar excess of pyruvic acid chloride 2,6-dinitrophenylhydrazone over the theoretical amount of alcohol was employed. The derivative was checked by thin-layer partition chromatography (*6*) against the expected authentic derivative.

[3] If the expected alcohol is known to be soluble in less polar solvents, e.g., hexane, benzene, CCl_4, etc., these may be used.

Procedure at the Microgram Level

Procedure 2 was modified so that it could be run on microliter volumes containing microgram or submicrogram amounts of carbonyls. A melting point capillary is dabbed into the Celite–$NaBH_4$ powder until a column of the mixture approximately 2.5 cm in length is retained. The powder is pressed into a compact column about 1.5 cm in length by using the ends of two paper clips or other suitable tampers. Up to 10 μl of a CCl_4 solution containing 0.5–5 μg of the carbonyl is injected onto the column and the wall washed down with 2 μl of CCl_4 from a clean syringe. After 10 min the column is eluted by injecting dichloromethane into the capillary. The first approximately 8 μl (about 8 mm) of solvent emerging contains the alcohol and is withdrawn with a hypodermic syringe. Light air or nitrogen pressure can be used to force the solution through the column.

Analysis of effluent for completeness of the reduction of carbonyls to the excepted alcohols was made by gas–liquid chromatography (glc). The conditions were: column, 4 ft × ⅛ inch silanized stainless-steel packed with 7.5% ethylene glycol adipate and 2% phosphoric acid on 90–100 mesh Anakrom ABS; instrument, Hewlett-Packard 5750; detector, flame; helium flow rate, 40 psi; injection port temperature, 230°C; detector temperature, 270°C; column temperature 55°–195°C programmed at 6°C/min. The range was set at 10 and the attenuation at ×4.

Retention time of authentic alcohols was used as evidence that the expected product had been obtained. When no authentic alcohol was available, a mass spectrum was used to identify the peak. The LKB-9000 gas chromatograph-mass spectrometer was utilized.

RESULTS AND DISCUSSION

The carbonyl compounds reduced at the micromole level using procedure 1 are listed in Table 1. Essentially identical results were obtained with Procedure 2 although not all of the compounds were investigated with Procedure 2.

The glc analysis of the alcohols produced in the reduction of the carbonyls at the microgram level indicated that all of the compounds examined were reduced to the expected alcohol with no or only traces of starting compound or extraneous peaks present. The following compounds were examined by this technique: acetophenone, benzophenone, 2,2-dimethyl-3-heptanone, furfural, 2-undecanone, 5-nonanone, menthone, 2-pentadecanone, 2-methylundecanal, 3,4-dimethoxybenzaldehyde, and cyclododecanone. The efficiency of the technique is illustrated in Fig. 1 which shows the reduction of tetradecanal, cinnamaldehyde, 2-undecanone, and benzophenone to the corresponding alcohols. The polyunsaturated fatty acid ester, methyl 5,8,11,14,17-eicosapentaenoate was included to see whether double bonds were

TABLE 1
CARBONYL COMPOUNDS REDUCED ON A CELITE–SODIUM BOROHYDRIDE COLUMN

Carbonyl compound	Amount over column (μmoles)	Alcohol found	Yield (%)
Aldehydes			
Benzaldehyde	2.6	Benzyl	100
Cinnamaldehyde	1.3	Cinnamyl	101
Crotonal	1.0	Crotonyl	67
2-Ethyl-2-hexenal	1.7	2-Ethyl-2-hexen-1-ol	90
Furfural	1.4	Furfuryl	63
2,4-Hexadienal	1.0	2,4-Hexadien-1-ol	104
5-Methylfurfural	0.5	5-Methylfurfuryl	65
2-Methylundecanal	1.0	2-Methylundecan-1-ol	104
Tetradecanal	1.3	Tetradecan-1-ol	100
Ketones			
Acetone	0.6	Isopropyl	80
Acetophenone	1.5	not identified	106
Benzophenone	0.8	Benzhydrol	95
Cholestan-3-one	1.3	Cholestan-3-ol	102
Δ5-Cholesten-3-one	0.8	Cholesterol	99
Cyclododecanone	1.0	Cyclododecanol	98[a]
Cyclohexanone	5.0	Cyclohexanol	101
Menthone	1.3	Menthol	86
3-Methyl-2-heptanone	0.9	3-Methyl-2-heptanol	99
Methyl-β-oxo-eicosanoate	0.8	not identified	107
Methyl-9-oxo-stearate	1.2	Methyl-9-hydroxystearate	93
5-Nonanone	2.2	5-Nonanol	100
2,7-Octanedione	0.5	2,7-Octanediol	96[b]

[a] Cyclododecanone gives a 40% yield of alcohol at a flow rate of 17 min/0.5 ml, a 98% yield in 37 min/0.5 ml.

[b] Determined by the procedure of Schwartz *et al.* (*5*).

reduced. The chromatogram indicated that double bonds are unaffected. Butyric acid was included in the mixture to demonstrate that organic acids are extracted but not reduced by the Celite–$NaBH_4$ column. Besides butyric acid the following acids were found to be completely extracted at the micromole level from a hexane or CCl_4 solution: stearic, tridecanoic, caproic, phenylacetic, benzoic, sorbic, 1-cyclohexene-1-carboxylic, and 3-cyclohexene-1-carboxylic.

Simple methyl and ethyl esters were not reduced at all under the prescribed conditions. However, some but not all Δ- and γ-lactones were partially or completely retained by the column, presumably after ring opening, but were not reduced. In addition, it was noted that some Δ-lactones were converted to γ-lactones by passage over the column. This interesting phenomenon is being further investigated.

In the micromole procedures, *n*-hexane as solvent gave better yields

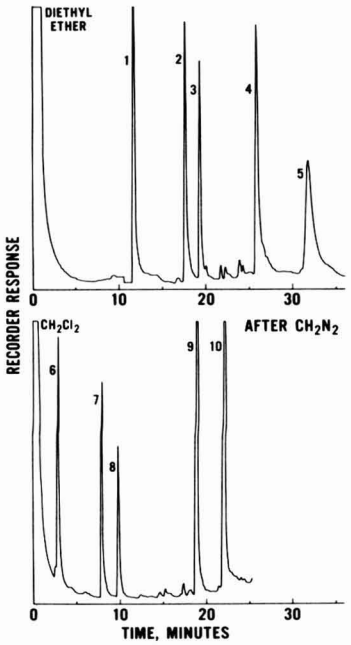

FIG. 1. Gas–liquid chromatograms of a mixture of aldehydes, ketones, an unsaturated ester, and an acid before (top) and after (bottom) contact with a micro column of Celite–$NaBH_4$.

of alcohol than did CCl_4, benzene or dichloromethane. In the microgram procedure, however, CCl_4 was substituted for hexane because of the tendency of the latter to tail and also to dirty the detector in glc.

Reductions with sodium borohyride are generally carried out in aqueous or alcoholic solution. With compounds that are poorly soluble in these solvents, dioxane–alcohol and ether–alcohol mixtures are sometimes used. Generally, regardless of the solvent used, the reduction products are isolated only after first decomposing the intermediate borates under acidic conditions. By using the column procedure, the product may be isolated directly, suggesting that no borate complex is formed under the prescribed conditions. The column procedure also offers an alternative to the use of oxygenated solvents.

SUMMARY

A Celite–sodium borohyride column has been used to reduce micromole amounts of a variety of simple carbonyl compounds to the corresponding alcohol in fair to excellent yields. The procedure is relatively rapid and easy involving only the application of a hexane solution of the carbonyl compound to the column and collection of the effluent containing the alcohol. A column constructed in a melting point capillary

suitable for the reduction of microgram amounts of carbonyls is also described.

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Auxiliary Conversion Tables for Sensitivity Performance Tests of Atomic-Absorption Instruments

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INTRODUCTION

Sensitivity values in atomic-absorption spectroscopy are frequently represented by percentual concentration limits (PCL) given in ppm of analyte, which are the ppm of analyte required to produce a signal of 1% absorption under given operating conditions. These are the values usually reported in journal articles and books when showing the sensitivity behavior of an analyte studied under specific experimental conditions.

From a practical standpoint, PLC values have several applications. One, of course, is their use in evaluating and comparing different instrumental systems. More importantly, however, PCL values can be used to estimate the signal level to be obtained for a given analyte at a given concentration. This information is helpful in evaluating the feasibility of a specific analysis and also as a guide to sample dilutions for optimal signals.

Such calculations are simple [only a few operations and perhaps the conversion of % ABSN absorption into absorbance or vice versa], but are tiring and time consuming when repeated many times.

To avoid this effort and consumption of time, conversion tables have been prepared which give signal size as a direct function of analyte concentration and PCL.

PREPARATION OF THE TABLES

Tables 1 and 2 were prepared by means of a FORTRAN program executed on a time-shared computer.

For each value of PCL and analyte concentration, absorbance was calculated from the equation:

$$A = 0.00436 \frac{C_{\text{ppm}}}{\text{PCL}_{\text{ppm}}},$$

where C_{ppm} = concentration expressed in ppm,

PCL_{ppm} = PCL expressed in ppm,

and % ABSN from:

$$\% \text{ ABSN} = 100\ (1 - 10^{-A}).$$

CONVERSION TABLES

Two tables have been prepared: Table 1 gives signals in % ABSN for given PCL values and analyte concentration in ppm; and Table 2 gives signals in absorbance units also for given PCL values and analyte concentration in ppm.

Both tables cover the ppm range between 0.001 ppm and 100 ppm in concentration steps normally used in the preparation of standard dilution series (1×10^n; 2×10^n; 5×10^n; etc.). PCL values (integer $\times 10^n$) also cover the range 0.001 ppm to 100 ppm. Values for intermediate concentration such as 0.025 ppm ($0.020 < 0.025 < 0.030$) can easily be interpolated either visually or by calculation, if necessary.

Table values are given with one to three significant figures (calculated values) for similarity with experimental values directly read from digital readouts, printers or charts.

Tables 1 and 2 can be used for absorbance or % ABSN signals obtained with any type of atomic-absorption equipment. As with any type of conversion tables, these do not serve to check instrumental performance by themselves, but are intended mainly as a means of simplifying some particular cases of numerical calculations.

The tables have been prepared with the assumption that the analytical system presents linear response. If a particular system provides linearity only at the lower concentration ranges, solutions of low concentration should be tested to obtain reference signals. However, whenever possible, signals corresponding to the best reading range (between 20–25 to 75–80% ABSN) should be chosen.

SUMMARY

Conversion tables have been prepared which give the signal (in absorbance units and percentage of absorption) corresponding to a given sensitivity level, expressed as concentration limit, when measuring the response of a solution of a given analyte concentration. These tables permit the operator a rapid check of the sensitivity performance of an instrument by comparing signals obtained under his conditions with the signal expected for a given concentration from concentration limits already published in the literature or previously obtained by himself in his laboratory with other operating conditions.

ACKNOWLEDGMENT

The author thanks Dr. R. L. Litle for the help received in the preparation of the final table presentation used in this communication.

TABLE 1

Absorbance Tables

ANALYTE CONC. PPM	PERCENTUAL CONCENTRATION LIMIT (PPM)									
	.0001	.0002	.0003	.0004	.0005	.0006	.0007	.0008	.0009	.0010
.001	.044	.022	.015	.011	.009	.007	.006	.005	.005	.004
.002	.087	.044	.029	.022	.017	.015	.012	.011	.010	.009
.005	.218	.109	.073	.055	.044	.036	.031	.027	.024	.022
.010	.436	.218	.145	.109	.087	.073	.062	.055	.048	.044
.020	.873	.436	.291	.218	.175	.145	.125	.109	.097	.087
.050	1.000	1.000	.727	.546	.436	.364	.312	.273	.242	.218
.100	1.000	1.000	1.000	1.000	.873	.727	.624	.546	.485	.436
.200	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.970	.873
.500	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
2.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
5.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
10.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
20.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
50.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
100.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

ANALYTE CONC. PPM	PERCENTUAL CONCENTRATION LIMIT (PPM)									
	.001	.002	.003	.004	.005	.006	.007	.008	.009	.010
.001	.004	.002	.001	.001	.001	.001	.001	.001	.000	.000
.002	.009	.004	.003	.002	.002	.001	.001	.001	.001	.001
.005	.022	.011	.007	.005	.004	.004	.003	.003	.002	.002
.010	.044	.022	.015	.011	.009	.007	.006	.005	.005	.004
.020	.087	.044	.029	.022	.017	.015	.012	.011	.010	.009
.050	.218	.109	.073	.055	.044	.036	.031	.027	.024	.022
.100	.436	.218	.145	.109	.087	.073	.062	.055	.048	.044
.200	.873	.436	.291	.218	.175	.145	.125	.109	.097	.087
.500	1.000	1.000	.727	.546	.436	.364	.312	.273	.242	.218
1.000	1.000	1.000	1.000	1.000	.873	.727	.624	.546	.485	.436
2.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.970	.873
5.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
10.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
20.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
50.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
100.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

ANALYTE CONC. PPM	PERCENTUAL CONCENTRATION LIMIT (PPM)									
	.010	.020	.030	.040	.050	.060	.070	.080	.090	.100
.001	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
.002	.001	.000	.000	.000	.000	.000	.000	.000	.000	.000
.005	.002	.001	.001	.001	.000	.000	.000	.000	.000	.000
.010	.004	.002	.001	.001	.001	.001	.001	.001	.000	.000
.020	.009	.004	.003	.002	.002	.001	.001	.001	.001	.001
.050	.022	.011	.007	.005	.004	.004	.003	.003	.002	.002
.100	.044	.022	.015	.011	.009	.007	.006	.005	.005	.004
.200	.087	.044	.029	.022	.017	.015	.012	.011	.010	.009
.500	.218	.109	.073	.055	.044	.036	.031	.027	.024	.022
1.000	.436	.218	.145	.109	.087	.073	.062	.055	.048	.044
2.000	.873	.436	.291	.218	.175	.145	.125	.109	.097	.087
5.000	1.000	1.000	.727	.546	.436	.364	.312	.273	.242	.218
10.000	1.000	1.000	1.000	1.000	.873	.727	.624	.546	.485	.436
20.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.970	.873
50.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
100.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

TABLE 1 (*continued*)

ANALYTE CONC. PPM	PERCENTUAL CONCENTRATION LIMIT (PPM)									
	.100	.200	.300	.400	.500	.600	.700	.800	.900	1.000
.001	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
.002	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
.005	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
.010	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
.020	.001	.000	.000	.000	.000	.000	.000	.000	.000	.000
.050	.002	.001	.001	.001	.000	.000	.000	.000	.000	.000
.100	.004	.002	.001	.001	.001	.001	.001	.001	.000	.000
.200	.009	.004	.003	.002	.002	.001	.001	.001	.001	.001
.500	.022	.011	.007	.005	.004	.004	.003	.003	.002	.002
1.000	.044	.022	.015	.011	.009	.007	.006	.005	.005	.004
2.000	.087	.044	.029	.022	.017	.015	.012	.011	.010	.009
5.000	.218	.109	.073	.055	.044	.036	.031	.027	.024	.022
10.000	.436	.218	.145	.109	.087	.073	.062	.055	.048	.044
20.000	.873	.436	.291	.218	.175	.145	.125	.109	.097	.087
50.000	1.000	1.000	.727	.546	.436	.364	.312	.273	.242	.218
100.000	1.000	1.000	1.000	1.000	.873	.727	.624	.546	.485	.436

ANALYTE CONC. PPM	PERCENTUAL CONCENTRATION LIMIT (PPM)									
	1.00	2.00	3.00	4.00	5.00	6.00	7.00	8.00	9.00	10.00
.001	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
.002	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
.005	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
.010	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
.020	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
.050	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
.100	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
.200	.001	.000	.000	.000	.000	.000	.000	.000	.000	.000
.500	.002	.001	.001	.001	.000	.000	.000	.000	.000	.000
1.000	.004	.002	.001	.001	.001	.001	.001	.001	.000	.000
2.000	.009	.004	.003	.002	.002	.001	.001	.001	.001	.001
5.000	.022	.011	.007	.005	.004	.004	.003	.003	.002	.002
10.000	.044	.022	.015	.011	.009	.007	.006	.005	.005	.004
20.000	.087	.044	.029	.022	.017	.015	.012	.011	.010	.009
50.000	.218	.109	.073	.055	.044	.036	.031	.027	.024	.022
100.000	.436	.218	.145	.109	.087	.073	.062	.055	.048	.044

ANALYTE CONC. PPM	PERCENTUAL CONCENTRATION LIMIT (PPM)									
	10.0	20.0	30.0	40.0	50.0	60.0	70.0	80.0	90.0	100.0
.001	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
.002	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
.005	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
.010	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
.020	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
.050	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
.100	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
.200	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
.500	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
2.000	.001	.000	.000	.000	.000	.000	.000	.000	.000	.000
5.000	.002	.001	.001	.001	.000	.000	.000	.000	.000	.000
10.000	.004	.002	.001	.001	.001	.001	.001	.001	.000	.000
20.000	.009	.004	.003	.002	.002	.001	.001	.001	.001	.001
50.000	.022	.011	.007	.005	.004	.004	.003	.003	.002	.002
100.000	.044	.022	.015	.011	.009	.007	.006	.005	.005	.004

TABLE 2

PERCENT ABSORPTION TABLES

ANALYTE CONC. PPM	PERCENTUAL CONCENTRATION LIMIT (PPM)									
	.0001	.0002	.0003	.0004	.0005	.0006	.0007	.0008	.0009	.0010
.001	9.6	4.9	3.3	2.5	2.0	1.7	1.4	1.2	1.1	1.0
.002	18.2	9.6	6.5	4.9	3.9	3.3	2.8	2.5	2.2	2.0
.005	39.5	22.2	15.4	11.8	9.6	8.0	6.9	6.1	5.4	4.9
.010	63.4	39.5	28.5	22.2	18.2	15.4	13.4	11.8	10.6	9.6
.020	86.6	63.4	48.8	39.5	33.1	28.5	25.0	22.2	20.0	18.2
.050	99.3	91.9	81.3	71.5	63.4	56.7	51.2	46.6	42.8	39.5
.100	100.0	99.3	96.5	91.9	86.6	81.3	76.2	71.5	67.3	63.4
.200	100.0	100.0	99.9	99.3	98.2	96.5	94.3	91.9	89.3	86.6
.500	100.0	100.0	100.0	100.0	100.0	100.0	99.9	99.8	99.6	99.3
1.000	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
2.000	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
5.000	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
10.000	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
20.000	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
50.000	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
100.000	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

ANALYTE CONC. PPM	PERCENTUAL CONCENTRATION LIMIT (PPM)									
	.001	.002	.003	.004	.005	.006	.007	.008	.009	.010
.001	1.0	.5	.3	.3	.2	.2	.1	.1	.1	.1
.002	2.0	1.0	.7	.5	.4	.3	.3	.3	.2	.2
.005	4.9	2.5	1.7	1.2	1.0	.8	.7	.6	.6	.5
.010	9.6	4.9	3.3	2.5	2.0	1.7	1.4	1.2	1.1	1.0
.020	18.2	9.6	6.5	4.9	3.9	3.3	2.8	2.5	2.2	2.0
.050	39.5	22.2	15.4	11.8	9.6	8.0	6.9	6.1	5.4	4.9
.100	63.4	39.5	28.5	22.2	18.2	15.4	13.4	11.8	10.6	9.6
.200	86.6	63.4	48.8	39.5	33.1	28.5	25.0	22.2	20.0	18.2
.500	99.3	91.9	81.3	71.5	63.4	56.7	51.2	46.6	42.8	39.5
1.000	100.0	99.3	96.5	91.9	86.6	81.3	76.2	71.5	67.3	63.4
2.000	100.0	100.0	99.9	99.3	98.2	96.5	94.3	91.9	89.3	86.6
5.000	100.0	100.0	100.0	100.0	100.0	100.0	99.9	99.8	99.6	99.3
10.000	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
20.000	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
50.000	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
100.000	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

ANALYTE CONC. PPM	PERCENTUAL CONCENTRATION LIMIT (PPM)									
	.010	.020	.030	.040	.050	.060	.070	.080	.090	.100
.001	.1	.1	.0	.0	.0	.0	.0	.0	.0	.0
.002	.2	.1	.1	.1	.0	.0	.0	.0	.0	.0
.005	.5	.3	.2	.1	.1	.1	.1	.1	.1	.1
.010	1.0	.5	.3	.3	.2	.2	.1	.1	.1	.1
.020	2.0	1.0	.7	.5	.4	.3	.3	.3	.2	.2
.050	4.9	2.5	1.7	1.2	1.0	.8	.7	.6	.6	.5
.100	9.6	4.9	3.3	2.5	2.0	1.7	1.4	1.2	1.1	1.0
.200	18.2	9.6	6.5	4.9	3.9	3.3	2.8	2.5	2.2	2.0
.500	39.5	22.2	15.4	11.8	9.6	8.0	6.9	6.1	5.4	4.9
1.000	63.4	39.5	28.5	22.2	18.2	15.4	13.4	11.8	10.6	9.6
2.000	86.6	63.4	48.8	39.5	33.1	28.5	25.0	22.2	20.0	18.2
5.000	99.3	91.9	81.3	71.5	63.4	56.7	51.2	46.6	42.8	39.5
10.000	100.0	99.3	96.5	91.9	86.6	81.3	76.2	71.5	67.3	63.4
20.000	100.0	100.0	99.9	99.3	98.2	96.5	94.3	91.9	89.3	86.6
50.000	100.0	100.0	100.0	100.0	100.0	100.0	99.9	99.8	99.6	99.3
100.000	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

TABLE 2 (*continued*)

ANALYTE CONC. PPM	PERCENTUAL CONCENTRATION LIMIT (PPM)									
	.100	.200	.300	.400	.500	.600	.700	.800	.900	1.000
.001	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
.002	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
.005	.1	.0	.0	.0	.0	.0	.0	.0	.0	.0
.010	.1	.1	.0	.0	.0	.0	.0	.0	.0	.0
.020	.2	.1	.1	.1	.0	.0	.0	.0	.0	.0
.050	.5	.3	.2	.1	.1	.1	.1	.1	.1	.1
.100	1.0	.5	.3	.3	.2	.2	.1	.1	.1	.1
.200	2.0	1.0	.7	.5	.4	.3	.3	.3	.2	.2
.500	4.9	2.5	1.7	1.2	1.0	.8	.7	.6	.6	.5
1.000	9.6	4.9	3.3	2.5	2.0	1.7	1.4	1.2	1.1	1.0
2.000	18.2	9.6	6.5	4.9	3.9	3.3	2.8	2.5	2.2	2.0
5.000	39.5	22.2	15.4	11.8	9.6	8.0	6.9	6.1	5.4	4.9
10.000	63.4	39.5	28.5	22.2	18.2	15.4	13.4	11.8	10.6	9.6
20.000	86.6	63.4	48.8	39.5	33.1	28.5	25.0	22.2	20.0	18.2
50.000	99.3	91.9	81.3	71.5	63.4	56.7	51.2	46.6	42.8	39.5
100.000	100.0	99.3	96.5	91.9	86.6	81.3	76.2	71.5	67.3	63.4

ANALYTE CONC. PPM	PERCENTUAL CONCENTRATION LIMIT (PPM)									
	1.00	2.00	3.00	4.00	5.00	6.00	7.00	8.00	9.00	10.00
.001	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
.002	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
.005	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
.010	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
.020	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
.050	.1	.0	.0	.0	.0	.0	.0	.0	.0	.0
.100	.1	.1	.0	.0	.0	.0	.0	.0	.0	.0
.200	.2	.1	.1	.1	.0	.0	.0	.0	.0	.0
.500	.5	.3	.2	.1	.1	.1	.1	.1	.1	.1
1.000	1.0	.5	.3	.3	.2	.2	.1	.1	.1	.1
2.000	2.0	1.0	.7	.5	.4	.3	.3	.3	.2	.2
5.000	4.9	2.5	1.7	1.2	1.0	.8	.7	.6	.6	.5
10.000	9.6	4.9	3.3	2.5	2.0	1.7	1.4	1.2	1.1	1.0
20.000	18.2	9.6	6.5	4.9	3.9	3.3	2.8	2.5	2.2	2.0
50.000	39.5	22.2	15.4	11.8	9.6	8.0	6.9	6.1	5.4	4.9
100.000	63.4	39.5	28.5	22.2	18.2	15.4	13.4	11.8	10.6	9.6

ANALYTE CONC. PPM	PERCENTUAL CONCENTRATION LIMIT (PPM)									
	10.0	20.0	30.0	40.0	50.0	60.0	70.0	80.0	90.0	100.0
.001	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
.002	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
.005	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
.010	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
.020	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
.050	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
.100	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
.200	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0
.500	.1	.0	.0	.0	.0	.0	.0	.0	.0	.0
1.000	.1	.1	.0	.0	.0	.0	.0	.0	.0	.0
2.000	.2	.1	.1	.1	.0	.0	.0	.0	.0	.0
5.000	.5	.3	.2	.1	.1	.1	.1	.1	.1	.1
10.000	1.0	.5	.3	.3	.2	.2	.1	.1	.1	.1
20.000	2.0	1.0	.7	.5	.4	.3	.3	.3	.2	.2
50.000	4.9	2.5	1.7	1.2	1.0	.8	.7	.5	.6	.5
100.000	9.6	4.9	3.3	2.5	2.0	1.7	1.4	1.2	1.1	1.0

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Potentiometric Microtitration of Cobalt[1]

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Our program of measuring cobalt isotope separation factors by ion-exchange chromatography generated many samples whose isotopic composition had to be determined. The number of such samples was sufficiently large so that their analysis by mass spectroscopy was prohibitively expensive. To reduce this cost, we developed an inexpensive alternate procedure in which ^{60}Co was determined by conventional γ-ray scintillation counting, and total cobalt ($^{59}Co + {}^{60}Co$) was measured by a potentiometric titration especially adapted for our samples which contained only 1–500 μg Co.

Lingane (*4*) determined that the titration of cobalt with ferricyanide in ammoniacal medium "is the best currently available method for the critical assay of cobalt materials." However, his study extended only to samples containing a minimum of 2 mg Co. Poppe and Den Boef (*6*) observed that "the determination of smaller amounts of cobalt by this method proves to be impossible, because of poor response of the Pt electrode." We substituted polarized bimetallic electrodes (anodized platinum indicating electrode and cathodized antimony reference electrode) for the platinum and calomel electrodes and observed excellent response at the end-point for the titration of microgram quantities of cobalt. Polarized platinum–antimony electrodes have been used in potentiometric titrations with good success (*3, 9*). Only empirical studies of electrode reactions have been made, (*3*) however, the phenomena on the electrode surfaces at the end-point discussed by Kekedy and Makkay (*1*) may be applicable. We also found a better end-point response when the sample was dissolved in concentrated ammoniacal ammonium chloride solution (4.5 *M* NH_4Cl in concd NH_4OH) instead of water followed by the addition of the ammine complexing solution. Concentrated NH_4Cl–NH_4OH was used to transfer the sample quantitatively to the titration vessel so that no extra water would dilute the sample.

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

MATERIALS AND METHODS

Mallinckrodt "AR" grade potassium ferricyanide was used to make the titrants (0.005–0.5 *M*). For the isotopic assay studies the absolute molarity of the titrants did not need to be known because cobalt concentration ratios were determined. It was required only that the same titrant be used for each set of samples. For the determination of the precision of the analyses, titrants were standardized against $[Co(NH_3)_5Cl]Cl_2$ which was prepared according to the procedure of Schlessinger (*7*). Reagent grade NH_4Cl and NH_4OH were used in the formation of the cobalt ammine complex for titration. Air was removed from the NH_4Cl–NH_4OH solution by bubbling NH_3 through the solution for a few minutes (*4*). Nitrogen was continuously passed over the surface of the sample during titration to prevent air oxidation.

Most of the cobalt samples titrated for isotopic assay were in the range of 50–500 μg Co in 5 ml of solution. These were titrated in a 20-ml vial. The plastic screw cap was drilled to receive the electrodes, the nitrogen flushing tube, and the buret. A magnetic stirrer was used to stir the solution. A Hewlett-Packard model 405BR automatic dc digital voltmeter was used to measure the electrode potential. The 0.25-ml microburet was an ultraprecision micrometer buret from Roger Gilmont Instruments, Inc.

Extremely small cobalt samples (1–50 μg Co) were titrated in smaller apparatus. One to two hundred microliter solutions were titrated in $\frac{1}{4}$-dram shell vials (Kimble Glass Co.) with electrodes sealed in 1-mm capillary tubing.

PROCEDURE

The Pt anode and Sb cathode were polarized by electrolyzing 5% H_2SO_4 for 2–3 min at 3 V. The electrodes were washed with distilled water. Polarizing the electrodes once a day was more than sufficient for good electrode response. The sample was transferred to the titra-

TABLE 1
ACCURACY OF THE TITRATION OF COBALT (1.5×10^{-4}–7.4×10^{-4} *M*) WITH $K_3Fe(CN)_6$ (0.25 *M*)

Co taken (μg)	Co found (μg)	Error (μg)	Error (%)
293.8	293.7, –	0.1	0.03
146.9	147.9, 146.8	0.5	0.34
117.5	116.7, 116.9	0.7	0.60
88.1	87.2, 88.9	0.1	0.11
58.8	59.5, 59.5	0.7	1.2

TABLE 2
ACCURACY OF THE TITRATION OF COBALT (3.8×10^{-5}–7.5×10^{-4} *M*) WITH $K_3Fe(CN)_6$ (0.005 *M*)

Co taken (μg)	Co found (μg)	Error (μg)	Error (%)
8.84	8.85, –	0.01	0.11
4.42	4.39, 4.41	0.02	0.45
3.54	3.38, –	0.16	4.5
2.65	2.50, 2.62	0.09	3.4
1.77	1.85, 1.78	0.05	2.8
0.88	0.85, 0.89	0.01	1.1
0.44	0.43, –	0.01	2.3

tion cell quantitatively by rinsing the sample tube four times with 1 ml of 4.5 *M* NH_4Cl in concd NH_4OH. Blanks were run for each set of samples. For the very small samples (<50 μg), the cobalt solutions were evaporated to dryness in a $\frac{1}{4}$-dram shell vial. The sample was dissolved in 50 μl of concd HNO_3 and just before titration 150 μl of concd NH_4OH was added very slowly. With nitrogen flowing, the titration was made and the end point determined by plotting $\Delta E/\Delta V$ vs V.

RESULTS AND DISCUSSION

Standardization of $K_3Fe(CN)_6$ against $[Co(NH_3)_5Cl]Cl_2$ illustrates the reproducibility of the titration: 0.05063 and 0.05065 *M*. Accuracy of the titration for the larger cobalt samples in the 20-ml vial are given in Table 1. The results for the smaller cobalt samples titrated in the $\frac{1}{4}$-dram shell vials are shown in Table 2. The results show that the ammoniacal ferricyanide method for the determination of cobalt can be extended to the microgram range with very good accuracy and precision. The use of bimetallic electrodes, antimony and platinum, has facilitated the method. We have been able to use this titration method together with γ scintillation counting to determine isotopic separation factors for cobalt isotope separation by ion exchange.

In this work the solutions analyzed contained only cobalt and ammonium salts. If cobalt must be separated from other metal ions, the solvent extraction method with 1-nitroso-2-naphthol (*5*) or the anion exchange method (*2*, *8*) are recommended.

SUMMARY

Microgram quantities of cobalt have been determined by potentiometric titration with potassium ferricyanide. The electrode response has been improved by the use of polarized Sb reference and Pt indicating electrodes. The titration method together with γ-scintillation counting has been used to determine isotopic assays of cobalt isotopes.

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An Improved Selective Determination of Mercury(II) by Complexometric Titration

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INTRODUCTION

The purpose of this work was to develop a convenient and accurate method for the complexometric determination of mercury in the presence of some cations. Mercury is not readily titrated directly with EDTA, but may be determined by back or by replacement titration (*9*). A large number of cations interefere and they must either be removed or masked (*5*). A number of analytical systems have been devised for mercury determination in the presence of one or more elements. Thus, simultaneous complexometric determination of copper and mercury has been reported (*7*) in which potassium iodide is used to decompose the mercury-EDTA chelate. In this procedure zinc, nickel, cobalt, and alkaline earth elements should be absent. Other methods include use of thiosemicarbazide (*4*) or cysteine (*1*) but copper interferes. The use of thiourea has been recommended (*6*) for the selective determination of mercury and a slightly modified procedure is used if copper, up to 12 mg, is present. 4-Chloro-2-(2-thiazolylazo)phenol was suggested (*2*) as a metallochromic indicator for the titration of mercury with EDTA, but nickel interferes. Another metallochromic indicator 5-ethylamino-2-(2-pyridylazo)-*p*-cresol was also suggested (*3*) and there was no interference only from silver, thallium, or platinum.

It was decided that the ease with which mercury-EDTA chelate is decomposed quantitatively by *N*-allylthiourea (*8*) at pH $\geq$ 9, could be the basis for a mercury determination, in the presence of a number of cations. Results of this work are given in the present paper.

EXPERIMENTAL

Reagents

Standard EDTA solution 0.01 M. This solution was standardized against the standard zinc solution using Eriochrome Black T as an indicator.

Standard zinc solution 0.01 M. An accurately weighted sample of 0.6538 g of analytically pure zinc was dissolved in 25 ml of 1:1 hydrochloric acid and diluted to 1 liter with distilled water.

Reagent solution. A 0.1 *M* solution of *N*-allylthiourea (ATU) was prepared by dissolving the requisite quantity of ATU in distilled water.

Metal ion solutions. Nitrates and chlorides of metals were used.

Indicators. Eriochrome Black T, 0.1% in absolute methanol. Zincon was prepared by dissolving 0.13 g in 20 ml of 0.1 *N* sodium hydroxide solution and diluting it to 100 ml with distilled water. Xylenol Orange, 0.1% aqueous solution. All the reagents used were Merck's G. R. grade.

Procedure

(a) *Titration at pH 8–11.* A sample containing 10–50 mg of mercury(II) is placed into a 250-ml Erlenmeyer flask, diluted to approximately 50 ml and, adjusted to pH 9–10 with NH_4OH–NH_4Cl buffer. An excess amount of EDTA is added, and the solution is back titrated with the standard zinc solution in the presence of 0.5 ml Eriochrome Black T indicator. Then the ATU solution is added, 1 ml for every 2 mg of mercury. By heating at 60°C for 5 min the mercury-EDTA chelate is decomposed, mercury sulfide is precipitated, and the equivalent metal-free EDTA is titrated again with the standard zinc solution. The formed precipitate doesn't interfere but it can be separated by filtration using a fine-porosity (G4) sintered glass (funnel) and a 250-ml suction flask. In this case precipitate is washed with 10–20 ml of hot water and the filtrate is titrated as above.

In the presence of lead the back and the final titrations were performed in the presence of zincon as indicator, while in the presence of iron(III) instead of EDTA, *N*-(2-hydroxyethyl)-ethylene diamine-*N*,*N*′,*N*′-triacetic acid (HEEDTA) was used, with Eriochrome Black T, as indicator.

(b) *Titration at pH 5–6.* If copper is present, then titration is per-

TABLE 1
DETERMINATION OF Hg AT DIFFERENT FINAL pH VALUES[a]

Sample no.	pH	Hg found (mg)	Error (mg)
1	8.1	10.23	+0.08
2	9.2	10.23	+0.08
3	9.9	10.06	−0.09
4	11.0	10.22	+0.07

[a] Mercury taken 10.15 mg.

TABLE 2
DETERMINATION OF VARIOUS QUANTITIES OF MERCURY[a]

Sample no.	Mercury		
	Added, mg	Found, mg	Error, mg
1	1.02	1.03	+0.01
2	2.03	2.01	−0.02
3	4.06	4.04	−0.02
4	10.15	10.21	+0.06
5	20.30	20.34	+0.04
6	50.75	50.951	+0.25
7	101.50	101.92	+0.42

[a] Solution's pH ~ 9.

formed at pH 5–6 with xylenol orange as indicator, hexamethylene tetraamine being used as a buffer.

At this pH range *N*-allylthiourea also decomposes selectively mercury-EDTA chelate but without formation of mercury sulfide and the metal-free EDTA is titrated by using Xylenol Orange as indicator.

RESULTS AND DISCUSSION

Samples containing known amounts of mercury were analyzed by this method at different pH values. The results are shown in Table 1.

It can be concluded that the determination of mercury can be performed at pH values from 8–11.

TABLE 3
DETERMINATION OF MERCURY[a] IN THE PRESENCE OF OTHER CATIONS

Sample no.	Cation added (mg)		Mercury found (mg)	Error (mg)
1	Mn^{2+}	0.55	4.00	−0.06
2	Ni^{2+}	0.59	4.01	−0.05
3	Co^{2+}	0.59	4.08	+0.02
4	Cd^{2+}	1.12	4.12	+0.06
5	Ag^{+}	1.07	4.02	−0.04
6	Ca^{2+}	0.40	4.03	−0.03
7	Mg^{2+}	0.24	4.02	−0.04
8[b]	Pb^{2+}	4.14	4.01	−0.05
9[c]	Fe^{3+}	0.56	4.00	−0.06
10[d]	Cu^{2+}	19.05	4.01	−0.05

[a] Mercury added 4.06 mg.
[b] Indicator Zincon.
[c] Titrated with HEEDTA.
[d] Titrated at pH 5–6, indicator Xylenol Orange.

The results of mercury determination in samples containing only mercury are given in Table 2 and the analytical results in the presence of some cations are shown in Table 3. Each result is the average of three determinations.

In the presence of copper the titration was performed at pH 5–6, without heating, and by using Xylenol Orange as an indicator. Results with a mean relative error of 1% was obtained even with a mercury to copper ratio of 1:4 by weight. By using the same conditions, i.e., at pH 5–6 all the reported determinations can be performed with the same accuracy.

When iron(III) is present, then HEEDTA solution was used for titration. At this pH value, iron-HEEDTA complex resists hydrolysis (*10*).

SUMMARY

A simple and accurate method has been developed for selective determination of mercury in alkaline solution. It involves the addition of an excess of EDTA to the mercury solution, titration of unreacted EDTA with a standard zinc solution, and then addition of *N*-allylthiourea solution at pH $\geq$ 9. By heating, the mercury–EDTA chelate is decomposed selectively, mercury sulfide is precipitated, and the EDTA freed is again titrated with standard zinc solution. Eriochrome Black T is used as indicator. Interference of some cations is discussed.

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Ultramicro Photometric Titrations Using a Long-Path Photometer

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INTRODUCTION

Recently a long-path photometer has been described (*2*) that allows photometric determinations with very small volumes required. As was established from some cursory investigations (*1*) the instrument could also serve well in photometric titrations. However, in order to assess its value as a phototitrator more closely, a series of experiments has been performed, some of which are reported here.

The instrument is especially suited for two cases. The first is one in which rather large volumes of solution to be titrated are available, but where the absorbance effects during the titration are small. In such a case the increase in path length, as can be readily deducted from Beer's law, will proportionally increase the absorbance. The special form of the long-path cell does not, of course, allow the whole solution to be contained. Instead, the titration has to be performed in an external vessel. Solution from that vessel can be transferred for absorbance measurement after each titrant addition, or else, a circulating system can be employed, pushing solution continously through the cell and thus making repeated transfers unnecessary. This would be a more routine application simply expanding the possibilities of such approaches already found in the literature (*5*). No tests in this direction have been performed.

The much more interesting application, however, is that of the second case, namely, where exceedingly small volumes of sample solution are at hand that are too small to be adequate for the cell assemblies found in commercial or user-constructed photometers or phototitrators. Of special interest are the cases where small volumes are coupled with small absorbance effects. In such a situation the long path photometer should be the remedy for both of these adverse situations. The modest volume requirement of the instrument remedies the volume situation, and the large cell length magnifies the absorbance effects.

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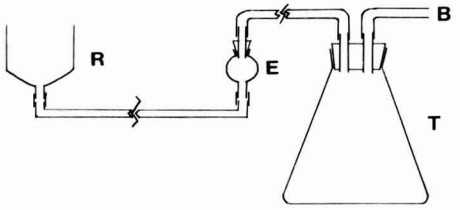

FIG. 1. Schematic diagram of titration cell assembly. See text for details.

Some method had to be found for achieving addition of titrant and mixing after each addition since the cell is not the usual open vessel type. The situation is however rather simply resolved as can be seen in Fig. 1. The titration itself is performed in the reservoir R which is selected according to size so that it fits the particular situation. The other cell end carries the overflow bulb, E, to which is attached the breathing assembly. This consists of the breathing tube, B, to which suction or pressure can be applied by mouth in order to move the sample liquid in and out of the cell proper. A trapping vessel, T, is inserted to protect the operator from accidental ingestion of corrosive or poisonous liquids. Where the composition of the breathed air is detrimental to the titration, the mouth aspiration can be replaced by use of a suction bulb or syringe. Stirring after each addition is achieved by blowing air through the solution in the reservoir via the cell; or in case carbon dioxide is detrimental, a small vibrating or rotating stirrer can be inserted. Contrary to the normal way of performing photometric titrations on the micro scale, the stirrer is no problem with regard to obstruction of the light path.

The following types of titrations were used to test the instrument: turbidimetric, redox, and complexiметric titrations. From the various cells at hand, both long path capillary cells with glass and windows (*2,3*) and "all-glass" cells (*3*) of appropriate path length were employed. For details on the features of construction and operation of the apparatus (*1,2*) and construction of the various cells, the original papers should be consulted.

EXPERIMENTAL

Chemicals

Reagent-grade metal salts were used throughout. Stock solutions. 10^{-2} *F*, were prepared using deionized water; for their standardizations well-established techniques were employed. The very dilute working solutions were prepared daily by dilution of the stock solutions.

Both the pH 6.0 acetic acid acetate buffer and the pH 9.5 ammonia chloride buffer were approximately 0.1 *F*.

Titrant solutions were prepared from reagent grade EDTA and EGTA, and were standardized with 1.57×10^{-2} F (1000 ppm) copper(II) using murexide indicator. The permanganate solution was prepared from reagent-grade potassium permanganate using distilled water, and was standardized against the ferrous stock solution used for the iron determination, after passing this stock solution through a Jones reductor.

The stock solutions of the chromogenic agents were approximately 10^{-2} F.

Deionized water was used throughout except for the redox titration of iron with permanganate, where distilled water was used.

Spectrophotometer

The original long path photometer was modified to accomodate a variety of long path cells, as previously described (*2,6*).

General Procedure

The following is a general procedure for titrations using the long path photometer:

(1) Pipet 2.00 ml (with the 40-cm capillary cell) or 3.00 ml (with the 20-cm "all glass" cell) of solution containing an appropriate amount of metal to be determined into the reservoir, R.
(2) Add buffer and chromogenic agent where appropriate and mix by gently blowing air through the solution via breathing tube B. Alternatively insert a vibrating or rotating microstirrer and activate.
(3) Set the appropriate wavelength, and then the transmittance to about 10–15% (for upscale titrations) or 90% (for downscale titrations).
(4) Add increments of titrant from a microburet and after each addition mix as described in (2). Then swish the solution several times in and out of the cell proper in order to obtain a uniform liquid in the light path.
(5) Read and record the transmittance of the solution.
(6) Repeat (4) and (5) until a few points beyond the end point have been obtained.
(7) Convert to absorbance units, plot, and evaluate data as usual.

RESULTS AND DISCUSSION

Turbidimetric Titrations

It was felt that an investigation of turbidimetric titrations of low concentrations of species to be determined might be profitable, since better titration curves are usually encountered under these conditions. Both silver and barium were titrated with standard chloride and sulfate solu-

tions, respectively. The results did not show the improvement expected from operating at very low concentrations. Instead, the increased path length served only to multiply the difficulties caused by nonreproducible precipitation conditions and variations due to changes in the vigorousness or amount of stirring. Since the results for these two well-known titrations were so erratic and only rough estimates of concentrations of sought-for species could be obtained, this type of titration was not further investigated.

Redox Titrations

In order to evaluate the feasibility of a redox titration on small samples with a low concentration of sought-for species, two different iron titrations were attempted. The first titration used ferroin as the indicator, and cerium(IV) as the titrant, a well-established technique. The red color of the ferroin was monitored at 510 nm. The results for this titration were not satisfactory at the part per million iron level, due in part to the slow reaction of the indicator. An additional problem encountered was the size of the indicator blank in relation to the volume of titrant needed to titrate the iron. For these reasons, this approach was not further pursued, and instead the self-indicating titration of iron(II) with permanganate was investigated, with excellent results at the part per million level and below. As previously discussed, a self-indicating titration offers the advantage of simplicity. Because of the relatively high absorptivity of the permanganate titrant, very small amounts of excess titrant can be easily detected, especially with the long path cell. When adequate precautions are taken to exclude oxidizable or reducible impurities from the titration system, there is no detectable indicator blank in the titration when performed as described. The only problem encoun-

TABLE 1
SELF-INDICATING TITRATION OF Fe(II) WITH PERMANGANATE.
TOTAL SAMPLE VOLUME 3 ML

μg Iron		
Taken	Found	Difference
5.8	5.7	+0.1
5.8	5.8	0.0
5.8	5.6	−0.2
2.9	2.8	−0.1
2.9	3.0	+0.1
2.9	2.9	0.0
1.4	1.3	−0.2
1.4	1.5	+0.1

tered was the air oxidation of iron(II), which was easily overcome by titrating the sample immediately after exiting from the reductor.

As a general practice in all the titrations the titrant was from 10 to 100 times as concentrated as the solution being titrated, making correction for dilution unnecessary.

Several iron "unknown" samples were taken through the titration procedure, and the results are given in Table 1.

As can be seen, the error is at most 0.2 μg of iron, corresponding to about 0.2 μl of 10^{-2} N permanaganate. These results seem quite good, especially when considering that the concentration in the two last titrations was not more than 0.45 ppm.

Chelometric Titrations

Complexation reactions are very readily applied to photometric titrations because there is usually no need to consider an indicator blank and the conditions for the titration are easily established and maintained. A great number of standard procedures have been developed with the conditions for titration (pH, masking, choice of indicator, range of applicability) specified. Since such a wealth of information is readily available, and since a wide variety of chelometric titrations have been performed in this laboratory, most of the test titrations were chelometric ones.

Studies by Flaschka and Sawyer (*4*) have shown that photometric titrations are especially advantageous with a self-indicating system created by the addition of an excess of chromogenic agent. In most of the examples presented, an excess of the appropriate agent was added, and the small change in absorbance was followed that occurred when during the titration the metal was removed from the metal complex formed.

An example of such a system is the titration of zinc with EGTA in the presence of excess Zincon. The spectral curves of the free Zincon and the zinc–Zincon complex are shown in Fig. 2. The titration was performed at two wavelengths, 560 nm and 620 nm. Actual titration curves

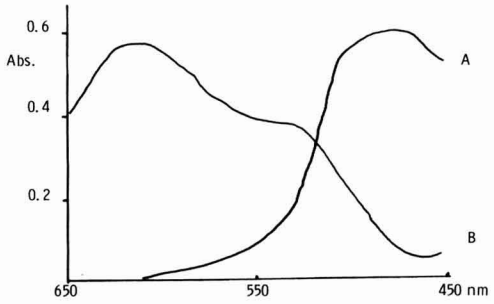

FIG. 2. Absorbance curves of Zincon (A) and Zn–Zincon (B). Approximate concentrations: 10^{-3} F.

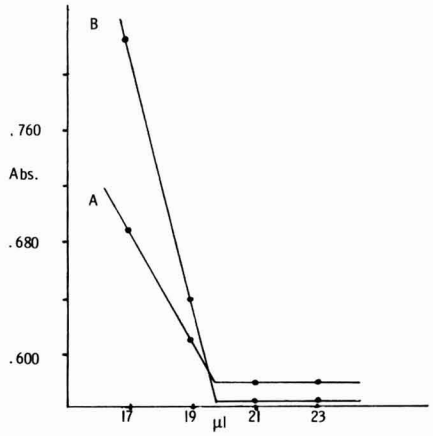

FIG. 3. Curves for the titration of zinc with EDTA in the presence of excess Zincon. A, at 560 nm. B, at 620 nm.

are shown in Fig. 3. The difference in the slope of the descending branch is due to the difference in absorptivity at the two wavelengths. The results for this titration are shown in Table 2. These data show that it is possible to successfully perform a photometric titration at a wavelength at which the absorbance curves of free and metalized forms of the chromogenic agent is quite moderate. In fact, in some cases where large absorbance values are encountered, it may be advantageous to operate at a wavelength at which the difference in absorbance of free and metalized agent is small and not maximum as usually intended.

Another example is the titration of copper(II) with EDTA in the presence of SNAZOXS. The curves for the free and metalized forms of the agent are shown in Fig. 4. The titration was followed at 550 nm, where the free indicator absorbs strongly. The amounts of copper titrated are very small, illustrating the potential of the long path photometric method for submicrogram amounts of material to be determined. Known amounts of copper were titrated to evaluate the method. The results are

TABLE 2
EGTA TITRATION OF Zn IN THE PRESENCE OF EXCESS ZINCON.
TOTAL SAMPLE VOLUME 3 ML

	μg Zinc		
Wavelength, nm	Taken	Found	Difference
560	13.1	13.3	+0.2
560	13.1	13.2	+0.1
560	13.1	13.1	0.0
620	13.1	13.2	+0.1
620	13.1	13.2	+0.1
620	13.1	13.0	−0.1

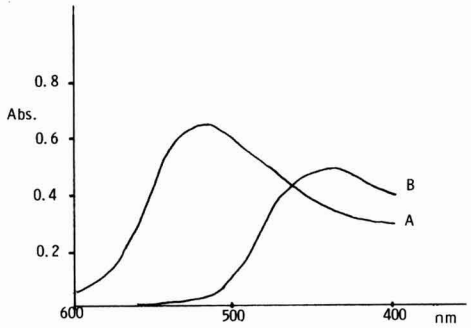

FIG. 4. Absorbance curves of SNAZOXS (A) and Cu-SNAZOXS (B).

given in Table 3. These results are very good for the level of concentration involved (9.5–13 ppb).

To illustrate the usefulness of the "all-glass" cell, a number of titrations were run with this type of cell. With only a small increase in the amount of liquid required to fill the cell (an additional milliliter is needed), virtually the same results can be obtained with the added benefit of the cell being resistant to strongly oxidizing reagents.

Calcium was titrated with EDTA using arsenazo(III) as the chromogenic agent using a 20-cm all-glass cell with a total volume of 3 ml. This system was chosen to examine the results of a titration where a significant amount of the material to be determined is present in water and reagents, i.e., where the blank is high and a blank titration is needed. Results of the titration of an "unknown" (0.13 ppm Ca) are given in Table 4. Good results are obtained also here, although the blank of 0.096 μg of calcium required about 25% of the total volume of 5.05×10^{-4} F EDTA consumed (4.8 μl for blank and 20.0 μl for the titration).

TABLE 3
EDTA TITRATION OF Cu IN THE PRESENCE OF EXCESS ZNAZOXS.
TOTAL SAMPLE VOLUME 2 ML

μg Copper		
Taken	Found	Difference
0.063	0.060	−0.003
0.063	0.063	0.000
0.063	0.062	−0.001
0.063	0.064	+0.001
0.038	0.037	−0.001
0.038	0.038	0.000
0.019	0.019	0.000
0.019	0.020	+0.001

TABLE 4
EDTA TITRATION OF Ca IN THE PRESENCE OF EXCESS ARSENAZO III.
TOTAL VOLUME 3 ML

μg Calcium		
Taken	Found	Difference
0 (blank)	0.09	+0.09
0.40	0.41	+0.01
0.40	0.42	+0.02
0.40	0.41	+0.01
0.40	0.39	−0.01
0.40	0.40	0.00
0.40	0.38	−0.02

Summary

The long-path photometer previously described was adapted for photometric microtitrations. The instrument was tested employing several types of titrations. Turbidimetric titrations of silver with chloride and of barium with sulfate did not yield good results. However, the following titrations gave excellent results down to the levels indicated in parentheses: iron(II) with permanganate (0.4 ppm), zinc with EDTA in the presence of excess Zincon (6 ppm), copper with EDTA in the presence of excess SNAZOXS (9 ppb !), and calcium with EDTA in the presence of excess arsenazo III (100 ppb). The last of these titrations gave good results although a blank of about 25% of the actual volume of titrant required was encountered.

ACKNOWLEDGMENTS

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Data on the Determination of Calcium and Phosphate in the Presence of Citric Acid

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INTRODUCTION

The determination of calcium and phosphate in citrate extracts is important because the fertilizer value of natural raw phosphates depend on their solubility in weak acids. Generally one uses 2% citric acid.

The natural raw phosphates contain for example $Ca_5(PO_4)_3OH$ (hydroxy apatit) and $Ca_3(PO_4)_2$ (phosphorit) which are soluble, or partly soluble in 2% citric acid, but the $Ca_5(PO_4)_3F$ is insoluble.

Hahn and Meyer (*3*) first described the precipitation titration of phosphate ions with magnesium sulfate solutions. This precipitation titration method combined with indirect complexometric calcium determination was very suitable for the assaying calcium in tablet form and in fertilizers (*2, 4–9*).

The principle of this method is as follows: EDTA, triethanolamine, and ammonia are added to a weakly acid solution containing calcium, phosphate, ferric, aluminium, and fluoride ions. Ferric and aluminium ions are complexed in this alkaline system, and calcium ions are masked by EDTA. Since methylthymol blue indicator functions in the presence of the triethanolamine–ferric complex, the excess EDTA may be titrated with standard magnesium chloride to the blue endpoint of this indicator. The calcium content can be calculated from this titration.

The resultant blue solution is then decolorized with pH 10 ammonia–ammonium chloride buffer and ethanol. Then titrated with standard magnesium chloride to the reappearance of the blue endpoint. Magnesium ammonium phosphate hexahydrate precipitates during this titration which measures the phosphate content.

This method is rapid and precise, but it can not be applied for the determination of calcium and phosphate of citrate extracts of the natural calcium phosphate raw materials (*2, 4*).

The authors have found that the above-mentioned complexometric–precipitation titration method of the calcium and phosphate is applicable to assay the citrate extracts of natural raw phosphates after the citric acid is oxidized by sodium hypochlorite.

It is well-known that citric acid reacts with hypohalogenite (*1*). Sodium hypochlorite and citric acid reacts according to the following equation:

$$C_6H_8O_7 + 4NaOCl + 4NaOH = CH_3—COONa + CHCl_3 + 3Na_2CO_3 + NaCl + 4H_2O$$

The excess of the sodium hypochlorite can be reduced by urea or ammonia, and subsequent complexometric–precipitation titration was utilized for the determination of calcium and phosphate (*8,9*).

The aforementioned method was utilized for the macro and micro determination of calcium in the presence of a relatively large amount of phosphate and iron. The authors assayed solutions which contained 6–30 mg of calcium, 50–150 mg of phosphate, and 5–10 mg of iron. The results of these determinations are shown in Table 1.

EXPERIMENTAL

Reagents

Ammonia, concentrated (sp.gr. 0.910)
Ammonia, diluted: 400 ml ammonia concentrated was diluted to 1000 ml
NaOH solution, concentrated
Hydrochloric acid, concentrated
Buffer pH 10; 80 g ammonium chloride and 200 ml of concentrated ammonia were dissolved in water and diluted to 1000 ml
EDTA, 0.05–0.1 *M*
Magnesium chloride, 0.05–0.1 *M*
Triethanolamine, 20%
Sodium hypochlorite solution, 1.5–2 *N*
Methylthymol Blue indicator, 1 : 100 in sodium chloride.

PROCEDURE

Add dropwise conc. sodium hydroxyde to 20–30 ml of a sample containing 400–500 mg of citric acid, 6–30 mg of calcium, 50–150 mg of phosphate, and 5–10 mg of iron, until permanent precipitate forms. Shake the mixture well. Add 15–20 ml 1.5–2 *N* of sodium hypochlorite solution and boil the mixture for 10 min. Cool the solution and add to the mixture 10 ml of diluted ammonia, then boil the mixture

TABLE 1

DETERMINATION OF CALCIUM AND PHOSPHATE IN THE PRESENCE OF IRON AND CITRIC ACID

		Calcium				Phosphate			
Present	No.	Found (mg)	Average (mg)	Difference (mg)	Standard deviation	Found (mg)	Average (mg)	Difference (mg)	Standard deviation
19.2 mg Ca 137.3 mg phosphate and 5.5 mg Fe	1	19.194		0.091		135.962		1.299	
	2	18.473		0.630		138.017		0.755	
	3	18.761		0.342		135.620		1.642	
	4	19.050		0.053		136.305		0.957	
	5	19.627		0.524		139.584		2.322	
	6	19.050		0.053		136.990		0.272	
	7	19.627		0.524		136.305		0.957	
	8	19.050		0.053		139.584		2.322	
	9					136.990			
			19.103		0.37		137.262		1.40
9.8 mg Ca 137.3 mg phosphate 5.5 mg Fe	1	9.758		0.067		136.843		0.859	
	2	10.062		0.371		138.330		0.628	
	3	9.758		0.067		136.843		0.859	
	4	9.758		0.067		137.586		0.115	
	5	9.453		0.236		138.330		0.628	
	6	9.453		0.236		139.074		1.371	
	7	9.453		0.236		137.884		0.181	
	8	9.758		0.067		136.843		0.859	
	9	9.758		0.067		137.586		0.115	
			9.690		0.19		137.702		0.74

6.65 mg	1	6.412		0.203		133.375	1.522	
Ca	2	6.716		0.101		134.096	0.801	
135 mg	3	6.412		0.203		133.375	1.522	
phosphate	4	6.716		0.101		135.538	0.640	
11.0 mg Fe	5	6.412		0.203		135.538	0.640	
	6	7.020		0.405		134.817	0.080	
	7	6.716		0.101		135.538	0.640	
	8	6.716		0.101		135.538	0.640	
	9	6.412		0.203		136.258	1.361	
			6.615		0.20		134.897	0.99
30.95 mg	1	30.757		0.034		54.792	0.881	
Ca	2	30.757		0.034		56.234	0.560	
55.80 mg phosphate	3	31.366		0.574		57.675	2.002	
11.0 mg	4	30.757		0.034		56.234	0.560	
Fe	5	30.757		0.034		55.513	0.180	
	6	30.757		0.034		55.513	0.180	
	7	30.453		0.338		54.792	0.881	
	8	30.757		0.034		54.792	0.881	
	9	30.757		0.034		55.513	0.180	
			30.791		0.22		55.673	0.78

again for 10 min. Cool, and dissolve the precipitate by the addition of a few drops of concd. hydrochloric acid. Add 5 ml of triethanol-amine (5 ml of triethanol-amine solution 20%, for each 10 mg of iron) and 10–15 ml of 0.05–0.1 *M* EDTA. Then, make alkaline the mixture with 15–20 ml of concd. ammonia solution.

The brown-colored solution becomes practically colorless after a few minutes. Add some cg methylthymol blue indicator and slowly titrate the pale-gray solution to blue by using 0.05–0.1 *M* magnesium chloride. The excess of EDTA is thus titrated. Calculate the calcium content from this titration.

Add 5 ml of buffer of pH 10 and 15–30 ml of ethanol to the blue solution which turns practically colorless. Titrate this solution with magnesium chloride to a blue end point. Magnesium ammonium phosphate hexahydrate is formed during the titration.

It is very advantageous to add about one-half of the calculated amount of magnesium chloride to the sample. This produces a strong blue color. After a minute of swirling, the blue fades and the precipitate forms in large crystals which settle easily. Then the titration is carried out as usual. The blue endpoint is not obscured by the quickly settling crystals.

Calculate the phosphate content from this titration.

SUMMARY

The determination of calcium and phosphate is possible in the citrate extracts of the natural raw phosphates with complexometric–precipitate titration as usual, after oxidation of citric acid with sodium hypochlorite.

The excess of sodium hypochlorite can be reduced by ammonia.

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Rapid Potentiometric Method for the Analysis of Mica, Talc, Feldspar, Nepheline Syenite, and Clay

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INTRODUCTION

Potentiometric titration of unconsumed EDTA (*4*) is applied to the analysis of mica, talc, feldspar, nepheline syenite, and clay. Nestoridis (*6*) suggested two rapid visual complexometric methods for determination of aluminium and titanium in common ores and silicates. Gillard (*1*) determined calcium and magnesium in raw materials potentiometrically using 1,2-bis-(2-aminoethoxy)-ethane-*N,N,N,N*-tetraacetic acid as a complexing agent. Naidu (*5*) described a complexometric method for determination of Fe, Al, Ca, and Mg in rocks, ores, minerals, cements, and water. Gladysheva (*2*) determined Al in cryolite, or aluminium fluoride and Grosskreutz (*3*) determined Al and Mg in spinels complexometrically. Khalifa and Ismail (*4*) suggested two simple and rapid potentiometric methods for Egyptian ilmenite.

EXPERIMENTAL METHODS

The water used was always deionized. The chemicals were all of the highest purity available.

Apparatus. The electrodes, potentiometer, and pH-meter used are essentially the same as those described before (*4*).

Solutions. All solutions were prepared and standardized by recommended procedures.

Procedures. In order to investigate the optimum conditions of analysis, synthetic mixtures containing Fe(III), Al, Ti(IV), Ca and Mg, having more or less the same ratio as in mica, talc, feldspar, nepheline syenite and clay were analyzed. Traces of titanium were determined in a separate mixture by the hydrogen peroxide colorimetric method.

1. *Analysis of Identical Mixtures to Mica and Nepheline Syenite*

(A) With Al + Fe + Ca, add to the mixture 10 ml 2% sodium borate, 5 ml 0.05 *M* EDTA, boil for 2 min to ensure complete

complexation of Al. Cool, buffer with 8 ml 10% urotropine, add 0.5 ml 0.05 *M* Hg(II), water to ~40 ml and back titrate at pH 6.5–6.8 with Bi(III) to obtain EDTA ≡ Fe + Al.

(B) Add 1.5 ml 10% NH_4F, leave for 30 min while stirring to ensure complete release of EDTA ≡ Al, as shown by return of potential to initial one and continue titration with Bi(III) to obtain EDTA ≡ Al. Find out EDTA ≡ Fe by difference.

(C) In a second identical mixture mask Al + Fe by (TEA) and titrate Ca with 0.01 *M* EDTA using calcein at pH 12.

2. *Analysis of Identical Mixture to Talc*

(A) With Al + Fe + Mg, proceed as under 1 (A) and 1 (B) to determine EDTA ≡ Al + Fe and EDTA ≡ Al, respectively, find out EDTA ≡ Fe by difference.

(B) In a second identical mixture precipitate Fe + Al as hydroxides in presence of a speck of NH_4NO_3 to keep Mg in solution and to prevent its precipitation as hydroxide, using dilute solution of NH_4OH, boil for 20 min to render $Al(OH)_3$ insoluble in dilute acids, alkalies, and EDTA, cool, adjust the pH to 9.5 to prevent the action of EDTA on $Fe(OH)_3$, add 5 ml 0.05 *M* EDTA, urotropine, and back titrate with Hg(II) to obtain EDTA ≡ Mg.

3. *Analysis of Identical Mixture to Feldspar*

With Al + Fe + Ca, proceed as under (1A) and (1C) to obtain EDTA = Fe + Al and EDTA = Ca. In a separate identical mixture, determine iron colorimetrically by the thiocyanate method (match the color with a standard ferric perchlorate solution containing sodium borate).

4. *Analysis of Identical Mixture to Clay*

With Al + Fe + Ti + Ca + Mg, follow (1A)—in the presence of a few drops of 1 : 1 lactic acid which is a specific masking agent for Ti (*8*), (1B), (2B), and (1C) to obtain EDTA = Fe + Al, Al, Ca + Mg, and Ca, respectively; find out EDTA = Fe and EDTA = Mg by difference. Alternatively in a separate identical mixture determine Mg alone potentiometrically in the filtrate—after separation of R_2O_3 as hydroxides, calcium as tungstate (using 5 ml 1% sodium tungstate)—at pH 11 by back titration of unconsumed EDTA with Hg(II) without interference from tungstate.

Analysis of Mica, Nepheline Syenite, Talc, Feldspar, and Clay

Fuse 0.5 g of the pulverized sample with 4 g anhydrous sodium borate, dissolve the melt into 50 ml (1 : 1) HCl, evaporate the re-

sulting solution to dryness, bake for 1 hr at 110°C to fix silica, cool, add 30 ml 1 : 1 HCl, digest for 10 min to dissolve the chlorides of Fe, Al, Ti, Ca, and Mg, filter through No. 40 Whatman filter paper. Wash silica (1) with 1% hot HCl, then with water till chloride-free, add to the filtrate and washings from silica (1) 1 ml HNO_3, 30 ml 70% $HClO_4$, evaporate to white fumes of $HClO_4$, reflux for 10–20 min to ensure complete conversion of chlorides to perchlorates. Then add 150 ml H_2O, boil, filter, wash silica (2) with 1% H_2SO_4 then with hot water, ignite (1) and (2), volatilize silica as $SiF_4(HF–H_2SO_4)$, find its weight by difference, fuse the occluded impurities with solid $KHSO_4$, dissolve the melt in the silica-free solution and make it with water up to 500 ml. Take aliquots of 20 ml and proceed as under (1), (2), (3), and (4) using 5 ml 0.05 *M* EDTA to obtain EDTA ≡ Fe, Al, Ti, Ca, and Mg. Find out the percentages of each component.

RESULTS AND DISCUSSION

Table 1 lists the results of analysis of various types of synthetic mixtures, which show that the suggested procedures are rapid, accurate, and reliable. The end point potential breaks, in majority of cases, are quite sharp and large in magnitude. With EDTA ≡ Fe + Al, Al, Ca, Ca + Mg, or Mg the potential jumps amounted to the average of 140, 90, 80, 63, or 48 mV/0.1 ml titrant, respectively. Table 2 lists the percentages of various constituents of a group of natural silicates as analyzed by the present and by the classical methods which are quite concordant.

In the course of analysis of the above-cited natural products the following comments are taken into account: 1) Reduction of the size of mica to an impalpable powder by ordinary grinding methods is a matter of difficulty; however, following the method cited in (7) elim-

TABLE 1
ANALYSIS OF SYNTHETIC MIXTURES

	Fe (mg)		Al (mg)		Ti (mg)		Ca (mg)		Mg (mg)	
	Taken	Found	Taken	Found	Taken	Found	Taken	Found	Taken	Found
1[a]	1.080	1.085	3.246	3.230	–	–	0.079	0.081	–	–
2	1.512	1.521	2.002	1.989	–	–	0.513	0.510	–	–
3	0.648	0.651	0.271	0.267	–	–	–	–	3.480	3.504
4	0.022	0.022	2.164	2.170	–	–	0.237	0.239	–	–
5	0.864	0.868	2.597	2.582	0.182	0.182	0.276	0.272	0.145	0.145

[a] 1 ± identical to mica; 2 ± identical to nepheline syenite; 3 ± identical to talc; 4 ± identical to feldspar; 5 ± identical to clay.

TABLE 2
ANALYSIS OF (I) MICA, (II) NEPHELINE SYENITE, (III) TALC, (IV) FELDSPAR, AND (V) CLAY

	Fe_2O_3 (%)	Al_2O_3 (%)	TiO_2 (%)	CaO (%)	MgO (%)
I[a]	5.7	33.1	–	0.5	–
I[b]	5.6	33.3	–	0.51	–
II[a]	9.1	18.7	–	3.6	–
II[b]	9	18.8	–	3.54	–
III[a]	4.7	2.3	–	–	30.2
III[b]	4.6	2.26	–	–	30.4
IV[a]	0.17	20.4	–	0.36	–
IV[b]	0.17	20.5	–	0.35	–
V[a]	6.3	24	1.8	2	1.1
V[b]	6.2	24.2	1.8	1.98	1.1

[a] By classical method.
[b] By the present method.

inates this problem. 2) Sodium borate used as a flux should be low in water ($Na_2B_4O_7 \cdot 5H_2O$) or preferably anhydrous to avoid frothing. 3) Several washings of silica occluded with boron do not lead to complete removal of boron, hence the need for its volatilization as methyl borate for very careful determination of SiO_2. 4) Applying Hg(II) as back titrant causes fading of potential in the vicinity of the end point which is attributed to the fair stability of Al-EDTA complex as shown by its log K value of 16.13. This phenomenom is remarkable in presence of earth alkalies or lactic acid. This is overcome by using $Bi(NO_3)_3$ as back titrant which leads to good potential breaks. With this back titrant it is recommended to add little Hg(II) to the solution prior to back titration. 5) Masking of Al in presence of Fe is more effective when NH_4F is added before the complexing agent as reversing of this order causes incomplete release of EDTA ≡ Al leading to an error (average 1.5%) with respect to Fe. However leaving the solution of the last succession for 30 minutes while stirring decreases to a reasonable extent this error (average 0.7% depending on the amount of Al present). Moreover this order is less time and reagent consuming, as it involves simultaneous determination of two components. 6) Iron and titanium hydroxides are insoluble in excess alkali or EDTA but that of Al forms aluminate which interferes in no small measure during estimation of alkaline earths at pH 11. However making the solution slightly alkaline with ammonia and boiling the aluminium hydroxide containing solution, renders aluminium completely inert towards acids, alkalies and EDTA, so that the A.E.M. can be titrated without the interference from Al.

SUMMARY

A simple rapid and reliable potentiometric method is given for the analysis of a group of Egyptian ores having the similarity of being insoluble silicates which contain more or less the same metallic components but with different ratios, by application of the method of back titration of unconsumed EDTA in hexamine-buffered media using Hg(II) and Bi(III) as titrants and silver amalgam as indicator electrode.

ACKNOWLEDGMENT

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Design and Construction of a Fluorotitrator[1]

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One can visually detect the end point in a fluorometric titration with relative ease when working in a darkened room or looking into a "titration box" (*15*). This holds for titrations involving a fluorescent indicator and for slope titrations [for definition see (*6*)], provided the decrease in fluorescence intensity terminates in complete disappearance of fluorescence at the end point. Thus, the situation is analogous to that in "color titrations," and, as with these, the application of an instrumental end point will greatly increase the range of titrations. An instrumental fluorescent end point can be obtained simply by performing the titration in any vessel and transferring a portion of the solution to a fluorometer after each addition of titrant.

It is more convenient to modify a fluorometer so that it facilitates fluorometric titrations, but even with extensive modifications, restrictions will remain and full practicality can hardly be achieved. The ideal solution, of course, is to design and construct an instrument specifically for fluorometric titrations. For the instrument to be fully analogous to a phototitrator, it must be stable and sensitive, accommodate vessels of a wide variety of shapes and sizes, and, most of all, operate with the titration vessel in the open, that is, uninfluenced by ambient light. Such an instrument would, of course, also permit fluorometric determinations and, with the added benefit of accommodating frozen samples, would be considerably more useful than conventional instruments.

Instruments of varying degrees of complexity have been described (*2, 3, 9, 10, 13, 14*). Two others are commercially available, one from Fiske Associates[3] (*1*), the other from Corning Instruments (*4*). Both are specifically designed to monitor the complexometric titration of calcium, with calcein used as indicator. None of these instruments operates in the

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[3] Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U. S. Department of Health, Education, and Welfare.

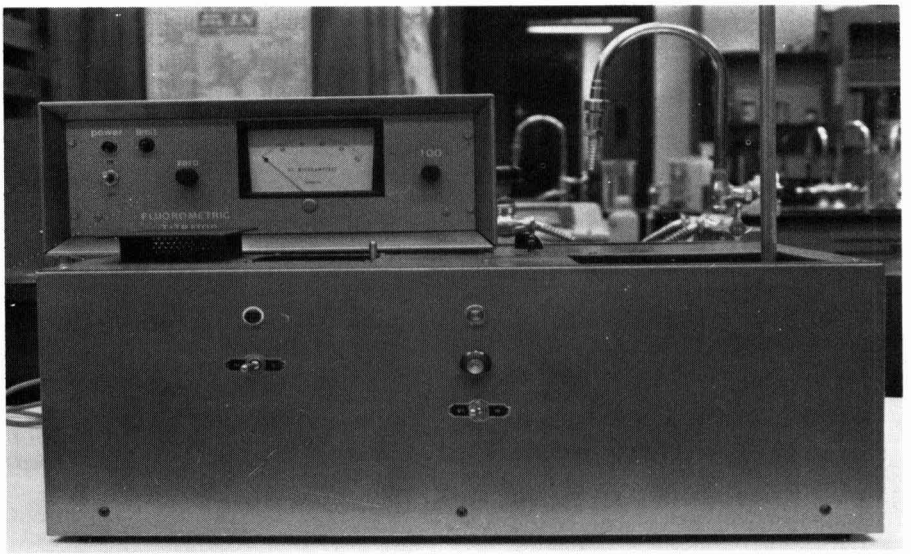

FIG. 1. The fluorotitrator.

open without interference by ambient light. The design and construction of an instrument that does operate without such interference is reported here.

Initially, it was hoped that the principle of geometric exclusion of the ambient light would work as well in this instrument as it does in phototitrators (*5, 7, 8, 11*). Unfortunately, this was not so because the fluorescent light emerges from a large area (or volume) and thus cannot be focused or collimated. Therefore, a simple design was tested, operating on the double-beam principle (sample and reference beam) with a light chopper. This system worked relatively well when the ambient light was constant, but most laboratory light is pulsed at 60 or 120 cycles, and under these circumstances the simple arrangement became inoperative. Many approaches were then tested until the instrument described here was developed. During the design and construction, these points were kept in mind: simple operation, low cost, and high performance. Whenever compromises were considered, they were made in favor of performance.

The final prototype is shown in Fig. 1. In describing the instrument, its parts, and their functions, the optical and electronic assemblys are discussed separately.

Optical Assembly

The optical portion of the instrument is shown schematically and in a photograph in Fig. 2 and 3, respectively. It consists of an 85-W mercury

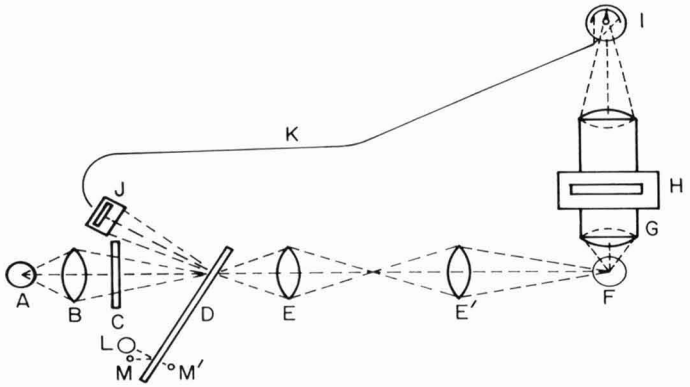

FIG. 2. Schematic of optical assembly. (A) Lamp; (B) lens; (C) primary filter; (D) chopper; (E,E′) lenses; (F) titration vessel; (G) collimating tube; (H) secondary filter; (I) photomultiplier; (J) optical occluder; (K) fiber optics; (L) 2.5-V lamp; (M,M′) photodiodes.

lamp (A), a focusing lens (B), a primary filter (C), a rotating chopper (D), which is detailed in Fig. 4, a lens system (E and E′), a titration vessel (F), a collimating tube (G) with a secondary filter (H) mounted midway between the collimating lenses, an RCA 931A photomultiplier (I), a variable occluder (J), which is detailed in Fig. 5, a fiber optic bundle (K), a 2.5-V prefocused lamp (L), and two photodiodes (M and M′).

A modified double-beam system is maintained in which the rotating chopper directs the light either into the sample pathway or the reference pathway. For the sample pathway, the light from the source passes

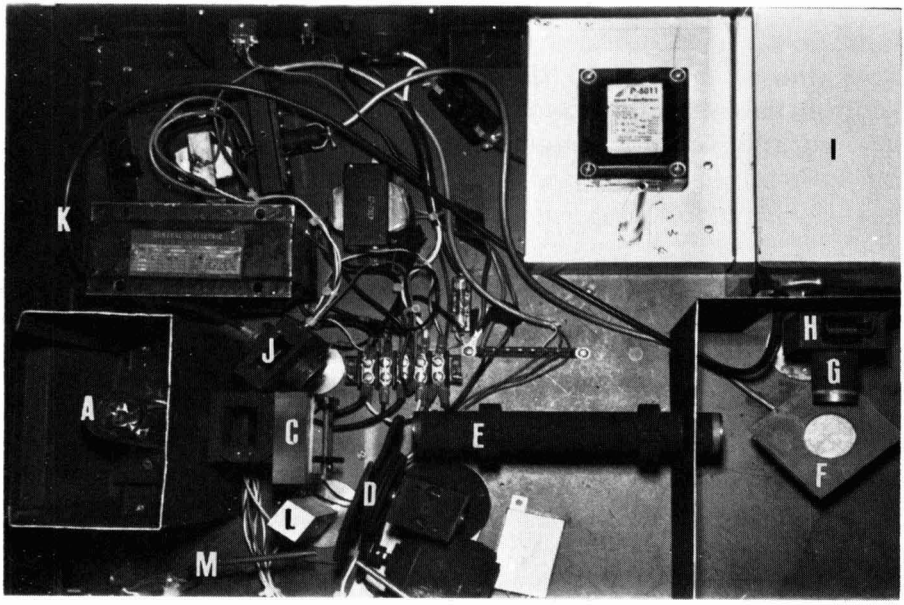

FIG. 3. Optical assembly. Letters refer to components listed in text and legend of Fig. 2.

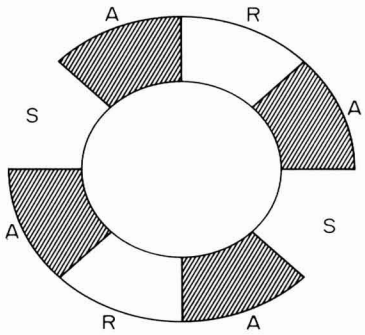

FIG. 4. Rotating chopper. Sectors S: open; allow exciter light to pass; Sectors A: nonreflecting; cut off exciter light; Sectors R: reflect exciter light into the reference path.

through an open sector of the chopper, through lenses E and E′, and is focused on the center of the titration vessel. The light emitted by the sample passes through the collimator and the secondary filter and is finally focused on the cathode of the photomultiplier. For the reference pathway, the light reflected from the polished sector of the chopper passes through the occluder and the fiber optic bundle and strikes the cathode of the photomultiplier. The occluder allows the intensity of the reference beam to be adjusted to the level of that produced by the sample.

Sample and reference beams strike the same spot on the photomultiplier surface alternately, and some technique must be used to maintain the proper phase relationship and to trigger the electronic operations. An optical system was selected which consists of a low intensity light source and two photodiodes with a very short response time. This system is placed parallel to the path of the exciting radiation so that every action taken on the exciting radiation is simultaneously taken on the phasing light. This arrangement guarantees that the whole system

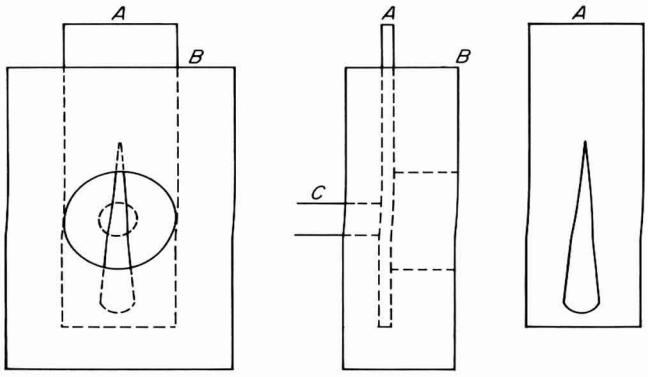

FIG. 5. Optical occluder. (A) Movable plate containing wedge-shaped aperture; (B) mounting block; (C) fiber optic bundle. Middle drawing shows the side view.

stays in phase no matter what changes occur in the chopper speed. The only requirement is that the chopper maintain its physical symmetry.

Electronic Assembly

The electronic portion of the instrument is shown schematically in Fig. 6. Its main components are a preamplifier, three integrating amplifiers, and three switches. The switches are tied into the optical phasing system, thus assuring that when a given sector of the chopper is in the exciting light beam, the proper integrating amplifiers are activated and function. The functions of the three integrating amplifiers are, respectively, to: (a) provide electronic compensation for ambient light that strikes the photomultiplier, (b) operate an automatic gain control on the preamplifier in order to compensate for random changes in source intensity and photomultiplier output, and (c) amplify the sample signal.

The most important features of this design are the four operational amplifiers (A1–A4). A1 is the preamplifier which serves as a current-to-voltage converter and receives all of the output of the photomultiplier tube. A2 is the central component of a negative feedback loop which compensates for ambient light. A3 amplifies the reference signal and provides a voltage output which compensates for fluctuations in the source and photomultiplier. A4 amplifies the signal from the sample and provides a signal output for a 100-μA meter and a 1-mV recorder, which can be operated simultaneously.

The functions of these systems can be best described by discussing the sequence of events which occur at each position of the chopper (Fig. 4). When the open sector of the chopper is in the incident beam, the exciting light strikes the sample and the emitted fluorescent light is picked up by the photomultiplier; the output of the photomultiplier is monitored by the current-to-voltage converter A1. Simultaneously, light from the phasing lamp passes through the second open sector and causes the photodiode PD2 to conduct and operate the switch driver Q7–8, which closes the signal switch Q4. The 2-μF capacitor C4 associated with the signal amplifier A4 then begins to charge up to the output of amplifier A1. At the next chopper position the dark sectors block off both the exciter and phasing lights. During this period the photomultiplier detects only the ambient light which passes through the collimating tube and its components. Neither photodiode is illuminated, and consequently, the outputs of both the sample and reference switch driver are −15 V. The like signals pass through an "AND" gate and operate the ambient switch driver Q9–11 which closes switch Q2 and allows the 0.1-μF capacitor C2 of amplifier A2 to charge to the output of A1.

Finally, when a reflecting surface of the chopper is in the beam, light is reflected through the occluder and fiber optic path to the photomul-

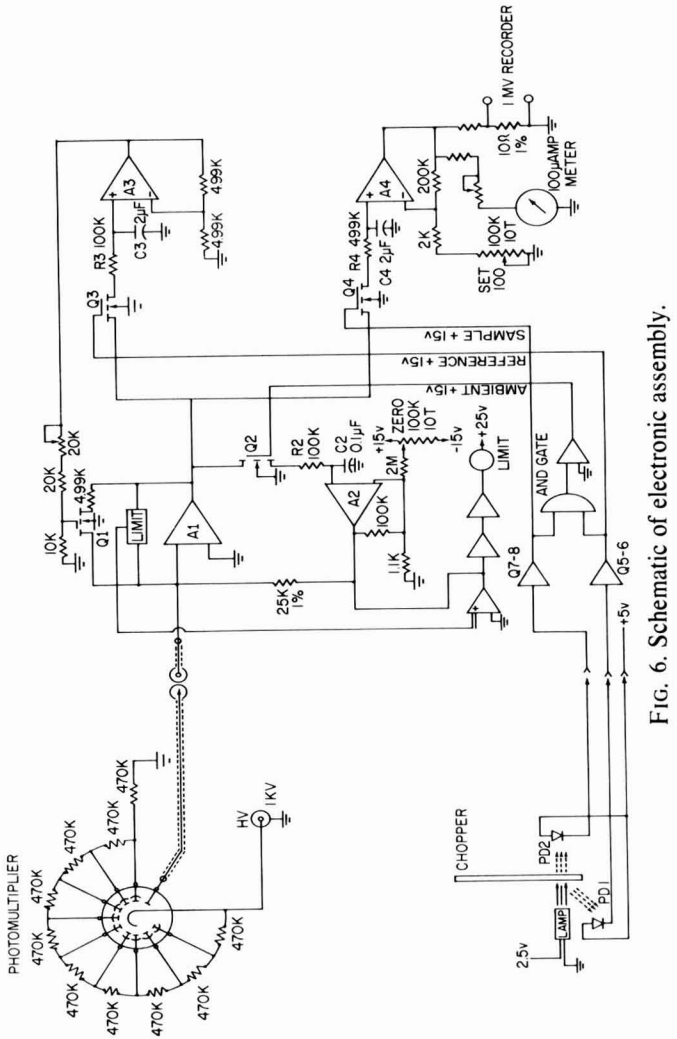

FIG. 6. Schematic of electronic assembly.

tiplier. Simultaneously, light from the phasing lamp is reflected to photodiode PD1, allowing it to conduct and drive the reference switch driver Q5–6. The 2-μF capacitor C3 associated with amplifier A3 begins to charge to the output of A1. As the chopper rotates, this sequence is repeated until each of the capacitors is charged to the output of the amplifier A1 for its respective phase of operation; this makes each of the operational amplifiers an integrator, with an integration period equal to the RC constant of the associated capacitor network.

The integration period of amplifier A2 is the shortest, approximately 0.01 sec. Its averaged signal is fed back to the summing point of the preamplifier A1, where the sum of the currents must equal zero. This cancels the effects of photomultiplier dark current and ambient light. The reference integrator A3 has a time constant of 0.2 sec. This system feeds back a constant voltage signal through resistor R1 and field effect transitor Q1 which acts as a voltage-controlled resistor. The output of A1 is given by the relation $E_0 = I_{pm}R_f$; here E_0 is the voltage out of A1, I_{pm} is the photomultiplier current, and R_f is the total feedback resistance, that is, 4.99 K + R_{Q1}. If the reference output is decreased, then R_{Q1} and E_0 are increased. Should the lamp intensity or the photomultiplier output vary, the gain on preamplifier A1 will be increased or decreased to such an extent that a constant reference output is maintained, and this in turn assures that the sample signal is independent of lamp and photomultiplier fluctuations. The capacitor associated with sample amplifier A4 is then charged to the output of A1 (this requires about 1 sec). This voltage is amplified and measured on a 0–100-μA meter or a 1-mV recorder. The gain of A4 is adjusted with the "SET 100" control and can be varied for amplification factors from 2 to 50.

A protection or limiting circuit is also included (see Fig. 4). In normal operations, it plays no role, but if the current from the photomultiplier exceeds the normal operating levels, the limiting network will conduct and protect A1.

Discussion of Design Parameters

The initial proposal for the fluorotitrator included a number of requirements necessary for an instrument to be of greatest usefulness. In most instances, a combination of optical and electronic approaches were taken to fulfill the requirements.

Stability

Long-term stability is assured because the light source and photomultiplier are constantly monitored as a unit, and any changes in output are automatically corrected by electronic adjustment of the preamplifier gain.

Stability of the instrument readout in terms of lack of random meter movement is achieved by the integration step built into the signal-handling system. The chopper rotates at 2440 rpm, which means that during the 1-sec integration period the output is composed of 81.$\dot{3}$ individual signals from the sample.

The combination of these two stabilizing features allows the use of a pulsed light source (the mercury lamp fires at 120 Hz), which would have been impossible with the simple double-beam design tested in earlier stages of the investigation.

Stray Light

Stray radiation from the exciter lamp that may reach the photodetector is minimized by the conventional arrangement of the photodetector at a 90° angle to the incident radiation and by the proper choice of primary and secondary filters.

Ambient Light

The ability to operate with the sample exposed to room light is one of the most significant features of the instrument. External light can only reach the photomultiplier by passing through the collimator tube (G) containing the interference filter (H). The instrument case prevents room light from entering the collimator directly. The inside of the collimator tube is painted with optical black to help exclude indirect radiation. The use of a narrow band-pass secondary filter further limits the amount of ambient light which reaches the photomultiplier, and thus the photomultiplier cannot be saturated. The chopper and electronic system sample and correct for this small portion of ambient light four times during each revolution of the chopper, or 162.$\dot{6}$ times each second.

Sensitivity

A satisfactory level of sensitivity was obtained by using an 85-W full-range mercury lamp, a photomultiplier detector with an amplified output, and filters of relatively broad band-pass. The signal from the photomultiplier goes through two amplification stages, first, the preamplifier, and then the sample integrator, before being displayed. The size of the first amplification depends on the reference signal and can be increased by a decrease in that signal. The second amplification can give a 50-fold increase in signal and is controlled by the "SET 100" control.

Convenience

The operational convenience of the instrument can be judged from the two procedures given below. Normally, the necessary adjustments for performing a titration even for the first time will not require more than a very few minutes.

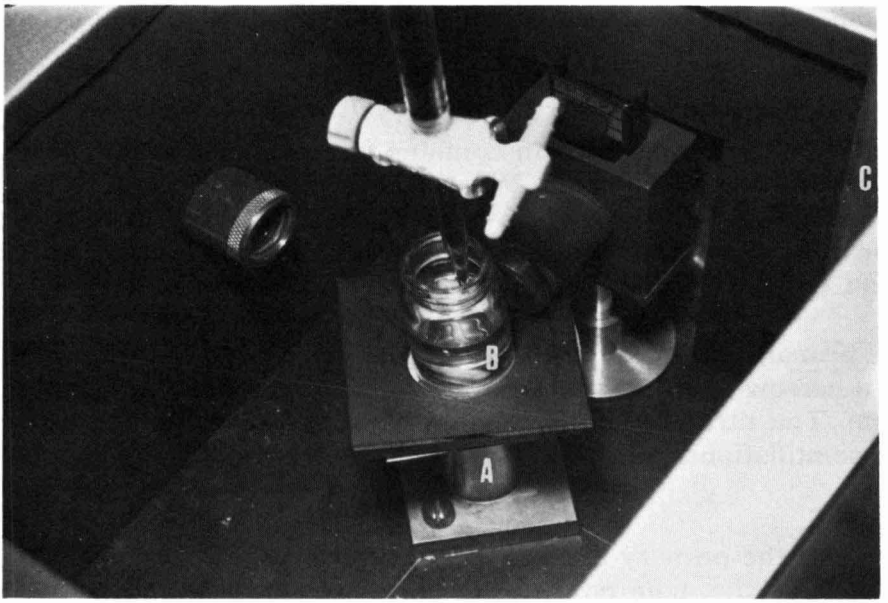

FIG. 7. View of sample compartment. (A) Magnetic microstirrer; (B) titration vessel; (C) mounting rod.

Several features make the instrument particularly easy to operate. The incident beam size allows measurements to be made with a sample vessel with an internal size as small as 3 × 8 mm and at the other extreme a 100-ml beaker. Rectangular cells with even larger volumes can be used. The size of the sample or titration area, 18 × 20 cm, shown in Fig. 7, allows the operator a great deal of latitude in handling titration vessels and/or mounting special apparatus such as temperature regulating devices. The high degree of accessibility along with the independence from ambient light offers a great deal of promise for use with automated systems. The micromagnetic mixer can be removed by unfastening a single screw and unplugging one electrical connection. Thus, a variety of ancillary apparatus can be accommodated.

Performance Evaluation

The performance of the instrument was evaluated in a number of tests. Two types of titrations were performed. The first was the titration of copper with EDTA, with calcein used as the indicator. Under the conditions selected it is an up-scale step titration, that is, the end point is signaled by an abrupt increase in fluorescence. The second was the titration of calcium with EDTA in the presence of excess calcein. This is a down-scale slope titration, that is, the fluorescence gradually decreases during the titration and at the end point disappears or becomes constant. Both systems have been chosen because their theoretical and

practical aspects are so well established (*12*) that any deviation from the expected course clearly could be blamed on the instrument.

Titration of Copper

Initially, the sample solution contains free copper and the nonfluorescent copper–calcein complex. When EDTA is added, the free copper is complexed. After all this copper is used, the EDTA attacks the copper–calcein complex, fluorescent calcein is freed, and the curve rises steeply until all copper is transferred to the EDTA. Then the curve levels off again.

The primary filter had a peak transmittance of 480 nm; the secondary filter, a narrow band-pass interference filter, had a peak transmittance of 520 nm. The titration vessels employed in this series of titrations were 20-ml scintillation tubes.

Procedure

1. Insert the primary and secondary filters.
2. Turn on the main power switch and wait 5 to 10 min for the lamp to reach maximum intensity.
3. Turn on the electronic power switch.
4. In one titration vessel place 1.5 ml of indicator solution and bring to 15 ml with ammonia buffer. Insert the vessel into the light path and adjust the reading to 80 with the "SET 100" control. Remove the vessel.
5. In another titration vessel place 1.00 ml of copper solution and 1.5 ml of indicator solution and bring to 15 ml with buffer. Add the stirring bar and insert the vessel into the instrument. Adjust the reading to 10 with the "SET ZERO" control.
6. Repeat steps 4 and 5 to correct for the small amount of interplay between the controls.
7. Start the stirrer and add titrant in small increments, noting the reading after each addition. Select particularly small increments when a slight rise in fluorescence is noticed.

A typical titration curve is shown in Fig. 8. It has the expected shape and a correctly located end point. Repeated titrations under the above conditions and with more concentrated and more dilute copper and EDTA solution showed satisfactory reproducibility, about the same as that shown in Table 1 for the calcium titration.

Titration of Calcium

In this titration calcein is again used, not as the indicator however, but rather as a fluorogenic agent to create a self-indicating system. At a pH >10 calcein does not fluoresce, although its calcium complex does. Thus, when calcein is added to a calcium solution in excess over the cal-

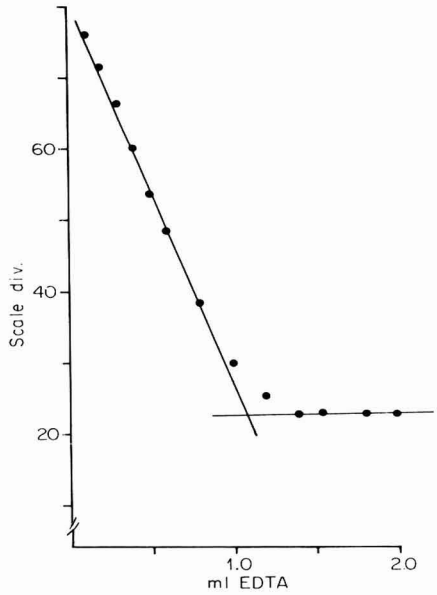

FIG. 8. Curve for the fluorometric step titration of 1 ml 0.01 *F* copper in 15 ml of solution with 0.01 *F* EDTA with calcein used as indicator.

cium, a fluorescence occurs that is proportional to the calcium content. In this case when the titration is started with EDTA, calcium is removed from the calcein complex, and the fluorescence intensity decreases linearly with the process. A straight line will, of course, only be obtained when the overall calcium concentration is low enough to be within the range where linearity between fluorescent intensity and the concentration of fluorescing species is maintained.

Primary and secondary filters were the same as in the copper titration, namely, those with peak transmittances at 480 and 520 nm, respectively. The concentration and amount of the calcium solution were selected to simulate a calcium determination in human serum (10 mg Ca/100 ml; 0.1-ml sample volume).

TABLE 1
RESULTS FOR REPLICATE DETERMINATIONS OF CALCIUM WITH THE FLUOROTITRATOR

Ca taken (mg)	EDTA used (ml 2.3×10^{-4} *F*)	Ca found (mg)
0.010	1.07	0.0098
0.010	1.08	0.010
0.010	1.07	0.0098
0.010	1.10	0.012

Reagents. Calcium 2.50×10^{-3} *F* (or 0.1 g/l); EDTA 2.30×10^{-4} *F*; potassium hydroxide 0.8 *F* containing 1 mg calcein in 100 ml.

Procedure

Steps 1 through 3 are identical to those described for copper.

4. Place a titration vessel containing water in the instrument and, using "SET ZERO" control, adjust the reading to 10.

5. In another, identical titration vessel place 0.100 ml of calcium solution and bring it to 15 ml with the calcein containing potassium hydroxide solution. Insert in the instrument and, using the "SET 100" control, adjust the reading to 80.

6. Repeat steps 4 and 5 to correct for the small amount of interplay between the controls.

7. Start the stirrer and add EDTA in increments, noting the reading after each addition. Proceed until the reading become constant.

A typical curve is shown in Fig. 9, and here too the shape is exactly as expected. Reproducibility was satisfactory, as indicated by the data in Table 1.

The sample size can be reduced by using a smaller titration vessel. A vessel containing 0.025 ml of sample and 5 ml of potassium hydroxide solution diluent is quite compatible with the instrument.

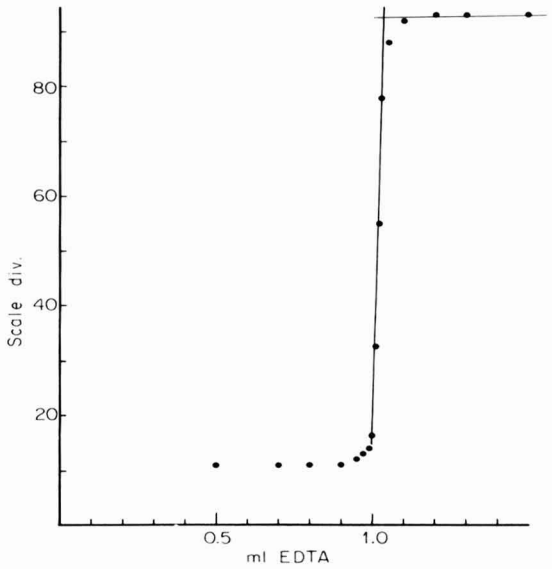

FIG. 9. Curve for the fluorometric slope titration of 0.1 ml of 2.5×10^{-3} *F* calcium with 2.3×10^{-4} *F* EDTA in the presence of excess calcein.

Linearity of Response

Figures 8 and 9 show that a linear response is obtained within the concentration range where such a relationship is supposed to exist. As is to be expected, nonlinearity is encountered in those portions of the titration curve representing higher concentrations of the fluorescing species. However, this does not make a titration impossible.

Stability

The stability of the instrument was checked over various time spans with settings for maximum sensitivity. Under these conditions the photomultiplier was operated at the top value of 1500 V, and the optical occluder was closed as far as needed to drive the current-to-voltage converter A1 to its maximum gain. A typical result showed a drift of about 6 scale divisions over a 3-hr period, which, based on an initial scale setting of 80, corresponds to a drift of approximately 2%/hr. Since a titration is completed in a much shorter time, this drift is of no consequence. Further, such extreme gain adjustments are seldom necessary.

The situation concerning short time, random fluctuations was also very satisfactory because with the same extreme settings, they never exceeded 1% of full scale.

SUMMARY

An instrument is described that permits fluorometric titrations in a large, open compartment that accommodates titration vessels of a wide variety of shapes and sizes. The influence of ambient light is excluded by applying geometric exclusion in combination with electronic monitoring and compensation. In test titrations of copper and calcium with EDTA and calcein, the instrument performed satisfactorily.

ACKNOWLEDGMENTS

Financial assistance was provided to J. M. W. by the U. S. Department of Health, Education, and Welfare through Public Law 85-507. Extensive help during the development of the electronic portions of the instrument was received from G. O'Brien, Ga. Tech., and J. W. Weaver, of CDC. J. A. Moore, of CDC, helped with mechanical and metal work. All of this aid is most gratefully acknowledged.

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Sodium–Lead Alloy in the Decomposition of Organic Samples for the Detection of Heteroelements[1]

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INTRODUCTION

Sodium fusion has long been used for the decomposition of organic samples, thereby allowing various heteroelements to be detected in an ionic form. Such fusion was originally used by Lassaigne (*6*) in 1843 for the detection of nitrogen. The history of the Lassaigne test has been reviewed by Tucker (*10*). Mulliken and Gabreil (*7*) have studied the recovery of heteroelements from the sodium fusion residue of organic compounds. Optimum techniques for the fusion and some associated detection tests have been reviewed by Gower and Rhodes (*5*).

Although the sodium fusion test is simple to conduct, there are problems and hazards attendant to the use of metallic sodium: difficulty in handling, instability in air that is not humidity controlled, hydrogen ignition, rapid reaction with some compounds with the contents of the tube being expelled, incomplete decomposition and subsequent poor results, and hazards of disposal.

The use of the binary 10% sodium–90% lead alloy for drying solvents was described by Soroos in 1939 (*8*). The alloy became commercially available in the late 1950's in a dry, granular form. The product is relatively stable in air, presents no difficulty in handling, and is useful as a safe and convenient drying agent for solvents commonly dried with sodium wire. Further, the alloy can be added directly to water or dilute acid to give a nonviolent reaction without hydrogen ignition, and consequently, its disposal is simple. The hazard-free use of this alloy in demonstrating the reaction of sodium with water and the reduction of copper (II) by sodium was delineated by Barnard *et al.* (*1*). The reduction of various cations and anions by the alloy was reported by Edge and Fowles (*3*). The alloy has found use in the preparation of organometallic compounds, notably lead alkyls, and the reduction of ketones (pinacol reaction).

[1] Paper I in the series "Studies of Laboratory-Use Reagents."

In all of these reactions, lead can be considered as the moderator of a sodium reaction. Consequently, it appeared interesting to explore the use of this alloy in the fusion of organic samples. It could be hoped that a safe, well-moderated, but complete, decomposition would be achieved. The study has taken several directions: (1) experiments to assess the value of the alloy in the decomposition of common compounds and the subsequent detection of halogen, nitrogen, phosphorus, silicon, and sulfur; (2) an extension to chlorine and nitro-bearing compounds reported to be potentially hazardous or to give poor results; (3) application to fluorine-containing compounds; and (4) the establishment of detection limits.

EXPERIMENTAL METHODS

Reagents

All reagents were of analytical grade or better. The sodium–lead alloy (dri-Na) is available from the J. T. Baker Chemical Co. ULTREX high purity compounds (J. T. Baker) were used for the establishment of most detection limits.

Procedure for Sodium–Lead Fusion

Note. In the following operations, common safety precautions, such as the use of an efficient fume hood and safety shield, should be observed.

Place about 0.5 g of sodium–lead alloy in a 5-ml borosilicate test tube. Carefully melt the alloy over a Bunsen flame, then heat until the fumes of sodium reach halfway up the walls of the tube. Remove the tube from the flame and add the requisite amount of organic sample, liquid or solid, in such a way that as little of the sample as possible adheres to the tube walls. Heat gently in the flame until reaction is initiated, remove until the reaction subsides, then heat to redness and cool. Add about 3 ml of water and allow to stand until the reaction of sodium and water is complete. If desired, the tube and contents may be warmed to 50°C to expedite the reaction. Usually, a clear solution is obtained, with a pellet of lead remaining at the bottom of the tube. Filter, if necessary, through a well-washed filter paper, and subject portions of the filtrate (or decanted liquid) to tests for the heteroelements of interest. Run a total blank if doubtful results are secured.

Test Methods for Heteroelements

The following test procedures are suggested for the detection of the heteroelements.

Test for Bromine, Chlorine

Acidify a portion of the filtrate with nitric acid, boil for several minutes, cool, and add 1 ml of 2% silver nitrate solution. A yellow-white (bromine) or white (chlorine) precipitate indicates the presence of the halogen.

Test for Fluoride

The detection of organic fluoride using alizarin fluorine blue, lanthanum derivative, has been discussed by Belcher and West (*2*) and others. Place 1 ml of the filtrate in the depression of a spot plate and add 1 ml of acetate buffer (10.5 g of sodium acetate trihydrate and 10 ml of glacial acetic acid diluted to 100 ml with water). Add 2 drops of a 10% water solution of alizarin fluorine blue, lanthanum derivative (available from J. T. Baker Chemical Co.) and after 10 min compare the color developed to that of a complete blank test. A blue or purple-blue color indicates the presence of fluorine.

Test for Nitrogen

A. Prussian blue test. Adjust 1 ml of the filtrate to pH 13 with 10% sodium hydroxide solution. Add 2 drops each of saturated ferrous ammonium sulfate solution and 30% potassium fluoride solution, and boil for 30 sec. Make the hot solution just sufficiently acid with 30% sulfuric acid to dissolve any hydrous iron(III) oxide. A Prussian blue color or precipitate indicates the presence of nitrogen.

B. Pyrazolone color reaction (*4*). Dilute 1 ml of the filtrate to 10 ml with water and adjust to pH 6.5 with 1:4 acetic acid. Add 0.2 ml of 1% aqueous chloramine-T solution and allow to stand for 2 min. Add 5 ml of "pyridine–pyrazolone solution" (0.25 g 3-methyl-1-phenyl-2-pyrazoline-5-one in 100 ml of water and 0.01 g of 3,3′-dimethyl-1,1′-diphenyl-[4,4′-bi-2-pyrazoline]-5,5′-dione in 20 ml of pyridine, the two solutions being mixed just before use). Allow the resulting solution to stand for 30 min. A deep blue color indicates the presence of nitrogen. A complete blank test should be run for comparison purposes. If quantitation is desired, dilute the solution with water to a known volume after addition of the pyridine–pyrazolone solution, and measure the absorbance at 620 nm (1-cm cells). The concentration may then be determined using a standard curve prepared from data obtained using sodium cyanide standard solutions.

Test for Phosphorus or Silicon

Dilute 1 ml of the filtrate to 10 ml with water and acidify with sulfuric acid solution (150 ml of sulfuric acid, 2 ml of nitric acid, diluted with water to 500 ml). Add 0.2 g of potassium persulfate and boil the solution

for 10 min. Cool, add 1 drop of phenolphthalein, and then aqueous ammonia until the solution is just alkaline. Add sulfuric acid solution dropwise until the pink color is discharged, and then 1 drop in excess. Now add 8 drops of ammonium molybdate solution (5 g of this salt in 40 ml of water added to a cooled solution of 60 ml of concentrated sulfuric acid and 80 ml of water, cooled, and diluted with water to 200 ml), and 2 drops of a 2.5% tin(II) chloride solution in glycerol. Mix well and allow to stand for 10 min. A *deep* blue color indicates the presence of phosphorus or silicon. A complete blank test may exhibit a *slight* blue color, especially on long standing.

Test for Sulfur

Place 1 drop of the filtrate, acidified with acetic acid, on lead acetate test paper or in 1 drop of 10% lead acetate solution. The immediate development of a black or brown-black color indicates the presence of sulfur (sulfide). Alternatively, boil 1 ml of the filtrate, [made acidic with hydrochloric acid (1 : 9) solution], with 1 ml of 30% hydrogen peroxide and 5 ml of water. Cool, add 1 ml of 12% barium chloride solution and allow to stand for 15 min. A white precipitate or turbidity indicates the presence of sulfur (sulfate). The presence of sulfur species in the sodium–lead alloy limits its usefulness in the detection of minute quantities of sulfur.

Detection Limits

To establish the merit of the above tests and to provide an indication of the detection limits, about 5 mg of a compound containing a heteroelement of interest was accurately weighed on a microbalance and subjected to the sodium–lead fusion procedure. After quantitative transfer of the filtrate to a 100-ml volumetric flask and dilution to mark, aliquots were subjected to the tests given above.

RESULTS AND DISCUSSION

Initial Study

Initial experiments were directed toward the detection of the common heteroelements in organic compounds (halogen, nitrogen, phosphorus, silicon, sulfur). Table 1 lists the compounds studied with the detection test applied. In all cases, with sample weights of 10 to 20 mg, tests for the relevant heteroelement were positive.

Study of Some Difficult Compounds

Some potentially hazardous chlorine compounds were subjected to the sodium–lead fusion and the halogen detected by silver chloride turbidity.

TABLE 1
COMPOUNDS AND RESULTS OF INITIAL STUDY

Compound	Element	Test	Result
m-Chlorobenzoic acid	Cl	$AgNO_3$ turbidity	Positive
8-Quinolinol	N	Prussian blue	Positive
Diphenylphosphinic acid	P	Molybdenum blue	Positive
Dibenzyl sulfide	S	Lead acetate paper	Positive
N-Bromosuccinimide	Br	$AgNO_3$ turbidity	Positive
	N	Prussian blue	Positive
Trichlorophenylsilane	Cl	$AgNO_3$ turbidity	Positive
	Si	Molybdenum blue	Positive
Pyridine	N	Prussian blue	Positive
Chloramine-T	Cl	$AgNO_3$ turbidity	Positive
	N	Prussian blue	Positive
	S	Lead acetate paper	Positive

No untoward reactions were observed, although some compounds burned incompletely, leaving sooty deposits on the test tube walls. In all cases, a positive test for chloride was obtained. The findings are summarized in Table 2.

The literature mentions nitro compounds for which nitrogen is only difficultly detectable after sodium fusion via the formation of Prussian blue. The results secured with sample weights of 10 to 20 mg for 5 nitro compounds are summarized in Table 3. In most cases, an almost instantaneous, but controlled, decomposition was noted, and all tests for nitrogen were positive. With a nitrophenyl-substituted tetrazolium com-

TABLE 2
STUDY OF POTENTIALLY HAZARDOUS CHLORINE-CONTAINING COMPOUNDS

Compound	Observed reaction	Result for chloride[a]
Carbon tetrachloride	No explosive or flammable reaction	Positive
Chloroform	No explosive or flammable reaction	Positive
Methylene chloride	Slight burning in mouth of tube	Positive
Chlorobenzene	Slight burning in mouth of tube; sooty deposit on walls of tube indicate incomplete combustion	Positive
Dichlorobenzene (mixed isomers)	Slight burning in mouth of tube; sooty deposit on walls of tube indicate incomplete combustion	Positive

[a] Determined by $AgNO_3$ turbidity.

TABLE 3
STUDY OF NITRO COMPOUNDS

Compound	Fumes observed on fusion	Result for nitrogen[a]
p-Nitrobenzaldehyde	Brown fumes	Positive
Nitrobenzene	Brown fumes	Positive
o-Nitrobenzoic acid	Brown fumes	Positive
Nitromethane	Few fumes	Positive
Nitro blue tetrazolium chloride[b]	Few fumes	Positive, slow reaction

[a] Detected by Prussian blue formation.

[b] 3,3′-(3,3′-Dimethoxy-4,4′-biphenylene)bis[2-(*p*-nitrophenyl)-5-phenyl-2*H*-tetrazolium chloride].

pound, the color development in the Prussian blue test was slow, and the test result was considered doubtful.

Application to Fluorine Compounds

Four fluorine compounds and a fluoropolymer were fused with sodium–lead alloy, and the fluoride was detected by the test given above. The findings are summarized in Table 4. In all cases, with samples of 10 to 20 mg, a positive test result was secured.

The contents of the tube were expelled when about 10 mg of 1-fluoro-2,4-dinitrobenzene were added to the molten alloy. Considerable sublimation occurred on reaction of Teflon with the molten alloy. It was found that filtration of the fusion solution is unnecessary, as the developed color is visible even in a turbid solution.

Detection of Nitrogen by Pyrazolone Color Reaction

As nitrogen in organic compounds is converted largely to cyanide on sodium fusion, it seemed of interest to study the application of the highly sensitive pyrazolone color reaction (*4*), now commonly applied in the photometric determination of trace cyanide in water (*9*).

TABLE 4
STUDY OF FLUORINE-CONTAINING COMPOUNDS

Compound	Remarks	Result for fluorine
p-Fluorobenzoic acid	Rapid color development	Positive
Fluorobenzene	Rapid color development	Positive
1-Fluoro-2,4-dinitrobenzene	Violent reaction![a]	Positive
Hexafluorobenzene	Extremely rapid color development	Positive
Teflon	Extremely rapid color development; sublimation occurred during fusion	Positive

[a] Contents of test tube were expelled on addition of 10 mg portion of compound. Reaction may be controlled by use of smaller additions.

TABLE 5
DETECTION LIMITS FOR HETEROELEMENTS

Heteroelement	Method of detection	Detection limit (μg)
Chloride (Cl)	$AgNO_3$ turbidity	2
Fluoride (F)	Alizarin fluorine blue	<1.7
Nitrogen (N)	Pyrazolone reaction	0.1
Phosphorus (P)	Molybdenum blue	1

Conventionally, the solution of the chromogenic agent is prepared daily, as deterioration occurs rapidly. The preparation of fresh reagents is time-consuming and does not lend itself to qualitative applications; an unsuccessful attempt was made to improve the stability of the final solution. It was found, however, that each of the component solutions is stable for a period up to 8 wk, the solution in pyridine being refrigerated. Consequently, the two solutions should not be mixed until the time of use. This method has the same degree of manipulative difficulty as the commonly used Prussian blue test, but exhibits greater sensitivity, and therefore was chosen for establishing the detection limit for nitrogen.

Detection Limits of the Heteroelements

The detection limits established by the above procedures (summarized in Table 5), involved the dilution of the filtered fusion solution to 100 ml, and are, consequently, based on concentrations about 20 times smaller than would normally be encountered, and therefore represent a conservative assessment.

To assure the validity of the findings, several crystals of chloramine-T ($\sim$0.3 mg) were subjected to the sodium–lead fusion and portions of the filtrate were tested for chlorine, nitrogen, and sulfur. For nitrogen, the Prussian blue test was negative; in contrast, the pyridine–pyrazolone test was positive. Positive tests were secured for chlorine and sulfur.

POSSIBILITIES FOR QUANTITATIVE APPLICATIONS

Bomb fusion with sodium or potassium metals has been utilized for the decomposition of organic samples and the determination of some heteroelements. Since open-tube fusion with sodium-lead alloy proceeds smoothly and usually without obvious loss of the fusion products, it appeared interesting to establish the degree to which the reaction might be quantified. The recovery of chlorine from *m*-chlorobenzoic acid was studied. Weighed amounts (5 to 13 mg) were carried through the sodium–lead fusion, and the total solution after filtration through well-washed paper was titrated with 0.01 *N* silver nitrate, using dichlorofluroescein indication. The results indicated better than 95% recovery

with good precision (±2%). The recovery of nitrogen from 8-quinolinol was also studied. Weighed amounts (about 5 mg) were subjected to the sodium–lead fusion. The cooled melt was treated with water and then filtered. The filtrate was diluted to 50 ml with 1 *N* NaOH, and 10-ml aliquots were taken through the pyridine–pyrazolone photometric finish. Recoveries of nitrogen from 100 to 104% were secured. Work on the quantitative aspects of open-tube sodium–lead alloy fusion is continuing.

SUMMARY

The decomposition of organic samples using open-tube fusion with sodium–lead alloy was demonstrated to be safe, complete, and controlled, in most cases. The detection of heteroelements in the fusion mixture of common organic samples was successful with samples of 10 to 20 mg. Detection of chlorine and nitrogen by classical methods in "difficult" compounds was secured, and the detection of fluorine in the fusion mixture of fluorocompounds extended the use of the alloy into this area. The use of the pyrazolone color reaction in the detection of nitrogen allowed the detection of more minute amounts of nitrogen than was previously detected using Prussian blue formation. The detection limits for heteroelements in organic samples were in the low microgram region. The application of sodium–lead alloy in quantitative work was briefly studied and will be considered in greater detail in the future.

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BOOK REVIEWS

Instrumental Analysis of Cotton Cellulose and Modified Cotton Cellulose. Edited by ROBERT T. O'CONNOR. Dekker, New York, 1972. x + 490 pp. $29.50.

This book constitutes Volume 3 of the Fiber Science Series. According to the editor its main function is to present an up-to-date description of the instrumental methods of analytical chemistry which may be used in the study of fibers from the qualitative, quantitative and structural standpoints.

The material covered in this particular volume is contributed by 11 individuals in the form of 8 chapters. It is interesting to note that 8 of the 11 authors are affiliated with the Southern Regional Research Laboratory in New Orleans.

Of the 98 theoretically possible spectroscopic methods, arrived at by considering the regions of the electromagnetic spectrum and the possible modes in which the radiation is used, only about 40 are employed in a way in solving problems dealing with investigations of cotton cellulose, chemically modified cotton cellulose and resin treated fibers. The instrumental methods described which are related to spectroscopic methods include: X-ray fluorescence, electron emission (spectrochemical analysis), atomic absorption, ir, X-Ray diffraction, wide line nuclear magnetic resonance, gamma ray and neutron absorption, and mass spectrometry. In addition to the above spectroscopic methods, microscopic techniques, both light and electron are used in the study of fibers. One chapter which deals with the study of oxidation, degradation and pyrolysis of cellulose, describes techniques such as paper and thin layer chromatography which are supplemented by instrumental methods such as gas chromatography, mass spectrometry, thermal and differential thermogravimetric analysis.

The authors, in addition to giving a brief theory of the instruments, also include in many instances specific instructions for carrying out the measurements. This reviewer finds that this particular volume represents an excellent contribution for not only researchers engaged in these types of investigations but, in general, for those individuals interested in the practical potentialities of some of the latest instruments. The chapters are well written and referenced. The book contains both an author and a subject index. In view of the abundant information and the superb presentation, this volume represents an excellent addition to the library of individuals engaged in this field as well as instrumentalists in general.

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Methods of Biochemical Analysis. Edited by DAVID GLICK. Wiley, New York, 1973. viii + 572 pp. $22.50.

This is Volume 21 in an obviously well-received series designed to cover the methods and techniques of biochemical analysis so that interested workers can keep abreast of the rapid and diverse developments. Some of the chapters serve as correlative recapitulations of well-established methods and techniques while others display new approaches of promise.

This volume consists of seven chapters plus separate author and subject indices for Volume 21 and for the series as a whole.

The first chapter discusses Techniques for the Characterization of UDP-Glucuronyltransferase, Glucose-6-phosphatase, and Other Tightly-Bound Microsomal Enzymes. Emphasis is placed on the problems encountered and ways in which they may be met. The two enzymes in the title are studied more extensively and in sufficient detail to permit extrapolation to other enzymes. Phospholipid–protein interactions are also reviewed in detail. The preparation of microsomes, and a discussion of interfering enzymes and substrate forms rounds out this practical chapter.

Determination of Selenium in Biological Materials is the subject of the second chapter. A review of both destructive and nondestructive methods is presented. In addition, detailed procedures are given for a method based on a wet digestion, and on an oxygen flask, as well as a gravimetric analysis of samples of very high selenium content.

The third chapter covers High-Performance Ion-Exchange Chromatography with Narrow-Bore Columns: Rapid Analysis of Nucleic Acid Constituents at the Subnanomole Level. It includes a discussion of the interpretation of the chromatogram. the use of resin coated beads, liquid chromatography, the application of ion-exchange chromatography to nucleic acids, the use of pellicular ion-exchange resins as well as other conventional resins for ultrasensitive analyses and a look at the methods of sample preparation. It is suggested that this techique is about ready to compete with the methods utilizing radioactive tracers.

Chapter four reports on Newer Developments in Enzymic Determination of D-Glucose and its Anomers. This is a how-to chapter and presents both detail and the author's critical comments.

Radiometric Methods of Enzyme Assay presents an encyclopedic approach. The general principles of enzyme assay are laid down, followed by a general discussion of the techniques used. A detailed cataloging of the radiometric enzyme assays reported comprises the bulk of this chapter which concludes with an extensive critique of the advantages and limitations of the method.

Chapter six is a review of Polarography and Voltammetry of Nucleosides and Nucleotides and their Parent Bases as Analytical and Investigative Tools. This is a comprehensive and critical survey of the electrochemical behavior of the subject compounds, with the goal of shedding light on biological phenomena and providing additional methodology for future investigators. It does.

The final chapter is entitled Integrated-Ion Current Technique of Quantitative Mass Spectrometric Analysis: Chemical and Biological Applications. The integrated-ion current technique described in this review seems to be the method of choice where unambiguous identification and quantification in the submicron region is required, e.g., in neurochemistry and neuropharmacology. The only snag suggested is the expense ($100,000-150,000) and the required personnel. The instrumentation, and a variety of applications are described.

This is a high-caliber practical book with clear and ample illustrations, tables and bibliographies.

BILL ELPERN, *9 Surrey Way, White Plains, New York 10607*

Conformational Properties of Macromolecules. By A. J. HOPFINGER, Academic Press, New York, 1973. x + 339 pp. $24.50.

There appear to be more and more scientists with the daring and the know-how to apply rather complex calculations to the conformational analysis of large molecules and polymers. The incredibly rapid advances made in computer technology no doubt play an important role in this rather new field of research.

The book here reviewed is concerned with such calculations and is a welcome addition to the "Molecular Biology" series. The author introduces us to all important aspects of initiating a study aimed at a definition of the conformation of large molecules. His approach is detailed enough to allow a novice (admittedly with a decent mathematical background) to plan his research keeping in mind the limitations of the approaches as well as the practicability of certain approaches to such problem. While, in principle, rigorous nonempirical (*ab initio*) quantum chemical methods can be applied to study any and all problems considered in this book, such a method has to date been applied only to the simplest ones. Certainly the semiempirical methods advocated by the author, for the moment, serve as the only practical approach to the solution of the problems outlined in the book.

The chapters include 1. definition and generation of macromolecular geometries; 2. conformational energys and potential function calculations (including nonbonded, electrostatic, induced dipole, torsional, hydrogen bonding, bond angle and bond length distortion potential functions along with a brief description of semiempirical quantum mechanical approaches), approaches to calculation of the interaction of solvent with the polymer, calculations of conformational entropy; 3. accuracy and refinement of the classical potential functions; 4. conformational transitions in macromolecules, a rationalization of experimental data with calculations; 5. application of the classical potential energy calculations to an explanation of other experimental results such as those from absorption and optical rotation, epitaxial crystallization, NMR, conformational fluctuations observations. There are also two appendices one on the empirical nonbonded potential function between ions and atoms the other on the "detailed balancing approach to equilibrium properties of linear chains."

While the author's approach is broad enough, the recent advances in organic conformational analysis employing similar functions (but highly refined) by Allinger and others are not mentioned nor could any reference be found to the numerous theoretical studies of nucleic acid conformations.

This volume is most useful to the researcher concerned with theoretical conformational analysis of macromolecules and should be available in most libraries since with its up-to-date (1972) reference collection it would be an invaluable literature search aid for anyone in the field.

Of particular interest is the substantial section devoted to a discussion of the theoretical attempts to calculate solute–solvent interactions.

FRANK JORDAN, *Rutgers University, Newark College of Arts and Sciences, Department of Chemistry, Newark, New Jersey 07102*

Nonaqueous Electrolytes Handbook. Vol. 2. Edited by G. J. JANZ AND R. P. T. TOMKINS. Academic Press, New York, 1974. xiii + 933 pp. $60.00.

This second volume brings to completion the authoritative and up-to-date, two-volume work of "Nonaqueous Electrolytes Handbook." It is intended to provide quick

and easy access to tabulated nonaqueous electrolyte data. It includes data for inorganic, organic, and organometallic solutes in some 310 of single nonaqueous and mixed nonaqueous–aqueous and nonaqueous–nonaqueous solvent systems.

In brief, the volume consists of 11 well-defined areas: solubilities of electrolytes; EMF and potentiometric titration; vapor pressures; cryoscopy; heat of solution calorimetry; polarography; ligand exchange rates and electrode reactions; electrical double layer; spectroscopy and structure of electrolytes; organic electrolyte battery systems; and additional references and data sources. Throughout the book, the authors have surveyed and compiled extensively from both earlier studies and the more recent contributions. However, for nonaqueous polarography and potentiometric titrations, the emphasis has been placed only on the more recent literature owing to the relatively vast number of publications since 1940. Moreover, as an aid to researchers, the final section presents additional data sources, reviews, and references that were received too late for inclusion in the earlier sections.

In format, there is a brief introduction in each section describing the method of presentation of material and the effective use of the tabulated information. In each classification or category, the information is always arranged alphabetically either in solvents or in solutes. In each table, additional information pertinent to the main electrolyte data such as concentration of solutes used, temperature, experimental conditions, measuring techniques, related references, etc., are also provided. In addition, bibliographies for the related area are given at the end of each section. A total of some 2100 references, covering the literature to 1973, has been cited. A complete compound index, subdividing into two groups of solvent and solute, is also included.

On the whole, the many authors are to be congratulated on having successfully done an excellent job summarizing and compiling such a great number of electrolyte data in nonaqueous solvent systems. This handbook will therefore certainly be considered as a very important and invaluable reference source of information on nonaqueous electrolyte solutions. Besides, like other existing handbooks of physics and chemistry, it will be very useful to all phases of sciences. The university, industry, government laboratories; scientific research institutions; libraries; and information analysis and data centers should keep one copy on the shelves for convenient and permanent use.

GEORGE W. C. HUNG, *The University of Tennessee, Medical Units, Memphis, Tennessee 38163*

Catalysis and Enzyme Action. By MYRON L. BENDER AND LEWIS J. BRUBACHER. McGraw-Hill, New York 1973. xiv + 210 pp. $5.95 ($3.95 in paperback).

This is the seventh in a series designed to bridge the gap between chemistry and biology for students at the elementary college level. As such it does well, although an undergraduate biology major might find it rough sledding in spots.

Following an introduction are chapters on enzymes as catalysts; catalysis involving acids and bases; metal-ion catalysis; catalysis by nucelophiles and electrophiles; and the basis of enzyme action. After each chapter, there are a few selected references for supplementary reading. The book has a good index and is free from errors. In the chapter on enzymes as catalysts, it is unfortunate that the authors did not use all the approved *Chemical Abstracts* abbreviations for the amino acids. However, the undergraduate student in both biology and chemistry will find it very useful.

DAVID B. SABINE, *484 Hawthorne Avenue, Yonkers, New York 10705*

Handbook of Micromethods for the Biological Sciences. By GEORG KELETI AND WILLIAM H. LEDERER. Van Nostrand Reinhold, New York, 1974. xiv + 166 pp. $12.50.

There is a tremendous amount of information in this small book. The authors have gathered from the literature a large number of methods for the analysis of biological materials—far too many to be enumerated here. By going directly to the heart of the procedure and dispensing with frills and unnecessary words, they have presented 106 methods, together with subject and author indexes in one hundred sixty-six 5 × 8-in. pages.

The procedures are grouped as follows: preparation of material (primarily microbiological), chemical microanalytical methods, and biological characterization. After a short explanation of the objective, the details are given in explicit steps, all reagents are specified, illustrations (line drawings) provided where such clarification is necessary, and one or two important references are supplied. Procedures in molecular biology have been purposely omitted.

The book is principally for microbiologists, biochemists, and immunochemists and is highly recommended as a handy compact laboratory tool.

DAVID B. SABINE, *484 Hawthorne Avenue, Yonkers, New York, 10705*

Survey of Progress in Chemistry, Vol. 6. Edited by ARTHUR F. SCOTT. Academic Press, New York, 1973. xii + 340 p. $29.50.

This book is a continuation of a series started in 1963 that aims to improve the transmission of new material to college teachers by improving the communication between the research frontier and the teacher. Such material should also be of interest to all chemists. Volume 6 contains 5 major subjects contributed by 6 authors. The topics are as follows: "Ruthenium(II) Ammines—A Study in Reactivity" by Henry Taube (46 p.), "Prebiotic Biochemistry (Chemical Evolution)" by Richard M. Lemmon (36 p.), "Rates of Ionization of Carbon Acids" by J. R. Jones (30 p.), "Pericyclic Reactions and Orbital Symmetry" by K. N. Houk (96 p.), and "Conformation–Function Relationships in Peptides and Proteins. Part 1. Naturally Occurring Peptides" by H. R. Wyssbrod and W. A. Gibbons (118 p.). An author index of 11 p. and a subject index of 3 p. concludes the work.

The subject titles and the page coverage gives the degree of emphasis on each point. The book might be of greater interest to those specializing in organic or biochemistry. The reviewer feels that the material would be of little interest to microchemists. As a professor of analytical chemistry he found it of little interest to him. Thinking that this might be a chance selection of one volume, a quick survey of the previous 5 volumes in the series appeared to the reviewer to show a limited scope of coverage with lack of material on current analytically related techniques that might be of interest to chemists in all disciplines for example, such chromatographic techniques as GLC, TLC, HPLC; electrophoresis; ESCA; organic analysis; polymer characterization; computer techniques; environmental analysis, etc. He was not impressed and, at the price, in his mind questions not only how many would buy the book as a personal holding, but also if it is indeed worth buying. The latter would apply particularly as a purchase for those of analytical orientation.

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

Excited States, Vol. 1. Edited by EDWARD C. LIM. Academic Press, New York, 1974. xii + 347 pp. $24.50.

This is the initial volume in a new series dedicated to providing an authoritative guide to the advances in fundamental research on the formation, properties and relaxation of electronically excited states of polyatomic molecules.

The six chapters in this volume reflect the wide ranging work in this field. In the first, Molecular Electronic Radiationless Transitions are treated historically but include a number of post-1970 references.

Double Resonance Techniques and the Relaxation Mechanisms Involving the Lowest Triplet Scale of Aromatic Compounds is as comprehensive as the title is long, covering both theoretical and experimental aspects.

The third chapter, Optical Spectra and Relaxation in Molecular Solids, presents both a qualitative account of phenomena using conventional techniques and an elaboration of problems involving phonons. The phonon data is treated in some detail.

Chapter 4, Dipole Moments and Polarizabilities of Molecules in Excited Electronic States, treats the subject comprehensively with many current references.

The next chapter, Luminescence Characteristics of Polar Aromatic Molecules, carves out a small segment for discussion, namely monosubstituted benzenes and naphthalenes with either electron donating or withdrawing groups. Focus is on the emission characteristics that differentiate polar from nonpolar molecules.

The last chapter, Interstate Interaction in Aromatic Aldehydes and Ketones, points up the importance of interstate interaction caused by vibronic and media effects to aromatic carbonyl spectra.

Each of the chapters has its own bibliography. The book has an author and subject index, and is well presented.

BILL ELPERN, *9 Surrey Way, White Plains, New York 10607*

Analytical Methods for Pesticides and Plant Growth Regulators. Vol. 7. Thin-Layer and Liquid Chromatography Analyses of Pesticides of International Importance. Edited by GUNTER ZWEIG AND JOSEPH SHERMA. Academic Press, New York, 1973. xvii + 729 pp. $45.00.

The first volume of *Analytical Methods for Pesticides and Plant Growth Regulators* was introduced over 10 yr ago. Subsequent volumes have expanded and updated the field by introducing the application of newer techniques and reviewing the current literature. Volumes 5, 6, and 7 present an overview of the chromatographic approach to the determination of pesticides and plant growth regulators. Volume 7 expands the thin-layer chromatographic technique (TLC) found in volume 5 and presents the application of high pressure liquid chromatography (HPLC) for pesticide determinations.

Chapter 1 reviews current developments in the techniques and equipment for TLC. The chapter deals with developments basically after 1967 and includes the application of TLC for the determination of many insecticides, herbicides, pesticides, and fungicides. Chapter 2 introduces the exciting new field of HPLC and discusses the theory and principles, detectors, chromatographic columns, and applications. Both chapters present an excellent literature review and discuss several applications of these chromatographic techniques.

The subsequent parts of this volume contain chapters on the formulations and residue analysis of individual compounds grouped according to uses, e.g., insecticides, herbicides,

fungicides, and rodenticides. These chapters contain general information; such as emperical formula, alternate names, sources of analytical standards, biological properties, history physical properties, chemical properties, and formulation. The chapters discuss analyses and includes reagents, apparatus, experimental procedures, and limits of detection. This volume stresses the importance of the international scope of pesticide analysis and includes over 40 new pesticides—mostly products of European and Japanese companies.

The chapters on new developments in TLC and HPLC should aid in familiarizing the pesticide researcher with these chromatographic techniques. Although only a very basic discussion of the theory and principles of these chromatographic techniques is present, the application of this fast growing methodology as applied to pesticide determinations should be extremely valuable to the pesticide chemist. Despite the thoroughness of the chapters on specific pesticides nearly all the compounds are not familiar in the U.S. and therefore will find little application in american laboratories. However, the book is well organized and written and should be a valuable reference for the pesticide researcher.

DAVID F. TOMKINS, *Corporate Research Dept., Monsanto Co., St. Louis, Missouri 63166*

Assay of Vitamins in Pharmaceutical Preparations. By MANZUR-UL-HAQUE HASHMI. Wiley (Interscience), New York, 1973. xv + 515 pp. $32.00.

In this book the following vitamins are individually dealt with in separate chapters: vitamin A, vitamin B_1 (thiamine), vitamin B_2 (riboflavin), pantothenic acid and pantothenyl alcohol, nicotinic acid and nicotinamide, vitamin B_6 (pyridoxine, pyridoxal, and pyridoxamine), folic acid, vitamin H (biotin), vitamin B_{12} (cyanocobalamin and aquocobalamin), vitamin B_{15} (pangamic acid), vitamin C (*l*-ascorbic acid), vitamin D (ergocalciferol or vitamin D_2 and cholecalciferol or vitamin D_3), vitamin E (α-tocopherol), different forms of vitamin K, *p*-aminobenzoic acid, vitamin P (rutin), choline, and inositol. An assay procedure is given for each vitamin by itself as well as in a finished pharmaceutical formulation in the presence of other vitamins.

The assay procedures are basically of chemical, microbiological, or biological nature. Chemical and physicochemical methods used in the assay procedures were the following: titrimetry (aqueous and nonaqueous), spectrophotometry (ultraviolet, visible, and infrared), fluorometry, potentiometry, polarography, radiometry, column-, thin layer-, paper-, and gas chromatography. A chapter on stability of vitamins in pharmaceutical preparations was treated mainly on the basis of the incompatibility of vitamins with other chemicals. This chapter should be also of interest for the pharmaceutical formulator. Other special chapters concern the simultaneous determination of vitamins by a single procedure applying chemical, physicochemical, or microbiological techniques. The chapter on automated analysis is based mainly on Technicon instrumentation.

The references cited go as far as 1971. This is probably the main reason why no high-efficiency liquid chromatography was mentioned at all. Since 1972 assay procedures concerning the use of high-efficiency liquid chromatography in the quantitative determination of hydrophobic as well as hydrophilic vitamins started to appear in the U.S. chemical literature. Prior to 1972 some procedures pertaining to vitamin analysis using high-efficiency liquid chromatography were communicated in the trade publications of commercial chromatographic equipment manufacturers and supply houses. High-efficiency liquid chromatography at present is a powerful, fast and ideal technique in pharmaceutical analysis and its use is expanding very rapidly. The absence of this discipline in this book points to the

fact that a publication of this nature can become obsolete in a short period. In the chapter on riboflavin, the reagent mercuric sulfate used in the spectrophotometric assay was referred to numerous times as Denige's reagent. This should be corrected to Denigès' reagent.

Overall the book is a handy one to be on the pharmaceutical analysts' desk, especially if one is engaged in vitamin analysis.

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Bacterial Membranes and Walls (Microbiology Series, Vol. 1). Edited by LORETTA LEIVE. Dekker, New York, 1973. xv + 495 pp. $38.00.

The book is divided into three sections: (1) Biosynthesis and Assembly; (2) Interaction with the Environment; (3) Morphogenesis and Reproduction. The publishers suggest that the book "not only discusses past and present developments in the field, but points the way to the design of future experiments." This is an accurate description of the book. The chapters are as follows: (1) Synthesis and Assembly of Bacterial Membranes by Leonard Mindich; (2) Biosynthesis of Peptidoglycan by Jean-Marie Ghuysen and Gerald D. Shockman; (3) Biosynthesis and Assembly of Lipopolysaccharide and the Outer Membrane Layer of Gram-Negative Cell Wall by Hiroshi Nikaido; (4) Present Status of Binding Proteins that are Released from Gram-Negative Bacteria by Osmotic Shock by Barry P. Rosen and Leon A. Heppel; (5) Bacterial Transport Mechanisms by H. R. Kaback; (6) Colicins by S. E. Luria; (7) Cell Surface Structures and the Absorption of DNA Molecules During Genetic Transformation in Bacteria by Alexander Tomasz; (8) Bacterial Division and the Cell Envelope by Arthur B. Pardee, Po Chi Wu, and David R. Zusman; (9) Determinants of Cell Shape by Ulf Henning and Uli Schwarz. Note that the subjects covered encompass most of the important areas of research concerned with bacterial membranes and walls and that the chapters are written by the world's leading experts in these areas. The chapters are extremely well written, comprehensive in the treatment of the material, and very importantly, reflect a critical evaluation of data and concepts. Of particular usefulness is the description and evaluations of some of the methodology used in studying cell membranes and walls. Students, researchers, and especially teachers should find the text invaluable if for no other reason than it represents a series of first-rate articles about many different aspects of bacterial membranes and walls in one text. However, the price of the book is very high. Individuals who contemplate purchasing it should realize that (1) the area is moving rapidly and that many aspects of the book will not remain up to date, (2) much of the information can be garnered from already published review articles, and (3) newer review articles on these subjects will no doubt appear in the near future.

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Methods of Media Preparation for the Biological Sciences. By JOYCE A. STEWART. Thomas, Springfield, IL, 1974. vii + 100 pp. $7.50.

This little book contains everything you need to know about media (but have forgotten where to look!). The author has done a service in gathering this information from nu-

merous sources and presenting it in such an easy-to-use form. No words are wasted. Equipment, procedures, and formulas are given clearly and concisely. There is an ample bibliography and a complete index. This conveniently sized and inexpensive volume will be highly useful for both neophyte and veteran. As such, it is unreservedly recommended.

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ANNOUNCEMENT

The 22nd Spectroscopy Symposium of Canada sponsored by the Spectroscopy Society of Canada in collaboration with the Analytical Section of the Chemical Institute of Canada and the Canadian Probe Users Group, will be held in Montreal, Quebec, Canada, 27–29 October 1975.

Location of the Symposium will be the Sheraton–Mount Royal Hotel situated in downtown Montreal. Papers are solicited for approximately twelve sessions on all phases of spectroscopy to be presented, which will be complemented by a full instrumentation exhibit area.

Information can be obtained from:

Mr. P. J. Skerry, Symposium Chairman
Northern Electric Co., Ltd. Dept. K311
P.O. Box 6124
Montreal, Quebec, Canada H3C 3J4

or,

Miss C. Ratzkowski, Program Chairlady
Hoffmann-LaRoche Control Laboratory
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