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Briefs

The Reactions of Nitric Oxide, Nitrous Oxide, and Nitrogen Dioxide with Lead Dioxide. H. EDWARD MISHMASH AND CLIFTON E. MELOAN, *Department of Chemistry, Kansas State University, Manhattan, Kansas 66502.*

Utilizing the gas chromatographic technique for the determination of gases, and standard analytical techniques for the examination of the products, the reactions of several nitrogen oxides with lead dioxide under the conditions employed in a C, H analysis were studied in detail. It was concluded that the presently accepted reactions are incorrect and new reactions are proposed.

Microchem. J. **14,** 181 (1969).

A Spectrophotometric Study of the Chelates of Chromotrope 2R with Scandium and Yttrium. SHRIKANT B. DABHADE AND SATENDRA P. SANGAL, *Laxminarayan Institute of Technology, Nagpur University, Nagpur, India.*

A detailed study of the metal chelates formed by Chromotrope 2R with scandium and yttrium by spectrophotometric methods was described. The true stability constants of the metal chelates were derived by maintaining different ionic strengths.

Microchem. J. **14,** 190 (1969).

The Determination of Metals in Organic Compounds by Oxygen-Flask Combustion or Wet Combustion. A. M. G. MACDONALD AND P. SIRICHANYA, *Chemistry Department, The University, Birmingham 15, England.*

Decomposition by the oxygen-flask method followed by titration with EDTA is suitable for the determination of the metal in organic compounds containing calcium, magnesium, zinc, barium, manganese, or cobalt. Compounds containing nickel, copper, iron, or bismuth are better decomposed by wet combustion processes, because of alloy formation or insoluble oxide formation. Optimal indicators for the EDTA titrations are discussed.

Microchem. J. **14,** 199 (1969).

Application of Back Titration of EDTA with Mercury (II) to the Analysis of Alloys. III. Analysis of Copper–Cadmium, Copper–Nickel, and Copper–Nickel–Zinc Alloys. H. KHALIFA AND I. EL-BARBARY, *Chemical Department, Ministry of Industry, Cairo, U.A.R.*

Eleven synthetic and 3 commercial alloys involving Cu–Cd, Cu–6Ni, and Cu–Ni–Zn combinations together with minor constituents of Pb, Fe(III), Al, Sn, C, and Si were successfully analyzed. The procedures applied involved potentiometric

back titration of excess EDTA or CDTA with mercury(II) together with the additional classical methods, with the purpose of simplifying and making rapid such an analysis.

Microchem. J. **14,** 207 (1969).

Qualitative Detection Methods Based on the Liberation of Ammonia from Hexamminecobalt(III) Perchlorate. EDWARD J. POZIOMEK, ETHEL B. HACKLEY, DANIEL J. HOY, AND H. GEORGE FRIEDMAN, JR., *Physical Research Laboratory, Edgewood Arsenal, Maryland 21010.*

A number of compounds were screened for their general ability to liberate ammonia from hexamminecobalt(III) perchlorate in aqueous buffer and dimethyl sulfoxide. The ammonia was detected by a strip of moistened pH paper hung above the solution in a closed vial. Various compounds were detected, e.g., sodium iodide, phenols, hydroquinone, sodium sulfite, trypsin, Malathion, DDT, and several amines.

Microchem. J. **14,** 217 (1969).

Titrimetric Microdetermination of L-Asparagine. A. K. SAXENA AND O. C. SAXENA, *Chemical Laboratories, University of Allahabad, Allahabad, India.*

L-Asparagine was determined in microquantities with thorium nitrate using bromocresol green as indicator. Range in which L-asparagine was estimated lies between 7.2×10^{-4} and 86.4×10^{-4} mg/liter. Maximum error was 0.9%. It was observed that L-leucine, DL-valine, glycine, and DL-alanine do not interfere.

Microchem. J. **14,** 224 (1969).

Rapid Micromethod for Locating the Oxirane Group in 1,2-Epoxides. G. R. MIZUNO, E. C. ELLISON, AND J. R. CHIPAULT, *University of Minnesota, The Hormel Institute, Austin, Minnesota 55912.*

A procedure for the analysis of micro amounts of epoxides is described. The method is based on the splitting of epoxides by periodic acid in a nonaqueous medium, followed by direct analysis of the reaction mixture by gas chromatography.

Microchem. J. **14,** 227 (1969).

Disc Electrophoresis of Subclasses of Human Serum Low Density Lipoproteins. K. ANANTH NARAYAN, G. ELIZABETH S. MARY, AND H. P. FRIEDMAN, *The Burnsides Research Laboratory, University of Illinois; and The Carle Hospital Clinic, Urbana, Illinois 61801.*

Serum chylomicrons, very low density lipoproteins ($d < 1.006$) and two classes of low density lipoproteins ($1.006 < d < 1.019$ and $1.019 < d < 1.050$) were

isolated from freshly collected normal human blood. Disc electrophoresis was performed with microquantities of these fractions using the standard spacer gel and with main gel concentrations of 1.9, 2.5, 3.0, 3.75, and 5.0%. Three different stains, namely, amido black 10B, Sudan black B, and oil red O were employed for detecting the lipoprotein components in gels of 3.0, 3.75, and 5.0% concentration and Sudan black B prestain was used for gels of 2.5 and 1.9% concentration.

Microchem. J. **14,** 235 (1969).

Barium Titration of Sulfate with Chlorophosphonazo III as Indicator. B. BUDESINSKY, *Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada.*

Chlorophosphonazo III may be used for visual indication of a sulfate preciptation titration with barium chloride, perchlorate or nitrate. Titration in acidic medium up to 0.1 *N* of strong mineral acid and application of EDTA masking are possible. The determination of organic sulfur by the combustion method is also possible with use of this indicator.

Microchem. J. **14,** 242 (1969).

Microcrystalloptic Tests for Some Minor Alkaloids of *Peumus boldus*. K. GENEST, LORNA J. LOWRY, AND D. W. HUGHES, *Research Laboratories, Food and Drug Directorate, Ottawa, Ontario, Canada.*

Microcrystal tests for reticuline, isoboldine, laurotetanine, laurolitsine and isocorydine-N-oxide are described. In addition to crystal habit, other microoptical data, such as birefringence, extinction, angle of extinction, signs of elongation and absorption, and pleochroism were measured.

Microchem. J. **14,** 249 (1969).

A Spectrophotometric Study of the Chelates of Chromotrope 2R with Praseodymium, Neodymium, Samarium, and Europium. V. L. SHAH AND S. P. SANGAL, *Laxminarayan Institute of Technology, Nagpur University, Nagpur, India.*

The formation of the violet colored complexes of 4,5-dihydroxy-3-phenylazo-2,7-naphthalenedisulfonic acid, di Na salt (Chromotrope 2R) and praseodymium, neodymium, samarium, and europium was reported.

Microchem. J. **14,** 261 (1969).

***N*-Bromosuccinimide as a Direct Titrant for Thiourea and Thioacetamide Using Bordeaux Red as an Indicator.** R. J. THIBERT AND M. SARWAR, *Department of Chemistry, University of Windsor, Windsor, Ontario, Canada.*

A rapid, precise, and accurate method for the determination of small amounts of thiourea and thioacetamide is described. Bordeaux red was a suitable indicator

when *N*-bromosuccinimide was used as direct titrant. From 5–0.3 mg of thiourea and thioacetamide were analyzed with an average standard deviation of about 0.005 mg.

Microchem. J. **14,** 271 (1969).

New Titrimetric Microdetermination of Acetoin (2-Butanone-3-hydroxy) and Tartronic Acid (Hydroxymalonic Acid). J. P. MALL AND S. CHANDRA, *Research Studies Pathology Centre, Allahabad-2, India.*

The method depends on the oxidation of the substances with copper(III).

Microchem. J. **14,** 275 (1969).

General Method for Microdetermination of Mercury in Organic Compounds. CHING SIANG YEH, *Department of Chemistry, Purdue University, Lafayette, Indiana 47907.*

A method was developed for the analysis of all types of organomercury compounds. The use of a strong acidic and powerful oxidizing digestion medium consisting of potassium permanganate, concentrated nitric, and concentrated sulfuric acids afforded rapid conversion to Hg(II) without loss of mercury by volatilization and with complete removal of halogen. By means of a second-derivative spectrophotometric titrator coupled to an automatic buret even dilute Hg(II) solutions obtained from micro-samples can be analyzed accurately by the Volhard method.

Microchem. J. **14,** 279 (1969).

New Titrimetric Microdetermination of Formic and Acetic Acids in Their Mixture. P. K. JAISWAL AND S. CHANDRA, *Research Studies Pathology Centre, Allahabad-2, India.*

Titrimetric microdetermination of formic and acetic acids is described. Formic acid required two equivalents of the oxidant for complete oxidation, whereas, acetic acid required eight equivalents of the oxidant. Ceric sulfate oxidized only formic acid but Cu(III) oxidized formic acid as well as acetic acid. Interference was observed if other organic compounds were present.

Microchem. J. **14,** 289 (1969).

Rapid Microdetermination of Hemoglobin Iron: Standardization of Hemoglobin. E. S. BAGINSKI, P. P. FOÁ, S. M. SUCHOCKA, AND B. ZAK, *Divisions of Laboratories and Research, Sinai Hospital of Detroit and the Departments of Pathology, Wayne State University School of Medicine and Detroit General Hospital, Detroit, Michigan 48207.*

A simple method for the determination of iron in hemoglobin is described. It includes a rapid (3-minute) digestion of red cell hemolysate by nitric acid and

subsequent analysis of the iron in the residue. Inclusion of phosphate in the nitric acid prevented apparent loss of standard iron by volatilization or formation of unreactive iron oxides. Accuracy is proven by recovery of standard iron, comparative analysis of hemoglobin iron, and iron additions to redetermined base values.

Microchem. J. **14,** 293 (1969).

Microdetermination of Thorium. R. D. THAKUR AND R. P. AGRAWAL, *Chemical Laboratories, University of Allahabad, Allahabad-2, India.*

Thorium was determined titrimetrically by dissolving the complex, formed by boiling thorium nitrate and sodium rhodizonate together, in dilute H_2SO_4. The dissolved complex in acid solution was titrated against standard ceric sulfate solution using *N*-phenylanthranilic acid as indicator. Complex is formed in the ratio of 1:2.

Microchem. J. **14,** 298 (1969).

The Microdetermination of Mercuric Mercury. MOHAMED ZAKI BARAKAT, AHMED SHAWKI FAYZALLA, AND RAGA RAGAB HASSANEIN, *Biochemistry Department, Faculty of Medicine, Azhar University, Madina Nasr, Cairo, U.A.R.*

A new titrimetric method for the microdetermination of mercuric mercury is described. The proposed method is based on the fact that aqueous L-ascorbic acid solution reduces mercuric mercury to mercurous mercury; itself being oxidized to dehydro-L-ascorbic acid.

Microchem. J. **14,** 302 (1969).

Use of Microcosmic Salt as a New Titrant for the Microdetermination of Benzoic, Salicylic and Phthalic Acids. A. K. SAXENA, M. N. SRIVASTAVA, AND B. B. L. SAXENA, *Chemistry Department, University of Allahabad, Allahabad, India.*

Benzoic, salicylic, and phthalic acids were determined in micro quantities with a new titrant, i.e., microcosmic salt solution, using bromcresol purple as indicator. Estimations were made in the range 0.126–1.257 mg of benzoic acid, 0.075–0.746 mg of salicylic acid, and 0.083–0.831 mg of phthalic acid with maximum error of ± 0.004 mg.

Microchem. J. **14,** 315 (1969).

The Extraction and Photometric Determination of Zinc in the Presence of Large Amounts of Cadmium Using 1-(2-Pyridylazo)-2-naphthol (PAN) and Employing Iodide Masking. H. FLASCHKA AND WEISS, *School of Chemistry, Georgia Institute of Technology, Atlanta, Georgia. 30332.*

The zinc in high purity cadmium is determined photometrically as the PAN complex after extraction into chloroform. Massive amounts of potassium iodide

are added to the sample solution to mask cadmium. Copper, mercury, lead, and silver are also masked by the iodide. Some cyanide is added which allows masking of nickel and cobalt. Tolerance limits for these and several other interferences are given. The results are reliable to several tenths of a microgram of zinc. Samples containing up to about 1 g of cadmium can be handled. Zinc can be determined even at a cadmium to zinc molar ratio of 500,000:1; although additional operations are required at ratios above 100,000:1.

Microchem. J. **14,** 318 (1969).

Human and Rat Serum Proteins, Lipoproteins, Ammonium Persulfate, Gel Concentration, and Disc Electrophoresis. K. ANANTH NARAYAN, *The Burnsides Research Laboratory, University of Illinois, Urbana, Illinois 61801.*

Extensive electrophoretic experiments were conducted with serum proteins and lipoproteins substituting riboflavin and light as a catalyst for ammonium persulfate at several gel concentrations, removal of ammonium persulfate from the gel by preliminary electrophoresis and incubation of serum proteins and isolated lipoproteins with ammonium persulfate prior to electrophoresis. It was established that ammonium persulfate does not produce artifacts during disc electrophoresis using the pH 9.5 system.

Microchem. J. **14,** 335 (1969).

I. Microdetermination of DL-alanine. II. Microdetermination of L-glutamic acid, DL-valine, and DL-alanine in the form of a Mixture in one solution without separating. O. C. SAXENA, *Chemical Laboratories, University of Allahabad, Allahabad-2, India.*

Sodium tungstate, gold chloride, and potassium tellurite form complexes in definite ratios with the various compounds to be determined. Potentiometric data support these complex ratios.

Microchem. J. **14,** 343 (1969).

MICROCHEMICAL JOURNAL **14**, 181–189 (1969)

The Reactions of Nitric Oxide, Nitrous Oxide, and Nitrogen Dioxide with Lead Dioxide

H. EDWARD MISHMASH AND CLIFTON E. MELOAN

Department of Chemistry, Kansas State University, Manhattan, Kansas 66502

INTRODUCTION

The equations for the reaction of the oxides of nitrogen with lead dioxide which have long been accepted by workers in the area of organic microchemical analysis are:

For nitric oxide

$$2NO + 2PbO_2 \rightarrow Pb(NO_2)_2 \cdot PbO + 0.5O_2, \quad (1)$$

and for nitrogen dioxide

$$2NO_2 + 2PbO_2 \rightarrow Pb(NO_3)_2 \cdot PbO + 0.5O_2. \quad (2)$$

These equations were prosed by Kirner (*1*) in 1938.

Until the work of Kirner on the reaction of the nitrogen oxides, NO and NO_2, with lead dioxide, there was much controversy and little experimental confirmation of the exact reactions. Dennstedt and Hassler (*2*) claimed that nitric oxide (NO) could pass over lead dioxide (sometimes incorrectly referred to as lead peroxide) without being absorbed. However, Auden and Fowler (*3*) stated that a basic lead nitrate is formed starting at 15°C, which reaches a maximum concentration about 130°C, with a trace of the nitrite also formed. Sabatier and Lenderens (*4*) suggested that the metallic peroxide was reduced and nitrogen dioxide (NO_2) formed. Moser (5) proposed the following equations for the results:

$$3PbO_2 + 2NO \rightarrow Pb(NO_3)_2 \cdot PbO + PbO \quad (3)$$

$$PbO_2 + 2NO \rightarrow Pb(NO_2)_2 \quad (4)$$

Muller and Barck (*6*) stated that nitric oxide is completely absorbed by lead dioxide at room temperature forming the nitrite, as in Eq. (1), and that above 200°C, oxygen is liberated from the lead dioxide. Lindner (*7*) found that at 180°C, 0.465 g of lead dioxide absorbed

32 ml of nitric oxide. Theoretically, according to Eq. (4), it should have absorbed 87 ml and according to Eq. 5, 29 ml. Thus, there was quite a discrepancy as to the actual products of the nitric oxide–lead dioxide reaction.

The exact interaction of nitrogen dioxide with PbO_2 was not known either. Kopfer (*8*), Dennstedt and Hassler (*9*), Friedrich (*10*), and Hermann (*11*) believed that the nitrogen dioxide reacted with the lead dioxide forming the lead nitrate according to the following equation:

$$PbO_2 + 2NO_2 \rightarrow Pb(NO_3)_2 \qquad (5)$$

There was, however, little or no experimental proof of Eq. (5) by any of the above workers.

Therefore, Kirner (*1*) decided to study the mechanism in detail. His procedure involved passing a measured amount of either nitric oxide or nitrogen dioxide through an absorption tube containing lead dioxide at the end of a combustion train and then measuring the change in weight of the tube. Nitrogen was used as the carrier gas for nitric oxide and oxygen was used for the nitrogen dioxide. From the weight gains that he obtained, Kirner proposed the presently accepted equations.

These equations were based on the fact that the weight gain was not equal to the weight of the particular nitrogen oxide added. Examination of the water in the collecting Mariotte bottle showed only a trace of any nitrogen oxide when the diphenylamine–sulfuric acid test was used *(12)*; Eq. (1) and (2) gave the best fit for the data obtained.

We had hoped to use the removal of the oxides of nitrogen and the corresponding evolution of oxygen in a reactive column gas chromatographic experiment. It was noted that the oxides of nitrogen were removed but that there was no oxygen peak. Since there were several other aspects of the proposed equations which did not seem correct, the incentive was provided to study the reaction of the nitrogen oxides, NO, NO_2, and N_2O, with lead dioxide. Gas chromatography, polarography, and qualitative chemical analysis techniques were used.

EXPERIMENTAL METHODS

The basic ideas was to attach a gas chromatograph to the PbO_2 portion of a C,H apparatus, and examine the gaseous products. Polarographic data and qualitative chemical analysis were used to examine

the solid residues. Because the reactions occurred only on the surface of the particles, quantitative data could not be obtained on the residue.

CHEMICALS

Lead dioxide. Fisher "special micro" no. L-100 lead dioxide was used as the packing of the reactive column.

Molecular sieve. A 5A molecular sieve, 30/60 mesh was used as the packing of the chromatgraphic column.

Nitrogen oxides. Nitric oxide, nitrogen dioxide, and nitrous oxide (ca. 99% pure) were obtained from the Matheson Company and used as received.

Helium. Helium was used as the carrier gas and inert atmosphere needed in this study.

Other chemicals. All other chemicals used were of a reagent grade purity.

APPARATUS

Constant temperature bath. A Sargent constant temperature water bath, model S–84805, was used to maintain a temperature of 25.0 $\pm$ 0.1°C on the separation column. This was external to the main body of the gas chromatograph (Fig. 1).

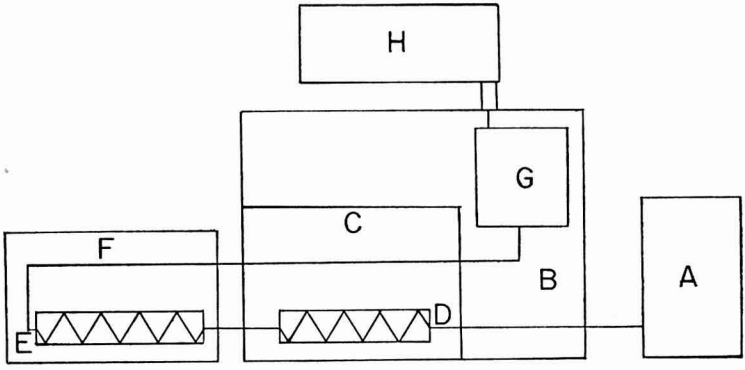

FIG. 1. Diagram of chromatograph medification: (A) helium carrier gas cylinder; (B) gas chromatograph body and controls; (C) gas chromatograph column even; (D) reaction column (PbO_2); (E) chromatographic column (molecular sieve); (F) constant temperature water bath; (G) thermal conductivity detector; and (H) recorder.

Nickel tubing. Because of the reactivity of the nitrogen oxides with copper, 0.25-inch o.d. nickel tubing was used for the reaction part of the column.

Aluminum tubing. The tubing used for the chromatographic part of the column was 0.25-inch o.d. aluminum. The cost and workability made it more desirable for this application.

Gas chromatograph. A Micro-Tek, model 2500R, gas chromatograph, utilizing a thermal conductivity detector, was used for all measurements.

Syringe. A Hamilton 50-μl, "gas-tite," syringe equipped with a Chaney adaptor was used for all samples injected into the gas chromatograph.

Recorder. A Sargent model SR recorder equipped with a disc integrator was used to record all the chromatographic measurements.

Polarograph. Sargent model XXI.

PROCEDURE

Column preparation. The reaction column was a 2-foot by 0.25-inch o.d. nickel tube that was packed with lead dioxide using a vibrator. Nitrogen dioxide will not pass through a 5A molecular sieve under normal conditions. However, when the sieve is treated with water, the NO_2 is converted to NO which will then be eluted through the column. There is a reduction factor of three in the volume of products formed. The equation for this transformation is:

$$3NO_2 + H_2O \rightarrow NO + 2HNO_3$$

A 10-foot by 0.25-inch o.d. aluminum tube was packed with 5A molecular sieve and 2 ml of water was added by means of a syringe. The maximum sensitivity of the column was achieved by conditioning it for the gas studied: several milliliters of NO or NO_2 were passed through it before starting an analysis.

Gas chromatographic studies. The reaction column was kept in the column oven portion of the gas chromatograph while the separation portion was external. This was emersed in the constant temperature water bath at 25°C. A 50-μl sample of nitrous oxide, nitric oxide, or nitrogen dioxide was then injected into the gas chromatograph and the

resulting chromatogram recorded. The temperature of the reaction column was varied, as was the flow rate of the carrier gas, to see if any changes in reaction would occur.

Product analysis. Qualitative techniques were used to study the products formed by the various gases and the lead dioxide and will be discussed later.

Reaction column check. In order to prevent acceptance of any data that might be the direct result of an interaction with the molecular sieve and not the reaction column, the gases were also determined with the reaction column removed.

RESULTS AND DISCUSSION

Since the accepted reactions have oxygen as the only gaseous product, gas chromatography was the choice of methods to check their validity. Gas chromatograms of the unreacted gases could be easily compared to the reacted samples, and the oxygen peak studied. The retention times of oxygen and nitrogen were obtained by running as chromatograms of air. This also served the purpose of being a check on the quality of the molecular sieve in its ability to achieve the separation.

A 50-μl sample was chosen for this investigation. According to Kirner (*1*) this should correspond to 12.5 μl of oxygen being formed from the reaction. The sensitivity was adjusted to give this amount nearly a full-sale deflection on the recorder.

Nitrous oxide (N_2O). Although nitrous oxide was not one of the gases studied by Kirner, it was decided that it might possibly react with lead dioxide, or be a product, and should also be investigated. When 50 μl of nitrous oxide was injected into the gas chromatograph, only one small peak was observed. This peak was less than 1% of the sample and a check of the "air chromatogram" revealed that this was nitrogen. No oxygen peak was present. During investigation of what caused the removal of nitrous oxide, a check with the lead dioxide column removed gave the same result. In another cross check of columns, the molecular sieve portion was removed, leaving only the lead dioxide section; N_2O came through in quantitative amounts, but no oxygen was observed. Therefore, it was concluded that the nitrous oxide was being retained on the molecular sieve and was not reacting with the lead dioxide.

Nitric oxide (NO). In the microdetermination of carbon and hydrogen, the lead dioxide is kept at 178°C. So, with the PbO_2 at 178°C, the molecular sieve at 25°C, and helium flow rate of 30 ml/minute, a 50-μl sample of nitric oxide showed only a minute air contamination of about 1%. The column check shows that nitric oxide will pass through molecular sieve but reacts with lead dioxide as expected. The important thing is that there is no oxygen peak. This reaction was studied at 178, 137, 67, and 35°C and at flow rates from 30 to 110 ml/min, all with the same results. The fact that the nitric oxide has reacted and that no oxygen was formed means that the presently-accepted reaction for NO with PbO_2 must be incorrect.

Nitrogen dioxide (NO_2). As mentioned previously, the molecular sieve was moistened prior to the analysis of nitrogen dioxide. A 50-μl sample gave the same results as with nitric oxide; NO_2 reacts and no oxygen is formed. The same results are obtained at various temperatures and flow rates. This leads to the conclusion that the reaction of nitrogen dioxide with PbO_2 must also be incorrect.

Water analysis. If water from the surface or entrapped moisture was a product of this reaction, it would not have been detected because of the molecular sieve. Also, the data of Kirner would permit substitution of water for 0.5 O_2 in Eq. (1) and (2) on the basis of molecular weight, with no problem. In order to assure that this was not the case, a polypropylene column was substituted for the molecular sieve. The results showed that no water is formed or released in the reaction.

Proposed reaction (NO_2). In order to substantiate an alternative reaction to the one in the literature, the products formed had to studied. To do this a 6 $\times$ 0.25-inch o.d. nickel tube was packed with lead dioxide. One end of this tube was attached to a cylinder to nitrogen dioxide and the gas was allowed to pass through this tube for about 30 seconds.

Since this is a surface reaction, exact combination data is impossible to obtain accurately. All of the lead dioxide cannot react because of its pellet size, which is about 20 mesh.

There exists, also, a discrepancy in the literature as to whether this is an absorption or actual chemical reaction. It was noted that when the nitrogen dioxide was passed through the packed tube the process was very exothermic. The evolution of large amounts of heat is not a

characteristic of a normal physical absorption. It was, therefore, assumed that the process is one of reaction.

This assumption was further supported by analysis of the products. If this was an absorption, the lead would all be in the 4+ valence state; while a reaction would probably take it to the 2+ state. There would also be NO_2 present and little or no NO_3^-. Adding the product to dilute sulfuric acid gave a white precipitate indicating the 2+ state ($PbSO_4$). No reaction was expected nor occurred when an unreacted lead peroxide control was added to the same dilute acid. The same 2+ state was found using polarography.

The anion analysis involved the brown ring test for nitrate. Several drops of the supernatant liquid from above were tested and a brown ring developed. This did not occur with the control. No PbO yellow color was formed.

Combining this information leads to the conclusion that lead nitrate is the product formed by the reaction of nitrogen dioxide with lead dioxide. This is represented quite simply by the following equation:

$$PbO_2 + 2NO_2 \rightarrow Pb(NO_3)_2.$$

Proposed reaction (NO). Nitric oxide was passed through a similar tube of lead dioxide. The process was again observed to be very exothermic. The products, however, had a different appearance. There was a yellow coating on much of the sample. The qualitative analysis of the product again showed the presence of lead nitrate. The yellow coating was identified as lead oxide, PbO. As was suggested by other workers, including Kirner, the nitric oxide reacts with the metallic dioxide causing its reduction to PbO and the formation of nitrogen dioxide. The equations for the reaction of nitric oxide with lead dioxide are then as follows:

$$2PbO_2 + 2NO \rightarrow 2PbO + 2NO_2 \quad (6)$$
$$PbO_2 + 2NO_2 \rightarrow Pb(NO_2)_2 \quad (7)$$
$$3PbO_2 + 2NO \rightarrow 2PbO + Pb(NO_3)_2 \quad (8)$$

which agrees completely with the gas chromatographic results.

CONCLUSION

Utilizing the gas chromatographic technique for the determination of gases, and standard analytical techniques for the examination of the

products, the reactions of several nitrogen oxides with lead dioxide under the conditions employed in a C,H analysis were studied in detail. It was concluded that the presently accepted reactions are incorrect and new reactions are proposed as follows:

Nitrous oxide (N_2O)

$$PbO_2 + N_2O \rightarrow \text{N.R.}$$

Nitric oxide (NO)

$$PbO_2 + NO \rightarrow PbO + NO_2$$
$$2NO_2 + PbO_2 \rightarrow Pb(NO_3)_2$$

Nitrogen dioxide (NO_2)

$$PbO_2 + 2NO_2 \rightarrow Pb(NO_3)_2$$

SUMMARY

Evidence is presented to show that the reactions

$$2NO + 2PbO_2 \rightarrow Pb(NO_2)_2 \cdot PbO + 0.5O_2,$$
$$2NO_2 + 2PbO_2 \rightarrow Pb(NO_3)_2 \cdot PbO + 0.5O_2,$$

which have been used for 30 years in conjunction with the micro carbon and hydrogen determinations are probably

$$2NO + 3PbO_2 \rightarrow 2PbO + Pb(NO_3)_2,$$

and

$$2NO_2 + PbO_2 \rightarrow Pb(NO_3)_2.$$

ACKNOWLEDGMENT

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A Spectrophotometric Study of the Chelates of Chromotrope 2R with Scandium and Yttrium

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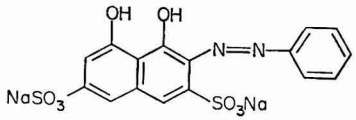
The azo chromotropic compounds offer an interesting field of study as reagents for spectrophotometric determination of metal ions. The present study of Chromotrope 2R (abbreviated as CTR) or phenylazochromotropic acid (disodium salt), designated as Acid red 29 according to the Colour Index has the following structure:



The dye was reported as reagent for the colorimetric determination of U^{6+} *(4)*, Be^{2+} *(5)*, La^{2+} *(7)*, Pb^{3+} *(6)* and Th^{4+} *(2)*. The preliminary studies of the reactions of CTR have been reported recently for various metal ions, viz, copper, zinc, gallium, aluminium, zirconium, hafnium, iron, cobalt, nickel, palladium, praseodymium, tin thorium and uranium *(9)*. The present study describes the reactions and formation of metal chelates and the characteristics of CTR chelates with scandium and yttrium.

EXPERIMENTAL METHODS

Instruments. Toshniwal's pH meter using glass calomel electrodes was used for adjusting pH of the solutions. The spectrophotometric studies were done on Beckman's model operated on stabilized voltage of 115 V. The blue sensitive phototube was used for observations below 600 mμ and the red sensitive phototube was used above 600 mμ. All observations were noted against distilled water blank using 10 mm cells.

Reagents. Standard solution of scandium was prepared to the desired concentration by dissolving specpure scandium chloride (J&M) in double distilled water and the concentration of the metal was checked by

complexometric titration with EDTA *(8)*. Standard solution of yttrium was prepared by dissolving specpure yttrium oxide in a small amount of HCl and the volume was made up by addition of distilled water. Fresh solutions of CTR were each time prepared by dissolving known amount of CTR in double distilled water. Sodium chloride solution was prepared by dissolving a known amount of sodium chloride (BDH AnalaR) in double distilled water.

Conditions of study. All experiments were conducted at 25 ± 2°C. The pH of the solutions was adjusted by addition of HCl or NaOH keeping the total volume of the solution 25 ml in each case. The ionic strength was maintained by addition of sodium chloride of the desired concentration and pH keeping the total volume 25 ml in each case.

RESULTS AND DISCUSSIONS

Absorption spectra of CTR with variation in pH and stability. Different solutions of CTR (5.0×10^{-5} *M*) were adjusted at different pH ranging from 1.5 to 12 and their absorption spectra were recorded. The λ_{max} of the solutions lies at 530 μ at all pH values (Fig. 1). The absorption of various solutions of CTR noted at various pH values at different intervals remained constant for indefinite time.

Range of pH for the formation of metal chelates of scandium and yttrium with CTR and the effect of time. Absorption spectra of the chelates of scandium and yttrium by taking equimolar proportions of the metal and the dye (5×10^{-5} *M*) were recorded from 400 to 600 mμ at different pH values. The change of λ_{max} and the absorbance with pH was noted and the results are recorded in Table 1 (Figs. 2 and 3). The absorbance of these solutions of 1:1(CTR:metal) at different intervals showed that the chelates are stable for indefinite time.

Absorption spectra of the chelates. The method of Vosburgh–Cooper *(10)* was applied for determining the nature of the complexes formed. The absorption spectra of these solutions were noted from 400 to 600 mμ and the results are included in Table 1 (Fig. 4).

Study of the effect of addition of electrolyte to the dye and the chelate. The effect of various electrolytes, viz., NaCl, KCl, $NaNO_3$, and KNO_3 was studied on the dye and the chelates and it was found that NaCl and KCl, gave consistent results. The electrolyte used in the studies is NaCl which is sufficiently soluble in water to give ionic media of the required concentration. It did not contribute to the measured physical

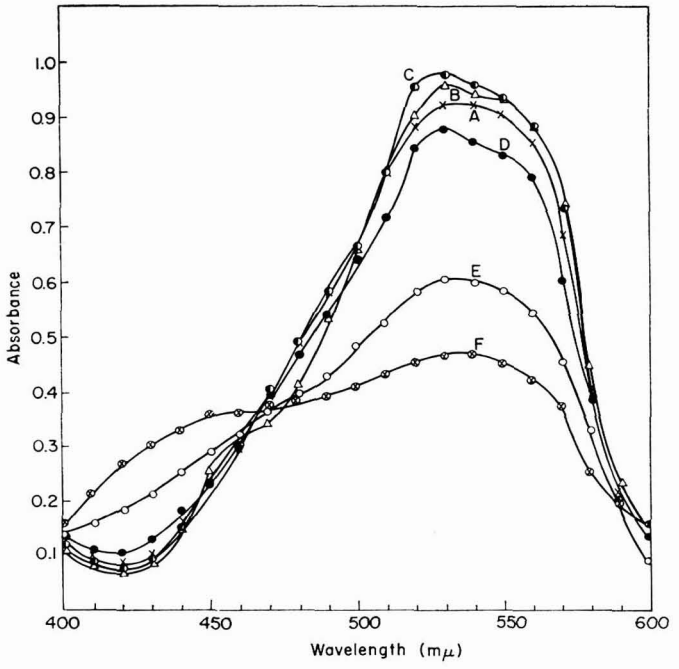


FIG. 1. Absorption spectra of CTR with variation in pH. Final concentration of CTR 5.0×10^{-5} *M*; pH values: Curve A, 2.0; Curve B, 6.0; Curve C, 8.0; Curve D, 9.0; Curve E, 10.0; and Curve F, 11.0.

TABLE 1

CHARACTERISTICS OF THE CHELATES OF SCANDIUM AND YTTRIUM WITH CTR

Metal	pH range of formation and stability of chelate	pH of study	λ_{max} of CTR (mμ)	λ_{max} of chelate (mμ)
Scandium	3.5–5.0	4.5	530	560
Yttrium	5.5–8.5	6.2	530	560

property, specially in the spectrophotometric studies, the absorbance being negligible at the wave lengths used. Further, since NaOH and HCl were used to maintain the pH of the solutions, NaCl was selected for maintaining the ionic strength.

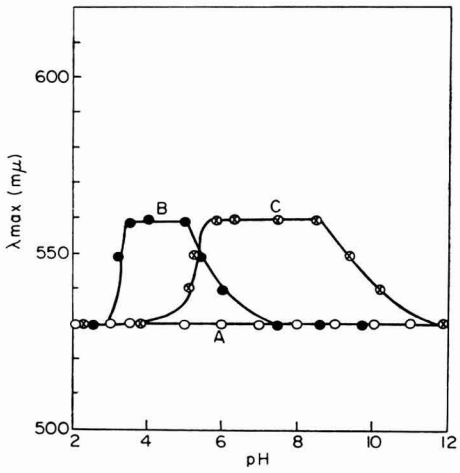


FIG. 2. Variation of λ_{max} with pH. Final concentrations of solutions 5.0×10^{-5} *M*; Curve A, CTR; Curve B, Sc:CTR Chelate; and Curve C, Y:CTR chelate.

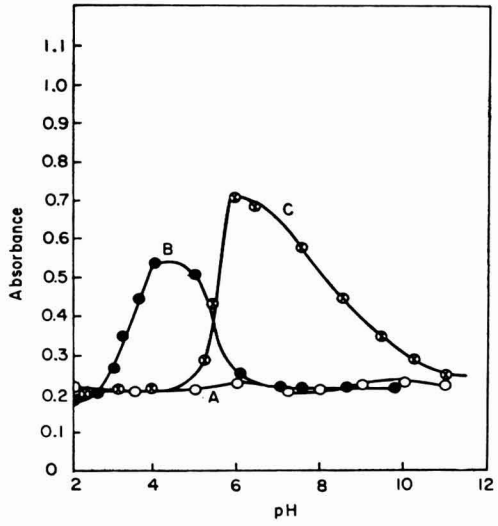


FIG. 3. Variation of absorbance of chelate and CTR with change in pH at 590 mμ. Final concentration of the solution 5.0×10^{-5} *M;* Curve A, CTR; Curve B, Sc:CTR chelate; and Curve C, Y:CTR chelate.

The stoichiometry of the components at various ionic strengths for the study of Sc:CTR and Y:CTR chelates. The continuous variation method was used for the determination of the composition of the metal chelates and the stability constant at various ionic strengths. The study was carried out by taking various concentrations of CTR, and metal solutions in various proportions so that the total volume does not exceed 20

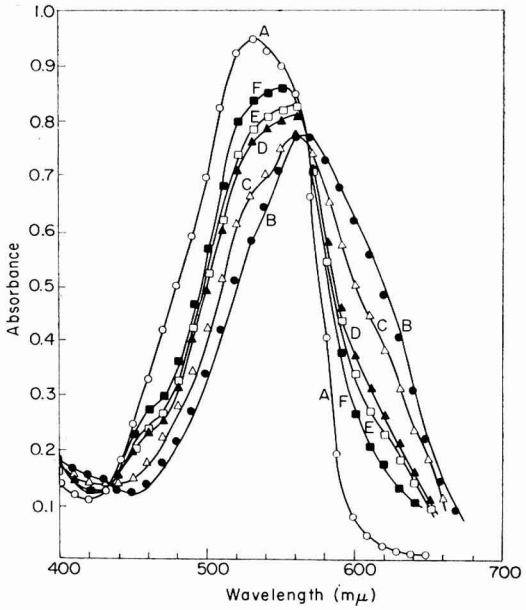


FIG. 4. Absorption spectra of Sc:CTR chelate, pH of study 4.5; CTR ($\times 10^{-5}$ *M*): Curve A, 5.0 CTR; Curve B, 5.0 CTR, and 1.0×10^{-4} *M* $ScCl_3$; Curve C, 5.0 CTR, and 5.0×10^{-5} *M* $ScCl_3$; Curve D, 5.0 CTR, and 2.5×10^{-5} *M* $ScCl_3$; Curve E, 5.0 CTR, and 1.66×10^{-5} *M* $ScCl_3$; and Curve F, 5.0 CTR and 1.25×10^{-5} *M* $ScCl_3$.

ml to which 5 ml of sodium chloride of various concentrations at the optimum pH of study was added, so that the resulting mixtures would be 0.2, 0.16, 0.12, 0.08, and 0.04 μ. The absorbances of these mixtures were noted at 590 and 600 mμ. At both the wavelengths the maximum difference in the absorbances was noted with the blank CTR and the chelate solution containing the same amount of CTR and the composition in both the cases was 1:1.

Evaluation of the stability constants. The values for the apparent stability constants for the chelates of Sc:CTR and Y:CTR were determined at various ionic strengths by the method of Mukherji and Dey *(3)*. The values of log K at various ionic strengths are shown in Table 2.

TABLE 2

STABILITY CONSTANTS OF Sc:CTR AND Y:CTR CHELATES [a]

Ionic strength (μ)	log K Sc:CTR chelate pH of study 4.5	log K Y-CTR chelate pH of study 6.2
0.20	3.69	3.65
0.16	3.80	3.96
0.12	3.92	3.92
0.08	4.23	4.15
0.04	4.04	4.52
0.00 (extrapolated)	4.80	5.25

[a] Composition in both cases 1:1; wavelength 590 mμ; temperature 25°C.

Equilibrium constants for the formation of complexes in solution at given temperature are usually reported as either activity quotients (thermodynamic stability constants) which should be independent of the ionic strengths and medium, or as concentration (stoichiometric stability constants which are valid for a standard defined state by the detailed composition of the solution. The experimental values of the activity quotients are seldom available for the direct substitution in the proper equations. Therefore, the concentration constants or the apparent stability constants are preferred to the activity quotients. However, since the concentration constant is not the real constant but depends on the medium it must be clearly and exactly defined. Any comparison, of course, refers to the values obtained at the same temperature in substantially the same media. In the present study, the stability constants for various ionic strengths were considered for the determination of the true stability constant at $\mu = 0$ by extrapolating the stability constants determined at various ionic strengths. Plots of log K against μ have been suggested *(1)* and the values thus obtained are included in Table 2 (Figs. 5 and 6).

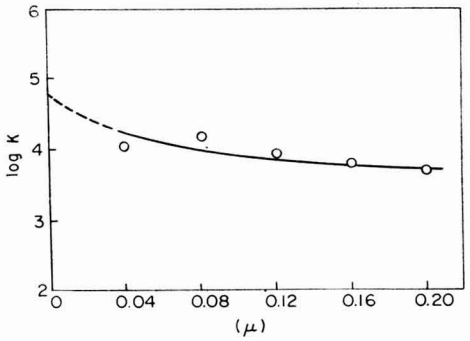


FIG. 5. Variation of log K with change in μ (ionic strength); Sc:CTR chelate.

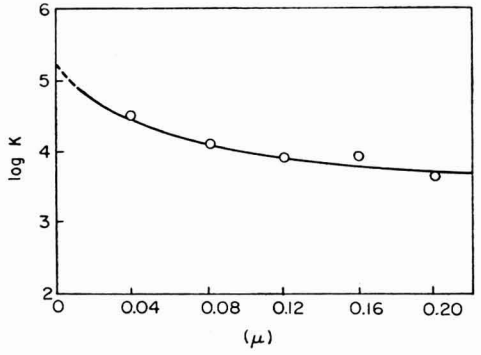


FIG. 6. Variation of log K with change in μ (ionic strength); Y:CTR chelate.

The thermodynamic relationship for the formation of a complex is given by the relation.

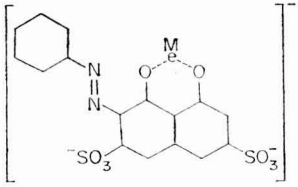
$$\begin{aligned}\Delta^0 F &= -RT \ln K \\ &= -2.303\ RT \log K\end{aligned}$$

Where $\Delta^0 F$ is the standard free energy change, R the gas constant, T the temperature in absolute, and K the stability constant. The values for the free energy change of Sc:CTR and Y:CTR are included in the Table 3 from the extrapolated values for the ionic strength $\mu = 0.0$.

TABLE 3

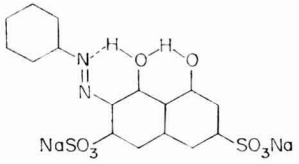
Metal	Wavelength of study (mμ)	pH	Temp (°C)	log K (μ = 0.0)	$\Delta\ ^0F$ at 25°C (kcal)
Scandium	590	4.5	25	4.80	−6.58
Yttrium	590	6.2	25	5.25	−7.20

Structure of the Sc:CTR and Y:CTR chelate. During the study of the dissociation constants of the dye the following structure was assigned for the dye *(2)*:



Where
Me = Sc or Y

In the present study the chelates of the two metals were tested for the nature of the ionic charge by using ion exchange resins Amberlite IR 120 and IR 45 and it was found that both the chelates were completely absorbed on the anion exchange resin. The ionic character suggests, that the following structure is applicable for the chelates of Sc and Y with CTR.



The above structure is tentative and further work on the structure and analytical application of the chelates is in progress in these laboratories.

SUMMARY

A detailed study of the metal chelates formed by Chromotrope 2R with scandium (pH 4.5 λ_{max} 560 mμ; composition 1:1) and yttrium (pH 6.0; λ_{max} 560 mμ; composition 1:1) by spectrophotometric methods was described. The true stability constants of the metal chelates were derived by maintaining different ionic strengths.

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The Determination of Metals in Organic Compounds by Oxygen-Flask Combustion or Wet Combustion

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Some years ago, a publication described the determination of zinc, cadmium, magnesium, and boron in organic compounds after combustion by the oxygen-flask method *(1)*. Surprisingly little further information has appeared since then on the determinations of metals after such combustions, though the literature on determinations of nonmetals has continuously increased. Apart from the few references mentioned later, only the determinations of boron, mercury, and selenium have received attention *(2)*.

Since it is rather unusual for an organic compound to contain more than one metal, high selectivity in the final measurement of the analyses is not normally essential and the convenience of a widely applicable titrant such as EDTA is seen to its full advantage. The EDTA titrations have been used for organometallic materials not only in our previous work *(1)*, but also by Tsuchitani *et al. (3)*. after decomposition by mixed acids. Decomposition by means of the oxygen flask is generally faster than the wet combustion techniques and so the work described below was undertaken in order to extend the applicability of the flask method of decomposition. Compounds containing calcium, barium, magnesium, zinc, bismuth, manganese, cobalt, iron, nickel, and copper were examined.

DETERMINATION OF METALS AFTER FLASK COMBUSTION

Calcium

In the previous work *(1)*, a back-titration method was preferred in order to have a uniform procedure for completing all determinations. However, during recent years, a wider selection of indicators for EDTA titrations has become available and so the use of various indicators for direct titrations was examined. A comparison of end-point sharpness

with murexide, calcein, calcichrome, glyoxal-bis(2-hydroxyanil), pyrocatechol violet, zincon, calmagite, and Eriochrome black T in either direct or replacement titrations for calcium *(4)* showed that the best precision of end-point detection after a flask combustion could be achieved with Eriochrome black T in the presence of zinc–EDTA complex at pH 10. Some results obtained by absorption of the combustion products in 0.2 *N* hydrochloric acid are shown in Table 1.

TABLE 1

ANALYSIS OF VARIOUS ORGANOMETALLIC COMPOUNDS

	Metal (%)	
	Theor.	Found
Calcium oxinate	12.2	12.4, 12.4
Calcium ferronate	5.0	5.0, 5.1
Zinc dibenzyldithiocarbamate	10.7	10.7, 10.75
Zinc lactate	22.4	22.2, 22.4
Barium phenol sulfonate	28.4	28.1, 27.9
Barium nitrobenzene sulfonate	25.4	25.1, 25.0
Manganese acetylacetonate	15.6	15.7, 15.9
Manganese lactate	19.1	18.9, 19.0
Cobalt *N*-benzoyl-*N*-phenylhydroxylamine	12.2	11.9, 12.0
Triethylenediaminocobaltibromide	12.0	12.0, 11.8
Bismuth oxinate	31.7	31.9, 31.6
Bismuth pyrogallate	62.9	62.7, 62.85
Iron(III) acetylacetonate	15.8	15.5, 15.5
Acetylferrocene	24.5	24.15, 24.2
Nickel diisopropyldithiophosphate	12.1	12.3, 12.3
Nickel ethylenediamine bis(acetylacetonate)	20.9	21.05, 21.2
Copper acetylacetonate	24.3	24.3, 24.2

In the case of magnesium, a direct titration with EDTA to an Eriochrome black T endpoint at pH 10 was satisfactory.

Barium

For the determination of barium ion, addition of excess of EDTA and back titration with magnesium or zinc solution in the presence of Eriochrome black T indicator gave the best end points. No difficulties were found unless the compound contained sulfur as well as barium; in such

cases, very low results were obtained because of the formation of barium sulfate. The difficulty could be overcome by adding 5 ml of 9 *M* ammonia and an excess of EDTA to the absorption solution, dipping the platinum sample holder into this solution, boiling for 15 minutes to dissolve barium sulfate *(4)*, and cooling before the back titration.

Zinc

Xylenol orange at pH 5 was found to give the best end points after the decomposition. Satisfactory results were readily obtained (Table 1). Cadmium could be determined in the same way as zinc, provided that 10 mg of zinc–EDTA complex were added to the solution before titration.

Manganese

Direct EDTA titration with Eriochrome black T or pyrocatechol violet as indicator or replacement titrations with the magnesium– or zinc–EDTA complexes gave sharp end points *(4)* on pure solutions, but after a flask combustion procedure, back titration was more satisfactory. When manganese compounds were burned, in the normal way, insoluble manganese oxides were formed which were difficult to dissolve by acid treatment. However, when sodium carbonate was added to the sample before the combustion, the simultaneous fusion–combustion process made it possible to avoid the formation of insoluble oxides and satisfactory results could be obtained (Table 1) with only a slight modification of the normal procedure.

Cobalt

Of the various indicators available for titrations of cobalt with EDTA *(4)*, murexide gave the best end points. In the flask combustions, cobalt behaved like manganese, forming insoluble oxides; again, mixing the sample with sodium carbonate before the combustion overcame the difficulty (Table 1).

DETERMINATION OF METALS AFTER WET COMBUSTION

When compounds containing the metals discussed above were analyzed by the flask method, any problems caused by formation of insoluble inorganic products could be readily overcome. However, when compounds containing nickel, aluminium, or copper were burned, the oxides formed could not be readily dissolved. Addition of sodium car-

bonate, bicarbonate, hydroxide, or peroxide, or of potassium nitrate or bisulfate provided only slight improvements and only treatment with concentrated acid dissolved the oxides. Accordingly, the flask combustion was unnecessary, since a straightforward wet combustion procedure provided greater simplicity. Many mixtures of acids and oxidants have been recommended in the literature for the decomposition of organometallic compounds *(3, 5, 6)*. One of the fastest and most convenient methods involves digestion with sulfuric acid and hydrogen peroxide and this mixture was preferred for the present work.

In none of the above cases was there any evidence of alloying of the metal in the organic sample with the platinum sample holder in the flask combustion and the low results obtained were caused simply by oxide formation. However, when compounds containing iron or bismuth were burned, alloy formation was immediately observed; even when silica sample holders were used in simultaneous fusion–combustion procedures, the insoluble residues could not be readily recovered from the sample holders. Accordingly wet combustion procedures were also preferred for the analysis of compounds containing these metals.

Nickel

Of eight indicators tested for the titration of nickel ion with EDTA *(4)*, murexide was by far the best, clear end points being obtained even in the presence of the large amount of sodium sulfate arising from neutralization of the digest containing sulfuric acid.

Copper

Excellent end points were obtained with several of the usual indicators for copper–EDTA titrations, but when the titrations were applied to sulfuric acid digests after appropriate adjustment of pH, the most accurate results were obtained with pyrocatechol violet. It has been reported *(7)* that copper can be determined without difficulty by the oxygen-flask method, but these results could not be confirmed in the present work.

Iron

There are few reasonably satisfactory indicators for the titration of iron(III) with EDTA even in pure solutions *(4)*; for titrations with 0.01 *M* EDTA in the presence of large amounts of neutral salts, no indi-

cator gave clear end points. A redox titration was therefore essential; titration of iron(III) with solutions of ascorbic acid *(8)* or mercury(I) *(9)* provides a straightforward determination which requires minimal protection of the reducing titrant against atmospheric oxidation. Mercury(I) was preferred in this work since its solutions are slightly more stable than those of ascorbic acid. The only problem encountered was in the determination of compounds containing iodine as well as iron; high results were obtained unless the sample was first boiled with sulfuric acid to remove iodine before the addition of hydrogen peroxide.

Bismuth

Sharp end points in titrations of bismuth with EDTA were obtained when pyrocatechol violet, xylenol orange, or pyrogallol red were used as indicators under suitable pH conditions; xylenol orange was slightly better than the other compounds. When organic bismuth compounds were decomposed by the oxygen-flask method, some alloying with platinum occurred even in the presence of fusion agents such as sodium peroxide. Wet combustion had, therefore, to be applied; digestion with nitric acid was preferable to the peroxide–sulfuric and mixture in this case.

EXPERIMENTAL METHODS

General Procedure for Flask Combustion

Burn a sample of the organic compound weighing 5–10 mg wrapped in filter paper in the usual way, in a 250-ml flask. If the sample contains manganese or cobalt, mix the sample with 15–20 mg of sodium carbonate on the filter paper before ignition and use a 500-ml flask to ensure a sufficient excess of oxygen. Absorb the combustion products in 5 ml of 0.2 *M* hydrochloric acid (or in 5 ml of 6 *M* hydrochloric acid when sodium carbonate has been used). After shaking the flask for ca. 10 minutes, rinse the gauze and stopper and neutralize the solution with 0.5 *M* sodium hydroxide solution, using a small drop of aqueous methyl red solution as indicator. If the compound contains nitrogen, sulfur, bromine, or iodine, boil gently for 1–2 minutes before neutralization to remove any oxidants or reductants which may affect the indicator. If sodium carbonate has been used, immerse the platinum gauze sample holder in the absorbent solution [an adapter head as suggested by Gedansky *et al. (10)* is suitable], boil for 10 minutes, and then neutralize with sodium hydroxide. In the case of manganese, add 30–40 mg of as-

corbic acid to the warm solution just before neutralization to ensure the presence of manganese(II).

General Procedure for Wet Combustion

Weigh a sample of 5–10 mg into a 50-ml digestion flask and add 3 ml of concentrated sulfuric acid (M.A.R. grade) and 3 ml of 100-vol. hydrogen peroxide (M.A.R. grade). Heat gently to fumes and then boil for 5–10 minutes, adding more hydrogen peroxide if necessary. Cool, carefully add a little distilled water and then neutralize with 6 *M* sodium hydroxide solution, cooling the mixture as required. Transfer to a titration flask.

In the case of bismuth, use 3 ml of concentrated nitric acid for the digestion and proceed as above.

Titration Procedures

Calcium. Add 10 mg of zinc–EDTA complex and 5 ml of 1 *M* ammonia solution and titrate with 0.01 *M* EDTA, using Eriochrome black T as indicator.

Magnesium. Add 5 ml of ammonia buffer pH 10, and titrate as for calcium.

Barium or manganese. Add excess of 0.01 *M* EDTA, 5 ml of buffer pH 10, and Eriochrome black T indicator, and titrate with 0.01 *M* zinc solution.

Zinc. Add 5 ml of buffer pH 5, and titrate with 0.01 *M* EDTA using xylenol orange as indicator.

Cobalt or nickel. Add 5 ml of buffer pH 10, and titrate with 0.01 *M* EDTA using murexide as indicator.

Iron. Use phenolphthalein as indicator in the preliminary neutralization of the digest. Then add 4.5 ml of 6 *M* nitric acid and 5 ml of 40% ammonium thiocyanate, dilute to 50 ml with distilled water, and titrate with 0.01 *M* mercury(I) nitrate solution *(9)*.

Copper. After neutralization of the digest, add 0.2 g of ammonium nitrate and pyrocatechol violet indicator and adjust the solution with 0.5 *M* ammonia solution until the blue color of the copper–indicator complex appears. Then add 0.5 g of sodium acetate and titrate with 0.01 *M* EDTA.

Bismuth. Adjust the digest to pH 1–3 with ammonia solution (pH meter), add xylenol orange indicator and titrate with 0.005 *M* EDTA. A weaker EDTA solution is advisable in this case, because of the high equivalent weight of bismuth.

DISCUSSION

The end points of the titrations with 0.01 *M* EDTA are sufficiently sharp for the accuracy of the final results to be within the normally accepted error range of $\pm 0.3\%$ in nearly all cases (Table 1).

When the organic compounds contain nitrogen, sulfur, bromine, or iodine, the combustion products from the oxygen-flask method may include nitrogen or sulfur oxides, bromine or iodine, which may react with the EDTA indicators used; these interferences can, however, be readily removed by boiling for a few minutes. Otherwise, no serious interferences were encountered. Bromine and iodine may also interfere when the compounds are decomposed by wet combustion but they can readily be split off by heating with sulfuric acid before the addition of hydrogen peroxide. The digestion method with sulfuric acid–peroxide mixtures is very simple and appears to be as effective as methods involving mixtures of concentrated acids.

The procedures which are described are simple, rapid, and suitable for routine work. The use of a single titrant makes the methods particularly appropriate when the analysis of a variety of organometallic compounds is required.

SUMMARY

Decomposition by the oxygen-flask method followed by titration with EDTA is suitable for the determination of the metal in organic compounds containing calcium, magnesium, zinc, barium, manganese, or cobalt. Compounds containing nickel, copper, iron, or bismuth are better decomposed by wet combustion processes, because of alloy formation or insoluble oxide formation. Optimal indicators for the EDTA titrations are discussed.

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Application of Back Titration of EDTA with Mercury (II) to the Analysis of Alloys

III. Analysis of Copper–Cadmium, Copper–Nickel, and Copper–Nickel–Zinc Alloys

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INTRODUCTION

The present work is an extension to the application of the potentiometric back titration of excess EDTA or CDTA with mercury(II)—which was recently applied to the determination of solders, bearing and type metals, stainless steels, bronzes, brasses and their special varieties, and lead pigments *(8–10)*—to the analysis of copper–cadmium, copper–nickel, and copper–nickel–zinc alloys.

About 70% of copper produced is used either pure or with the addition of small amounts of other elements intended to deoxydize it or increase its strength, while retaining its high electrical and thermal conductivity. Of the elements that may be added to copper, cadmium is the most effective. In practice the amount added is from 0.8 to 1.0%. Copper–cadmium alloy is used extensively in the form of trolly wire, line wire, and also used in the manufacture of brazing nonconsumable welding electrodes.

Copper–nickel alloys have no critical limits of composition. The principal characteristic of alloys containing between 40 and 50% nickel is their high electrical resistance and low temperature coefficient, in addition to high resistance to corrosion. The most important of all copper–nickel alloys is that marketed under the name Monel metal. The precipitation-hardening alloys are comparatively new development and are produced by the addition of Al, Si, or Be to copper–nickel alloys.

Copper–nickel–zinc alloys are widely used as the base of electroplating and for electrical resistances. The resistance to corrosion rises with the nickel content.

The most important separation methods of copper, when present in other than micro amounts are precipitation methods, viz. electrodeposition, precipitation as the sulfide and precipitation with organic reagents. The separation of copper by electrodeposition affords a convenient means for the determination of the element particularly amongst other alloys constituents.

In determination of cadmium with EDTA, interference from other cations is overcome by the judicious use of masking and demasking agents Cadmium can be determined in Cu–Cd alloys by a direct titration using Eriochrome black T as indicator, after a preliminary extraction as the thiocyanate with 1:1 methyl ethyl ketone–*n*-butyl phosphate mixture *(11)*. Direct titration can also be carried out with pyrocatechol violet *(12)*, or with 1-(2-pyridylazo)-2 naphthol as indicator *(2)*. The demasking action of chloral hydrate, formaldehyde, or acetone *(6)* on the cadmium cyanide complex is the basis of a titrimetric method for the determination of Cd in presence of Cu, Ni, and Co. Flaschka *(4)* reported a method for the determination of Cd or Zn in presence of Fe(III) and Mn(II).

Pyrocatechol violet *(12)*, pyrogallol red *(14)* and PAN *(3)* are recommended as indicators in determining nickel with EDTA. Nickel and cobalt are simultaneously determined by back titration of excess EDTA with $Bi(NO_3)_3$ solution using pyrocatechol violet as indicator *(5)*. Nickel is determined potentiometrically by back titration of excess EDTA with standard ferric solution *(13)*.

Direct potentiometric titration of Fe(III) with EDTA provides a highly accurate method of determining iron *(13)*. When aluminium is masked with fluoride, copper can be determined in its presence with EDTA, using pyrocatechol violet as indicator (*15*, p. 243).

Pribil *et al. (13, 15)* determined Zn potentiometrically at pH 5–6 using Fe(III) as back titrant for excess EDTA. Most of the above-mentioned heavy metals have been recently determined by potentiometric back titration of excess EDTA or CDTA with mercuric ions *(6, 7)*.

EXPERIMENTAL

The water used was always twice distilled. All the materials used were of the highest purity grade. These involved nitrates of copper(II), mercury(II), and ammonium; sulfates of iron(III), cadmium, and nickel; metallic lead, aluminium, and zinc; potassium and sodium hydroxides;

potassium cyanide; nitric, sulfuric, hydrochloric, hydrofluoric and tartaric acids; diammonium hydrogen phosphate. Ethyl alcohol, formaldehyde, acetone, urotropine, ammonia, ammonium chloride, and thiocyanate; EDTA; CDTA; dimethylglyoxime; hydrogen sulfide gas; Eriochrome black T, murexide, methyl orange, and phenolphthaline indicators.

Solutions

The 0.2578 *M* copper solution (1 ml = 16.3800 mg) was prepared from cupric nitrate $Cu(NO_3)_2 \cdot 3H_2O$, mol.wt. 241.6, and standardized electrolytically.

The 0.01147 *M* cadmium solution (1 ml = 1.28934 mg) was prepared from cadmium sulfate. 3 $CdSO_4 \cdot 8H_2O$, mol.wt. 769.56 and standardized potentiometrically.

The 0.052018 *M* nickel solution (1 ml = 3.05294 mg) was prepared from nickel sulfate $NiSO_4 \cdot 7H_2O$, mol.wt. 280.87 and standardized potentiometrically.

The 0.0250 *M* lead solution (1 ml = 5.1803 mg) was prepared by dilution from 0.05 *M* lead solution prepared from lead sheet of 99.99% purity and standardized potentiometrically.

The 0.0500 *M* mercuric nitrate solution was prepared as previously mentioned.

The 0.0500 *M* zinc solution (1 ml = 3.269 mg) was prepared from zinc metal of the laboratory chemical B.D.H. grade (As.T.).

The 0.01002 *M* aluminium solution (1 ml = 0.27027 mg) was prepared by dilution from 0.1 *M* aluminium solution. (prepared from aluminium of hilger high purity metal 99.96%) and standardized potentiometrically.

The 0.005025 *M* iron solution (1 ml = 0.2812 mg) was prepared by dilution from 0.0773 *M* iron solution (prepared from iron metal of H.H.P. metal) and standardized potentiometrically.

The 0.05 *M* EDTA solution was prepared in the usual way and standardized against a standard zinc solution.

Lower molarities of the above mentioned solutions were prepared by appropriate dilutions.

The 0.05013 *M* CDTA solution was prepared as previously mentioned and standardized against a standard zinc solution using murexide as indicator.

The ammoniacal buffer, pH 10, was prepared by making 54 g of NH_4Cl plus 350 ml of conc. NH_4OH with water to 1 liter.

The urotropine buffer solutions, pH 8 and pH 9, were prepared and checked as previously mentioned.

The diammonium hydrogen phosphate and the ammonium thiocyanate solutions were each 100 g/liter.

The alcoholic solution of dimethylglyoxime was 10 g/liter.

The titration cell consisted of a 150-ml beaker, a $\frac{1}{50}$ graded microburette; a mechanical stirrer; calomel and silver amalgam electrodes. The pH and potentiometer were PYE Dynacap, catalogue number 11072.

RESULTS AND DISCUSSION

1. Analysis of Copper–Cadmium Alloys

These involved Cu–Cd alloys which contain Ni either as alloying element or as impurity. The amount of Ni added does not exceed 0.1% as in brazing welding nonconsumable electrodes in order to increase the hardness of the alloys.

Synthetic mixtures containing varying percentages of Cd and Ni were analyzed in order to investigate the optimum conditions of analysis of such alloys. Copper is always separated by electrodeposition.

PROCEDURES

A. For total. Transfer a volume containing milligram amounts of Ni and Cd equivalent to those present in 10 ml of an alloy solution (5 g/liter) into 150-ml beaker, followed by a measured excess of 0.05 *M* EDTA; add 40 ml of buffer, pH 9, and back titrate with 0.05 *M* Hg(II) using the silver amalgam as indicator electrode. This titration gives ml of 0.05 *M* EDTA equivalent to Ni + Cd.

B. For Cd alone. To another identical mixture add 10 ml of ammonia buffer, pH 10, 5% KCN solution till the color changes to yellow, a spatula end of EBT, 25 ml of acetone, and titrate the liberated Cd immediately with 0.01 *M* EDTA.

This titration gives milliliters of 0.01 *M* EDTA equivalent to Cd. Table 1 lists the results of analyses of synthetic mixtures of nickel plus cadmium. The data indicate that the potentiometric method of determining nickel and cadmium in copper-cadmium alloys is quite accurate, simple, rapid and less reagent consuming.

TABLE 1

No.	Cd (mg)		Ni (mg)	
	Taken	Found	Taken	Found
1	0.5673	0.5676	0.0083	0.0081
2	1.1346	1.1353	0.0201	0.0197
3	1.7010	1.6974	0.0319	0.0320
4	2.2692	2.2651	0.0390	0.0387

a. The potentiometric procedure adopted for analysis of commercial alloys. One-g sample is dissolved in 10 ml of 1:1 HNO_3, The product is evaporated to 5 ml, diluted with 5% H_2SO_4 to 100 ml. The copper is determined by electrodeposition. The electrolyte is diluted with water up to 200 ml. Nickel and cadmium are determined in aliquot portions by procedures A and B.

b. The wet chemical analysis. Separate copper by electrolysing a 10-g sample solution, and determine as mentioned before. Separate cadmium in the electrolyte as cadmium sulfide by double precipitation and finally determine it by the electrolytic method *(1)*. In the filtrate and washings from cadmium sulfide separation determine nickel by dimethylglyoxime.

The results of analysis of a commercial alloy following, for the sake of comparison, the two above procedures are listed in Table 2. The data in Table 2 show that the present method is so accurate that it rivals the wet chemical one which is much more tedious. The results are easily reproduced.

II. Analysis of Nickel–Copper Alloys

These involved Monel metal and the precipitation hardening corrosion resistant alloys, which contain in addition to Cu and Ni, Al, Fe, C, and Si as alloying elements. In order to investigate the optimum conditions

TABLE 2

Cd (%)		Ni (%)	
a.	b.	a.	b.
1.596	1.603	0.021	0.022

of analysis, a variety of synthetic mixtures containing Cu, Ni, Fe, and Al covering their ranges in such alloys were analyzed. Si is separated as SiO_2.

PROCEDURES

A. For total. Transfer a volume containing milligram amounts of Cu, Ni, Fe and Al, equivalent to those present in 5 ml of an alloy solution (2 g/liter) into a 150-ml beaker, followed by a measured excess of 0.05 *M* EDTA, boil for 10 minutes, cool, add 40 ml of buffer, pH 8 (NaOH-hexamine), and back titrate with 0.05 M Hg(II) using the silver amalgam as indicator electrode. This titration gives milliliters of 0.05 *M* EDTA equivalent to Cu + Fe + Ni + Al.

B. For Cu + Ni + Fe. To another identical volume, add 5 g of NH_4F and boil for about 15 minutes; to the cold solution add a measured excess of 0.05 *M* EDTA followed by 40 ml of buffer, pH 8, and back titrate with 0.05 *M* Hg(II).

C. For Cu + Ni + Al. To a third identical volume, add a measured excess of 0.05 *M* EDTA, boil for 15 minutes, cool, add few drops of 0.05 *M* NaOH, just enough to precipitate $Fe(OH)_3$; digest on a water bath for 15 minutes at 60°C; filter through a medium texture filter paper; wash with water; add to the combined filtrate and washings 40 ml of buffer, pH 8, and finally back titrate the excess EDTA with 0.05 *M* Hg(II).

D. For Cu alone. Cu is electrodeposited in an identical volume and the milliliters of 0.05 *M* EDTA equivalent to the amount of Cu deposited is calculated.

The milligram amount of each constituent is thus easily computed.

Tables 3 and 4 list the results of analyses of synthetic mixtures of Cu, Ni, Fe, and Al covering their ranges in such alloys. The data indicate that it is possible by the present method to determine Cu, Ni, Fe, and Al in mixtures covering their ranges in Monel metal and similar alloys with fair accuracy, and that the method is quite reliable, simple, and less time and reagent consuming.

a. The potentiometric procedure adopted for analysis of commercial alloys. Dissolve 1-g sample in 20 ml of conc. HCl; after complete dissolution oxidize with few drops of 25% HNO_3 solution then boil off the brown fumes; add 25 ml conc. H_2SO_4 and evaporate on a hot plate (avoid boiling) to fuming; cool; and dilute with water up to 500 ml. If

TABLE 3

No.	Ni (mg)		Cu (mg)	
	Taken	Found	Taken	Found
1	3.9994	3.9950	4.9959	5.0000
2	5.0068	5.0036	2.9975	2.9975
3	6.9912	6.9907	1.9984	1.9983
4	9.0062	9.0064	7.9934	7.9938

TABLE 4

No.	Fe (mg)		Al (mg)	
	Taken	Found	Taken	Found
1	0.3993	0.3998	0.5000	0.5000
2	0.5005	0.5046	1.2000	1.2014
3	0.1997	0.2001	0.7000	0.7002
4	0.1012	0.1012	0.1000	0.1001

any precipitated SiO_2 was formed, it must be filtered off before dilution and determined. Determine Ni, Cu, Fe, and Al in 5-ml aliquots by procedures A, B, C, and D mentioned above.

b. The wet chemical analysis. Determine silicon as SiO_2 and Cu by electrodeposition; dilute the electrolyte up to 200 ml; determine Fe volumetrically in a 50-ml aliquot; in another 50-ml portion precipitate Fe and Al as hydroxides; ignite and weigh the R_2O_3; compute Al by difference. In the remaining 100 ml determine Ni as dimethylglyoximate.

Table 5 lists the results of analysis of a commercial alloy following, for the sake of comparison, the two above procedures a and b. The data in Table 5 show that the present method is extremely reliable, simple, less time consuming and fairly accurate.

TABLE 5

	Ni (%)	Cu (%)	Fe (%)	Al (%)
a.	41.50	46.01	4.36	7.02
b.	41.72	45.67	4.32	6.91

III. Analysis of German Silver

In order to investigate the optimum conditions of analysis we analyzed synthetic mixtures containing percentages of Fe, Ni, and Zn covering their ranges in such alloys. Tin is separated as metastannic acid, Pb and Cu by simultaneous electrodeposition. For the reasons mentioned before *(10)* CDTA is preferred to EDTA in analysis of zinc-containing mixtures.

Procedures

A. For total. Transfer a volume containing milligram amounts of Fe, Ni, and Zn equivalent to those present in 10 ml of an alloy solution (5 g/liter) into a 150-ml beaker, followed by a measured excess of 0.05 *M* CDTA, add 40 ml of buffer, pH 8, and back titrate with 0.05 *M* Hg(II) using the silver amalgam as indicator electrode. This titration gives milliliters of 0.05 *M* CDTA equivalent to Fe + Ni + Zn.

B. For Ni + Zn in presence of Fe. To another identical mixture, add a measured excess of 0.05 *M* CDTA, few drops of 0.05 *M* NaOH, just enough to precipitate the hydroxide of Fe; digest on a water bath for 15 minutes at 60°C; filter through a medium texture filter paper; wash with water; add to the combined filtrate and washings 40 ml of buffer, pH 8, and finally back titrate the excess CDTA with 0.05 *M* Hg(II) using the same electrode. This titration gives milliliters of 0.05 *M* CDTA equivalent to Ni plus Zn.

C. For Zn alone. To a third identical mixture, add NaOH to neutralize the acid, a spatula end of tartaric acid, 10–20 ml of ammonia buffer, pH 10; 5 ml of 5% KCN solution, and a spatula end of ascorbic acid. Boil up to a pure yellow color, dilute to about 100 ml, cool, add a spatula end of EBT indicator followed by 25 ml of acetone; titrate the liberated Zn with 0.05 *M* EDTA.

Table 6 lists the results of analyses of synthetic mixtures of Fe, Ni, and Zn covering their ranges in such alloys. The data indicate that the present method of determining Fe, Ni, and Zn in german silver is quite simple, rapid, reliable, and less time consuming.

a. The potentiometric procedure adopted for analysis of commercial alloys. Dissolve 1-g sample into 25 ml of 1:1 HNO_3; after decomposition boil gently to expell the brown fumes; add 50 ml of hot water, allow to stand on a steam bath for 1 hour, filter off the metastannic acid if present. Determine Cu and Pb by electrodeposition; dilute the electro-

TABLE 6

No.	Fe (mg)		Ni (mg)		Zn (mg)	
	Taken	Found	Taken	Found	Taken	Found
1	0.2776	0.2786	5.6174	5.6174	19.6110	19.6110
2	0.1380	0.1400	9.1588	9.1596	11.4400	11.4397
3	0.1380	0.1410	12.7613	12.7613	4.9030	4.9030

lyte and washings of the electrodes with water up to 200 ml; determine Fe, Ni, and Zn in aliquots by above procedures A, B, and C.

b. The wet chemical analysis. Separate Sn as metastannic acid and ignite it to the oxide, determine Pb in the filtrate either as $PbSO_4$ or simultaneously with copper by electrodeposition; dilute the electrolyte with water up to 500 ml; precipitate Fe in 100-ml aliquot and determine it colorimetrically. In the filtrate from Fe separation, determine Zn as $ZnNH_4PO_4$. Determine Ni in another aliquot by the dimethylglyoxime method. Zn may be computed by difference.

The results of analysis of a commercial alloy following, for the sake of comparison, the two above procedures a and b are listed in Table 7. The data show that the present method is extremely reliable, simple, less time and reagent consuming.

With all types of alloys typical titration curves (not represented) were obtained with sharp inflections lying in the immediate vicinity of the expected end points and amounting to an average of 184 mV per 0.1 ml of 0.05 *M* titrant.

TABLE 7

	Fe (%)	Ni (%)	Zn (%)
a.	0.066	12.55	19.03
b.	0.064	12.84	19.03

SUMMARY

Eleven synthetic and 3 commercial alloys involving Cu–Cd, Cu–Ni, and Cu–Ni–Zn combinations together with minor constituents of Pb, FbIII, Al, Sn, C, and Si were successfully analyzed. The procedures applied involved potentiometric back titration of excess EDTA or CDTA with mercury(II) together with the addi-

tional classical methods, with the purpose of simplifying and making rapid such an analysis. The potentiometric method proved superior especially with minute amounts of alloying elements. Moreover, its introduction with the additional use of masking and demasking agents proved much more promising than classical methods.

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Qualitative Detection Methods Based on the Liberation of Ammonia from Hexamminecobalt(III) Perchlorate

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INTRODUCTION

The present paper describes research in which a qualitative test was developed for various compounds based on the liberation of ammonia in substitution and reduction reactions of hexamminecobalt(III) perchlorate. The primary application is in being able to detect relatively nonvolatile substances by converting them to readily detectable ammonia vapor.

METHODS AND MATERIALS

A. Reagents.

Hexamminecobalt(III) perchlorate. This reagent was prepared by an adaptation of the method of Bjerrum and Reynolds *(1)*. The procedure is as follows: 23.5 g (0.2 mole) of NH_4ClO_4 were dissolved in 150 ml of water by heating on a steam bath. To this was added a solution of 36.6 g (0.1 mole) of $Co(H_2O)_6(ClO_4)_2$ in 25 ml of water, followed by 1 g of activated charcoal, and then by 45 ml of concentrated ammonium hydroxide. The precipitate which formed was disregarded, and 20 ml of 30% H_2O_2 (0.2 mole) was added slowly with stirring. The mixture was heated 5 minutes on a steam bath and left to stand for about 1 hour. The precipitate, containing the product and the charcoal, was filtered off and washed with cold (5°) water. The product was then dissolved in about 1500 ml of water (containing sufficient $HClO_4$ to give a pH of between 3–5) by heating on a steam bath. The charcoal was filtered off while the solution was still hot. To the hot solution was added 100 ml of concentrated $HClO_4$, which precipitated the product. The solution was cooled to 5°C and the product was filtered off, washed with cold water and ethanol, and dried. The yield was not recorded. The salt was recrystallized from water.

Anal.: Calc. for $Co(NH_3)_6(ClO_4)_3$ (%): N, 18.3; Cl, 23.2; Co, 12.8. Found (%): N, 18.8; Cl, 23.1; Co, 12.5.

Borate buffers. Harleco, pH 10.00, reference buffer solution (boric acid, potassium hydroxide) was used as obtained. Buffers of pH 10.15 and 9.55 were made according to general directions for Clark and Lubs buffer mixtures *(2)*.

Miscellaneous. Dimethyl sulfoxide was used as obtained from Matheson Coleman and Bell. All test reagents were of the highest purity grade available commercially. Most were Eastman chemicals.

B. Test Procedures.

Borate buffer system. The test compound (1–10 mg), 1 ml of buffer solution and 1.0 ml of 0.02 *M* aqueous hexamminecobalt(III) perchlorate were mixed in a 4-dr glass vial. Hydrion paper (pH range 6–8 for pH 10.15 and 10.00 buffers; pH range 1–10 for pH 9.55 buffer) was moistened with distilled water and suspended on a glass hook less than 1 cm above the surface of the liquid. (The glass hook was positioned by inserting it into a hole drilled in the Teflon stopper.) Blanks included a solution of the test compound in the absence of cobalt salt and a solution of cobalt salt in the absence of test compound. The capped vials were allowed to stand at room temperature. The time for the pH paper to turn color was recorded.

Dimethyl sulfoxide system. The same procedure as described for the borate buffer system was used except that dimethyl sulfoxide was substituted for the buffer. Also dimethyl sulfoxide instead of water was used to make up the cobalt salt solution.

Reagent stability. Buffered solutions of hexamminecobalt(III) perchlorate gave positive tests for ammonia evolution in less than 15 minutes to hours depending on age and pH. Stock solutions which gave blank tests in less than 25 minutes were discarded.

Stock solutions of the aqueous hexamminecobalt(III) perchlorate were tested every day. A sample was made up containing equal volumes of buffer and cobalt salt solutions. The test was run in the usual manner but at 40°C to give a faster response. The time to a distinct color change of the pH paper was noted. A straight line relationship, $t = t_0 - 3.0D$, was found. (t = time in minutes for the pH paper to change color; t_0 = time in minutes for the pH paper to change color on the first day of observation, D_0; D = the number of days after the first day of observation, D_0).

Attempts were made to find an organic solvent for the metal complex

to both increase stability and perhaps provide better specificity. Acetonitrile and methanol did not dissolve the complex to any noticeable extent. *N,N*-Dimethylformamide dissolved the cobalt salt but interfered in the test for ammonia. Dimethyl sulfoxide dissolved the salt without appreciable decomposition. Solutions of the salt in pure dimethyl sulfoxide were stable (no ammonia detected above the solution surface for at least 8 days at room temperature. Solutions of the complex in combinations of dimethyl sulfoxide–water (3:1 and 1:1) were more stable (13 and 17 days, respectively).

Test sensitivity and reproducibility. The sensitivity of the test procedure for detecting ammonia was determined by checking a solution of NH_4Cl adjusted to pH 9. At least 17 μg of ammonia could be detected unequivocally and immediately at room temperature. Actually the limit of identification of ammonia using a similar technique has been reported to be 0.01 μg at 40°C and 5 minutes *(3)*. The higher sensitivity can be obtained by using an apparatus with little air space above the test solution.

The reproducibility of the time of color change was determined at 40° by five experiments with 1,6-hexanediamine. The average time of a definite color change was 4.6 $\pm$ 0.6 minutes [99% confidence limits *(4)*].

RESULTS AND DISCUSSION

A variety of compounds (mostly amines, and several insecticides were tested for their ability to liberate ammonia from hexamminecobalt(III) perchlorate in buffer and dimethyl sulfoxide solutions (Table 1). Two mechanisms may account for the observed formation of ammonia, Eqs. (1) and (2) and it appears that both are operative.

Substitution

$$[Co(NH_3)_6][ClO_4]_3 + \underset{\text{Compound tested}}{(A)} \rightarrow [Co(NH_3)_5(A)][ClO_4]_3 + \boxed{NH_3}. \quad (1)$$

Redox

$$[Co(NH_3)_6][ClO_4]_3 + (A) \rightarrow [Co(NH_3)_6][ClO_4]_2 + ACl O_4$$

$$\downarrow \text{ solvent} \quad (2)$$

$$[Co(solvent)_6][ClO_4]_2 + \boxed{6\ NH_3}.$$

TABLE 1

DETECTION OF AMMONIA VAPOR AT ROOM TEMPERATURE FROM TEST SOLUTIONS OF HEXAMMINECOBALT(III) PERCHLORATE [a]

Compound tested	Borate buffer,[b] pH 9.55	DMSO
Adenine sulfate	N	N
p-Aminoacetanilide	N	W
4′-Aminoacetophenone	N	S
3′-Aminoacetophenone	N	S
o-Aminobenzenesulfonic acid	N	N
o-Aminobenzoic acid (anthranilic acid)	N	N
p-Aminobenzoic acid	N	N
m-Aminobenzoic acid	W	N
2-Aminobiphenyl (*o*-phenylaniline)	N	W
(±)-α-Aminocaproic acid	S	N
4-Amino-2,6-dibromophenol	S	N
5-Amino-1,3-dimethylbenzene (3,5-xylidine)	S	W
2-Amino-4-nitrophenol	W	N
2-Amino-5-nitropyridine	W	—
m-Aminophenol	S	N
p-Aminophenol	S	S
2-Aminopyrimidine	N	—
2-Aminoresorcinol	S	N
p-(1-Ethylpropyl)aniline	S	N
Aniline	W	N
2-Methoxyaniline (*o*-anisidine)	S	N
o-Bromoaniline	N	N
p-Bromoaniline	N	N
m-Bromoaniline	N	W
o-Chloroaniline	N	—
m-Chloroaniline	N	N
p-Chloroaniline	N	N
p-Chlorophenol	N	N
2,6-Dichloroindophenol sodium salt	S	—
Didodecylamine	N	S
N,*N*-Diethyl-2-mercaptoethylamine	W	—
N,*N*-Diethyl-*p*-phenylenediamine	S	N
N,*N*-Dimethyl-*p*-phenylenediamine	S	N
α,α-Dipyridyl (2,2′-bipyridine)	N	N
Ethyl *p*-aminobenzoate	N	W
Glycine	N	—
1,6-Hexanediamine	W	—
Hydroquinone	S	—

TABLE 1—(*Continued*)

Compound tested	Borate buffer,[a] pH 9.55	DMSO
p-Nitroaniline	N	N
Pancreatin	S	N
Peptone	S	N
Phenol	W	N
o-Phenylenediamine	N	N
m-Phenylenediamine	N	N
p-Phenylenediamine	S	S
Potassium iodide	N	S
Pyrazine	N	—
Pyridazine	N	—
Pyrrole-2-carboxaldehyde	N	—
3-Quinuclidinol	W	—
Sodium sulfite	S	S
Starch	W	N
Sulfanilic acid	N	N
N,N,N′,N′-tetramethyl-*p*-phenylenediamine	W	N
Tribenzylamine	N	N
Triheptylamine	N	N
2,4,6-Triaminopyrimidine	N	—
Thiourea	N	N
Trypsin	S	N
S-Tyrosine	S	N
Urea	N	N
Uric acid	W	N
Malathion	S	—
Parathion	W	—
EPN	S	—
DDT	S	—

[a] N = no ammonia detection in 30 minutes; W = ammonia was detected in 15–30 minutes; and S = ammonia was detected in less than 15 minutes.

[b] Buffers of pH 10.00 and 10.15 were also tried but were found to be less suitable because of higher blanks.

Immediate tests were obtained with compounds such as 4-amino-2,6-dibromophenol and hydroquinone. This is attributed to the reduction of cobalt(III) to cobalt(II); ammonia is easily displaced from the labile complex, Eq. (2). Potassium iodide gave a weak test in buffered solution but a strong one in dimethyl sulfoxide. Many of the easily oxidizable amines gave negative tests in dimethyl sulfoxide. These results

appear to indicate that displacement reactions of the complex occur more readily in dimethyl sulfoxide and redox reactions occur more readily in aqueous buffer.

Addition of water to test solutions made with dimethyl sulfoxide led to a general decrease in sensitivity for compounds which gave a test in the absence of water (Table 2). However, strong reducing agents such as *p*-aminophenol, *p*-phenylenediamine, and sodium sulfite gave a strong positive test irrespective of solvent combination.

The detection response time can be improved as shown with several compounds in Table 3. We have illustrated that substitution and oxidation–reduction reactions of metal complexes offer a simple approach in the conversion of chemicals to more volatile and more easily detectable materials. It is attractive to consider transitional metal complexes as such conversion reagents because of the available choice and the published data on their rates of hydrolysis, displacement and oxidation–reduction. Liberation of ammonia in substitution and reduction reactions of hexamminecobalt(III) perchlorate can be used for the qualitative detection of a number of nucleophiles and reducing agents. Specificity can be achieved by varying the reaction solvent.

TABLE 2

EFFECT OF WATER ON THE DETECTION OF AMMONIA VAPOR FROM TEST SOLUTIONS OF HEXAMMINECOBALT(III) PERCHLORATE IN DIMETHYL SULFOXIDE [a]

Compound tested	Water added (%)		
	0	25	50
p-Aminoacetanilidine	W	N	N
3-Aminoacetophenone	S	S	N
4-Aminoacetophenone	S	S	N
2-Aminobiphenyl	W	W	N
5-Amino-1,3-dimethylbenzene (3,5-xylidine)	W	W	N
p-Aminophenol	S	S	S
m-Bromoaniline	W	N	N
Di-*n*-dodecylamine	S	N	N
p-Phenylenediamine	S	S	S
Potassium iodide	S	W	N
Sodium sulfite	S	S	S

[a] N = no ammonia detection in 30 minutes; W = ammonia was detected in 15–30 minutes; and S = ammonia was detected in less than 15 minutes.

TABLE 3

EFFECT OF TEMPERATURE ON THE DETECTION OF AMMONIA VAPOR FROM HEXAMMINECOBALT(III) PERCHLORATE IN pH 9.55 BUFFER

Compound tested	Time (minutes)	
	Room temperature	40°C
2-Amino-5-dinitropyridine	16	3
Aniline	17	3
N,N-Diethyl-2-mercaptoethylamine	20	2
1,6-Hexanediamine	25	4
3-Quinuclidinol	25	5

SUMMARY

A number of compounds were screened for their general ability to liberate ammonia from hexamminecobalt(III) perchlorate in aqueous buffer and dimethyl sulfoxide. The ammonia was detected by a strip of moistened pH paper hung above the solution in a closed vial. Various compounds were detected, e.g., sodium iodide, phenols, hydroquinone, sodium sulfite, trypsin, Malathion, DDT, and several amines. It is concluded that the liberation of ammonia in substitution and reduction reactions of hexamminecobalt(III) perchlorate can be used for the qualitative detection of a number of nucleophiles and reducing agents. Specificity can be achieved by varying the reaction solvent. The primary application is in detection of relatively nonvolatile substances by converting them to readily detectable ammonia vapor.

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MICROCHEMICAL JOURNAL 14, 224–226 (1969)

Titrimetric Microdetermination of L-Asparagine

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Literature concerning the determination of L-asparagine is not very plentiful. L-asparagine is determined chromatographically *(1, 2)*; manometrically *(5)*; microbiologically *(6)*; enzymically with bacterial aspartic acid decarboxylase *(7)*; titrimetrically *(3, 4)*; and by oxidizing with gold chloride in alkaline medium *(8)*.

The present paper deals with the direct determination of L-asparagine in micro amounts with thorium nitrate at room temperature using bromcresol green as indicator. Potentiometric titration and analysis of the complex shows that the ratio between thorium and L-asparagine is 1:4. Probably the following reaction takes place:

$$4C_4H_8O_3N_2 + Th(NO_3)_4 \rightarrow Th(C_4H_7O_3N_2)_4 + 4HNO_3.$$

EXPERIMENTAL METHODS

Reagents used: L-asparagine (E.Merck grade); thorium nitrate (ANALAR, B.D.H. grade); bromcresol green (L.R; B.D.H. grade).

Bromcresol green (o.1 g) is dissolved in 100 ml of distilled water; it is then ready for use as indicator.

PROCEDURE

A known standard solution of L-asparagine is taken in a beaker and the volume is raised to about 25 ml. Two to five drops of bromcresol green solution is added when a blue color is developed with L-asparagine. The above mentioned solution is titrated against a standard solution of thorium nitrate with constant stirring. The end point is marked by a sharp change in color from blue to a light yellowish green.

RESULTS

The results are given in Table 1. Range in which L-asparagine was estimated vary from 7.2×10^{-4} to 86.4×10^{-4} mg/liter.

TABLE 1

MICRODETERMINATION OF L-ASPARAGINE

$C_4H_8O_3N_2$ 0.019 *M* (ml)	$Th(NO_3)_4$ 0.002 *M* (ml)	Indicator	L-asparagine		Error (%)
			Taken ($\times 10^4$ mg/liter)	Found ($\times 10^4$ mg/liter)	
0.25	0.06	Bromcresol	7.131	7.2	
0.75	0.18	Green	21.393	21.4	
1.00	0.24	From blue	28.524	28.8	0.9
1.50	0.36	to light	42.786	43.2	
2.00	0.48	yellowish	57.048	57.6	
3.00	0.72	green	85.572	86.4	

DISCUSSION

Since the ratio of the complex between thorium and L-asparagine is 1:4, in the determination of L-asparagine whatsoever is the value from calculation is multiplied by 4. Maximum error in these experiments was 0.9%. It was observed that L-leucine, DL-valine, DL-alanine, and glycine do not interfere. This method is the quickest possible for the determination of L-asparagine.

SUMMARY

L-Asparagine was determined in microquantities with thorium nitrate using bromcresol green as indicator. Range in which L-asparagine was estimated lies between 7.2×10^{-4} and 86.4×10^{-4} mg/1. Maximum error was 0.9%. It was observed that L-leucine, DL-valine, glycine, and DL-alanine do not interfere.

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Rapid Micromethod for Locating the Oxirane Group in 1,2-Epoxides

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INTRODUCTION

The position of epoxide groups in aliphatic compounds is usually determined by either hydrolysis of the group followed by splitting of the resulting 1,2-glycol with sodium periodate *(2)* or, according to a more recent procedure, by a one-step oxidative splitting of the epoxide using periodic acid *(4)*. In both cases the resulting aldehydes are identified. Usually the reactions have been performed in aqueous dioxane to facilitate solution of the organic epoxides and the inorganic reagents, and are followed by extraction, washing, and drying of the products before they are analyzed. The procedures require substantial amounts of starting material, are time consuming and are subject to losses of products, particularly of short-chain aldehydes.

MATERIALS AND METHODS

Starting Materials. All the starting epoxides were gas chromatographed during the study and the results of these analyses are discussed in some details below. Additional information about these compounds follows.

The 3,4-epoxyheptane was prepared from 3,4-heptene (Aldrich Chemical Co.) by epoxidation with peracetic acid and was purified by distillation. The product contained both *cis* and *trans* isomers as indicated by peaks at 810 and 890 cm^{-1} in the infrared spectrum.

The 2,3-epoxyoctane was a commercial sample of "Octylene oxide" obtained in 1958 through the courtesy of Becco Chemical Division of Food Machinery and Chemical Inc. The sample had been kept in the laboratory since that time and was used without further treatment.

The 1,2-epoxydodecane was kindly donated by Dr. W. E. Link, Archer Daniels Midland Co. The material had an oxirane value of 8.54 (theory 8.68) and was used as received.

Methyl *cis*- and *trans*-9,10-epoxystearates were prepared by epoxidation, with peracetic acid, of methyl oleate and elaidate, respectively. The epoxystearates were purified first by recrystallization from petroleum ether (bp 60–70°C) at −20°C for the *cis* isomer and 0°C for the *trans* compound and by column chromatography on silicic acid. The two compounds had the following characteristics: *cis:* mp 15.9–17°C; TLC, one spot; GLC, 98.5%; infrared, peak at 830 cm^{-1} *(5); trans:* mp 25.5–26.5°C; TLC, one spot; GLC, 99.9%; infrared, peak at 885 cm^{-1} *(5).*

Reagents. Diethyl ether used as a solvent was anhydrous analytical reagent. It was used without further treatment.

Periodic acid was purchased from G. F. Smith Co., Columbus, Ohio, and was ground to a fine powder in a mortar before use.

Sodium bicarbonate was of analytical reagent grade.

Apparatus. The reaction was carried out in micro test tubes, 50 × 6 mm o.d. The test tubes were made from 5 mm glass tubing and the lower 20 mm were tapered to a bottom diameter of 3 mm by drawing the tubing before sealing it. The test tubes were closed with Teflon plugs (for standard Luer hypodermic needle hubs; from Perkin-Elmer Corporation or Beckman Instruments, Inc.).

Gas chromatography was performed with an F & M model 810 instrument equipped with hydrogen flame detectors. Two columns, each 6 ft. × ⅛ in. o.d. were used. One, consisting of 10% EGSS-X on 100–120 mesh Gas Chrom P, was temperature programmed from 60–165°C at 10°/min and permitted separation of aldehydes with more than 6 carbon atoms. The second column made of 20% Carbowax 20M on 60–80 mesh Gas Chrom P was temperature programmed from 70–210°C at 6°/min and allowed the separation and identification of aldehydes with 3 or more carbon atoms.

Procedure. Ten to 50 μg of sample was introduced into the test tube and dissolved in 25 μl of ether. Finely powdered periodic acid (1.5–2.0 mg) was added from the bent tip of a microspatula and the test tube was immediately stoppered and mixed vigorously for about 30 seconds with the help of a vibrator. The reaction was allowed to proceed at room temperature for 20–40 minutes, depending on the compound, and the tube was shaken vigorously 4 or 5 times during that period. At the end of the reaction, 3–4 mg of powdered sodium bicarbonate was added,

the tube was again stoppered, shaken gently for a few seconds and the stopper removed once or twice to release the carbon dioxide formed. When gas evolution had ceased, 1 μl or more of the clear supernatant fluid was injected directly into the gas-liquid chromatograph.

RESULTS

2,3-Epoxyoctane. This material was the least pure and most complex of the compounds studied. It is discussed in detail to illustrate the results that may be expected. The gas-liquid chromatograms of the sample and of its periodic acid oxidation products are shown in Fig. 1. The epoxide (Fig. 1A) shows four peaks. The first three (peaks 3, 5, and 6) disappear on treatment with periodic acid and may be assumed to be epoxides. The fourth peak (peak 7) does not react with the periodic acid reagent and is not an epoxide. The first two peaks are due to the *trans* and *cis* isomers of 2,3-epoxyoctane, respectively, and their total area constitutes 96.6% of the total peak area of the sample. On periodic acid oxidation the 2,3-epoxyoctanes give rise to hexanal (peak 2) which is the major peak of Fig. 1B and amounts to 96% of the aldehyde peaks present. The third epoxide peak in Fig. 1A (peak 6) shows no evidence of *cis, trans* isomerism and is very likely due to 1,2-epoxyoctane. Terminal epoxides are slightly more polar than the internal isomers and therefore would be expected to have slightly longer retention times. This peak represents 3% of the epoxides in the sample and its expected oxidation product, heptanal (peak 4), amounts to 2.8% of the aldehydes in the chromatogram of the oxidation products. In addition, Fig. 1B shows a peak corresponding to pentanal (peak 1) and representing 1.3% of the total aldehydes. This indicates that a small amount of a third isomer, 3,4-epoxyoctane, was also present in the sample. The polarity of the 2,3- and 3,4-isomers would be expected to be very similar and the two compounds are not resolved in Fig. 1A. However, the shoulder on the leading edge of peak 3 may be due to the 3,4-epoxide.

3,4-Epoxyheptane. Gas chromatography of this material on a Carbowax column showed two major peaks corresponding to the *trans* and *cis* isomers and 2 small peaks (less than 1%) with slightly longer retention times. The latter did not react with periodic acid. The periodic acid oxidation products contained large amounts of the expected propanal and butanal with small quantities of residual unoxidized material and the

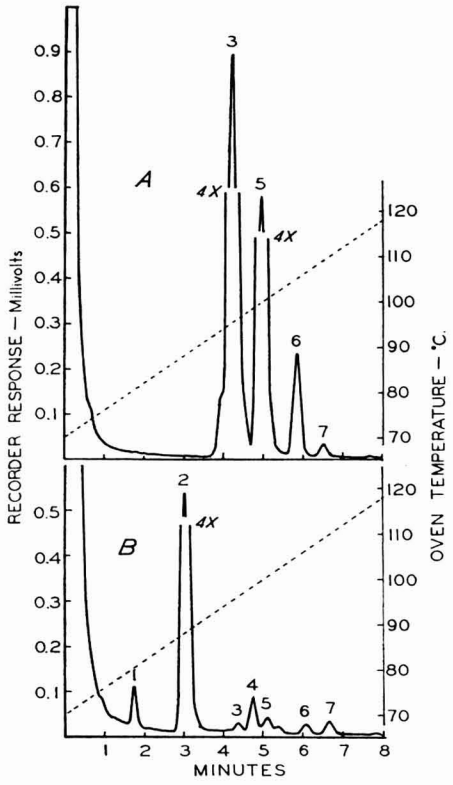

FIG. 1. Gas chromatograms of (A), 2,3-epoxyoctane, and (B), its periodic oxidation products. Conditions: 20% Carbowax 20M on 60–80 mesh Gas Chrom P 6 ft. × ⅛ in. o.d. column, temperature programmed 70–210°C at 6°/minute. Peak identification: (1) pentanal; (2) hexanal; (3) *trans*-2,3-epoxyoctane; (4) heptanal; (5) *cis*-2,3-epoxyoctane; (6) 1,2-epoxyoctane; (7) non-epoxide impurity.

impurities contained in the original sample. No other aldehydes were present.

1,2-Epoxydodecane. Epoxydodecane and its oxidation products were chromatographed on the EGSS-X column. The original material showed one large peak attributed to the 1,2-epoxide and two small unresolved peaks with slightly shorter retention times which disappeared on periodic acid oxidation and are probably due to small amounts of *cis*- and *trans*–2,3-epoxydodecanes. One additional small peak was unaffected by

periodic acid treatment and, therefore, is considered to be a non–epoxide impurity. Altogether the area of the minor peaks was less than 5% of the total area. The oxidation products consisted largely of undecanal (86%), a small amount of decanal from 2,3-epoxydodecane and several additional small peaks accounted for by unreacted epoxydodecane and the small amount of impurities present in the original sample.

9,10-Epoxystearates. The epoxystearates and their oxidation products were chromatographed on the EGSS-X column. The *trans* isomer gave a single peak while the *cis* compound showed a second peak corresponding to ketostearate (1.5%). The *trans* compound has a shorter retention time than the *cis* isomer. The oxidation products of both compounds consisted only of equal amounts of nonanal and methyl azelaaldehydate. Under the conditions used to analyze the oxidation products, the epoxystearate and ketostearates have long retention times and the chromatograms were not continued long enough to detect their presence in the reaction mixture.

Mixture of epoxides. Figure 2 shows a chromatogram, on the Carbowax column, of the periodic acid oxidation products of a mixture of all the epoxides studied. It is evident that the scission products expected from the epoxides are present in unmistakably large amounts, are well separated and easily identified. All the minor peaks not attributable to oxidation products are easily accounted for as unreacted epoxides or impurities present in the original materials.

DISCUSSION

Maerker and Haeberer *(4)* found that the splitting of epoxides with periodic acid took place very rapidly at room temperature in the aqueous dioxane medium they employed. They reported also that diethyl ether was not a satisfactory solvent and that water was required for the reaction to proceed. Using microgram quantities of epoxides and anhydrous diethyl ether as a solvent, we found that, although the reaction was slower than reported by Maerker and Haeberer, it was sufficiently rapid to insure completion of analysis within a reasonable time. The rate of the reaction appears to depend on the molecular weight of the epoxides. Table 1 shows that with epoxydodecane a reaction time of 15 minutes resulted in the oxidation of only ⅔ of the material while 94% was reacted after 35 minutes. Other experiments showed that 15 minutes was sufficient to oxidize more than 95% of epoxyheptane or epoxyoctane

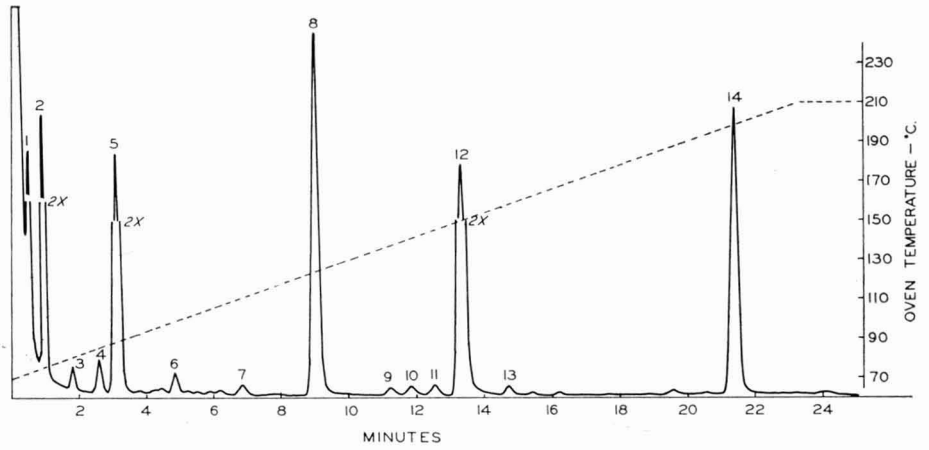

FIG. 2. Gas chromatogram of the periodic oxidation products of a mixture of epoxides. Conditions: same as Fig. 1. Peak identification and origin: from 3,4-epoxyheptane: (1) propanal; (2) butanal; (4) unreacted epoxyheptane; from epoxyoctane: (3) pentanal (from 3,4–); (5) hexanal (from 2,3–); (6) heptanal (from 1,2–); (7) impurity; from expoydodecane: (9) decanal (from 2,3–); (10) and (11) impurities; (12) undecanal (from 1,2–); (13) unreacted epoxydodecane; and from epoxystearate: (8) nonanal; (14) methyl azelaaldehydate.

but that the maximum yield of oxidation products from epoxystearate required 40 minutes.

Table 1 also shows that the addition of only 1 μl of water to the mixture retarded the reaction to such an extent that 90% of the epoxydodecane remained unreacted after 20 minutes while only 33% remained unreacted after 15 minutes in absence of added water. Water would be

TABLE 1

EFFECTS OF TIME AND WATER ON PERIODIC ACID OXIDATION OF EPOXYDODECANE

Reaction time (min)	Water added (μl)	Composition of products (%)	
		Undecanal	Epoxydodecane
15	0	67	33
35	0	94	6
20	1	10	90

required for the reaction if it is assumed that the first step is an acid catalyzed hydrolysis of the epoxide to a 1,2-glycol which is then split by periodic acid. However, Maerker and Haeberer *(4)* suggested that the splitting of epoxides with periodic acid does not require the formation of a glycol intermediate, but occurs directly through the acid catalyzed formation of a penta coordinated cyclic iodine complex as proposed by Buist *et al. (1)* during the periodate splitting of 1,2-glycols. Such a reaction does not require water and our observation, therefore, appears to support this proposal. However, with the small amounts of epoxides employed, enough water for hydrolysis of the epoxide may be available from the periodic acid used ($HIO_4 \cdot 2H_2O$).

Under the conditions employed, the compounds studied have retention times on the Carbowax column 3–4 times greater than on the EGSS-X substrate. The Carbowax column is necessary to detect aldehydes as small as propanal which appears as a definite peak but not completely resolved from the solvent peak. With the EGSS–X column the first aldehyde to separate from the solvent front is hexanal. When no oxidation products lower than hexanal are expected this column is preferred since it shortens analysis time appreciably. In some cases, however, the use of both columns with the same sample may be desirable to detect both short- and long-chain oxidation products.

In addition to the epoxides, vicinal glycols, amino alcohols, ketols, and hydroxy aldehydes react with periodic acid to give aldehydes also *(3)*. Obviously, the epoxide preparation examined must be substantially free of these substances. Usually this can be ascertained easily by thin-layer or gas chromatography since the interfering compounds contain hydroxyl groups and are more polar than the epoxides.

On the other hand, the procedure described here should be useful also for determining the structure of micro amounts of these compounds. In case of α-ketols and α-hydroxy aldehydes, however, only the aldehyde group resulting from the oxidation of the hydroxyl would be determined since the carboxylic acid derived from the carbonyl function would be neutralized to its sodium salt by the bicarbonate added. For the same reason the procedure is not suitable for the analysis of α-diketones or α-keto aldehydes, which are split by periodic acid also but do not yield aldehydes.

The results obtained with epoxyoctane (Fig. 1) show that, in addition to its use to determine the structure of relatively pure epoxides, the

method is sensitive enough to detect and identify small amounts of isomeric epoxides and non-epoxide impurities.

SUMMARY

A procedure for the analysis of micro amounts of epoxides is described. The method is based on the splitting of epoxides by periodic acid in a nonaqueous medium, followed by direct analysis of the reaction mixture by gas chromatography. The procedure is simple, rapid and requires only a few micrograms of material. The reaction is straightforward and yields no secondary products. The gas chromatographic analysis is sufficiently sensitive to detect small amounts of epoxy and non–epoxy impurities in addition to the major epoxide being analyzed. The method is not specific for epoxides, and its application to other substances capable of being split by periodic acid is discussed.

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Disc Electrophoresis of Subclasses of Human Serum Low Density Lipoproteins

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Disc electrophoresis is a highly sensitive electrophoretic technique that has been successfully used to obtain a high degree of resolution of the major classes of human serum lipoproteins *(10)*. This technique has, however, not been extended to the various subclasses of human serum low density lipoproteins. It is well known that serum low density and very low density lipoproteins are elevated among patients with coronary heart disease as compared with matched control *(4)*. It was therefore considered worthwhile to develop techniques to obtain optimum resolution between various subclasses of human serum low density lipoproteins. We report here the disc electrophoresis of normal human serum chylomicrons, very low density lipoproteins and the two classes of low density lipoproteins using several main gel concentrations.

MATERIALS AND METHODS

Fresh fasting blood was obtained from one of the authors (K.A.N.) and the serum derived from it was used in the isolation of very low density lipoprotein, $d < 1.006$ (VLDL), two low density lipoproteins, $1.006 < d < 1.019$ (LDI_1) and $1.019 < d < 1.050$ (LDL_2). In order to obtain fresh human serum chylomicrons, the fasting subject (K.A.N.) was given orally a corn oil emulsion and the blood was obtained after 3 hours.

Chylomicrons and very low density lipoproteins were isolated from fasting and nonfasting serum by ultracentrifugation of the serum without density adjustment at 208,314 *g* for 14 hours, using a type 65 rotor in a Spinco model L2-65 ultracentrifuge. The top fraction was resolved into chylomicron and VLDL species by centrifugation at 114,480 *g* for 45 minutes. The bottom fraction, which contained the crude VLDL, was recentrifuged twice at 114,480 *g* for 45 minutes in order to remove the

chylomicron impurity. The bottom fraction from the final centrifugation constituted the purified VLDL which was used in all subsequent experiments. The top fractions from above were pooled and centrifuged twice at 114,480 *g* for 45 minutes to obtain the final floating fraction which constituted the purified chylomicrons. As compared to the previous method *(8)* the present procedure decreased the loss of serum proteins and lipoproteins from the bottom serum fraction into the chylomicron fraction.

The bottom serum fraction obtained after removal of chylomicrons and VLDL was used in the isolation of LDL_1 and LDL_2, according to the method of Havel *et al. (3).*

The procedure employed for disc electrophoresis was described before *(6,7).* The quantity of lipoprotein fraction loaded on the gel generally varied from 50–200 μg and depended upon the sample and stain employed. The equipment used in this investigation was the model 12 disc electrophoresis unit (Canalco, Rockville, Maryland). Gels of several pore sizes were employed in this study and in case of "soft" gels which cannot be easily removed from glass tubes, the technique described by Narayan *(5)* was used. The procedures used for staining and destaining the gels with amido black 10B, oil red O, and Sudan black B were described earlier *(9).* Where relative migration distances of the components were desired, the tracking dye was permitted to penetrate the same, fixed distance into each gel.

RESULTS AND DISCUSSION

The present results conclusively showed that VLDL, LDL_1 and LDL_2 migrated through the spacer gel. On the other hand, the bulk of the chylomicron fraction failed to enter the spacer gel.

Resolution with 3.75% gel and standard spacer gel. The chylomicron fraction exhibited an intense band on top of the spacer gel and a band of light medium intensity near the spacer gel–main gel interface with oil red O stain (Fig. 1a). A few specks or particles were also noted inside the spacer gel but were not as numerous as those observed earlier with rat serum chylomicrons *(8),* possibly because the blood was collected 3 hours after ingestion of emulsion in the present experiment. Since the blood was obtained 8 hours after administration of the emulsion in the previous experiment, part of the chylomicrons may have been metabolized to smaller sized macromolecules. With amido black 10B stain, the

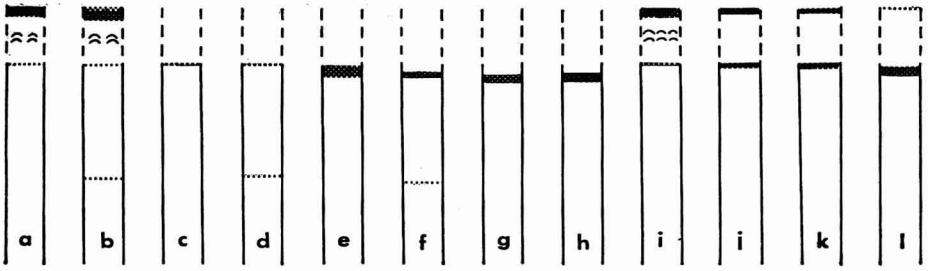

FIG. 1. Disc electrophoretic patterns using a standard spacer gel and a main gel of 3.75% polyacrylamide concentration. a, b, i, chylomicrons; c, d, j, VLDL; e, f, k, LDL_1; g, h, l, LDL_2. Tubes a, c, e, g, oil red O stain. Tubes b, d, f, h, amido black 10B stain. Tubes i, j, k, l, Sudan black prestain. For Figs. 1, 2, and 3 migration is from top to bottom and 2.5 mA/tube was used in all cases. The spacer gel is indicated by broken lines.

heavy spacer band appeared to be composed of two bands (Fig. 1b). In the case of VLDL, LDL_1, and LDL_2 fractions, no spacer bands could be detected with either oil red O or amido black 10B stain (Fig. 1c–h). A nonmigrating main gel component was, however, observed in the VLDL fraction. Both the LDL_1 and LDL_2 components migrated slightly into the 3.75% gel with the LDL_2 component migrating somewhat farther than the LDL_1 component. A fast component (presumably albumin contaminant) of faint to light medium intensity was observed with all fractions using amido black 10B stain.

Detection of lipoprotein components in 3.75% gel using different stains. As discussed earlier *(8),* it is preferable to use either oil red O or amido black 10B stain rather than Sudan black B stain for the identification of spacer gel components. Oil red O stains lipid intensely and, thus, chylomicron components which are predominantly composed of lipids are readily detected with this stain. The top spacer chylomicron bands were detected with amido black 10B as two bands, one staining very lightly and the other staining intensely (Fig. 1b). On the other hand, with oil red O stain, the resolution between the two bands was low because their intensities were approximately the same with this stain (Fig. 1a).

Oil red O is a very sensitive stain for lipids and, therefore, the absence of any spacer bands with VLDL, LDL_1, and LDL_2 fractions indicated that these macromolecules were not large enough to be retarded

by the spacer gel. With Sudan black B stain, a spacer "band" was observed with VLDL, LDL_1 and occasionally with LDL_2 (Fig. 1j,k,l). The presence of these spacer "bands" in VLDL and LDL_1 with Sudan black B stain indicated that these bands were the result of precipitation of unbound dye on top of the spacer. Similar results have also been observed with Sudan black B-prestained serum fractions, which were devoid of lipoproteins *(7)*. Hence meaningful results concerning spacer bands cannot be obtained solely with Sudan black B stain.

With respect to lipoprotein components in the main gel, the patterns observed with the three dyes were similar and showed that differences in staining technique did not significantly affect the detection of low density lipoprotein bands under the present conditions.

Resolution at different gel concentrations: 5, 3.75, 3.0, 2.5, and 1.9%. At 5% gel concentration, the low density lipoproteins did not penetrate the main gel (Fig. 2g,h) and hence lower gel concentrations were used to resolve these lipoproteins. The patterns in the main gel using 3.75% gel were not dependent on the type of dye used and the LDL_1 component was intermediate in mobility to that of VLDL and LDL_2. However, when 3.0% main gel and either amido black 10B or oil red O stain were used, VLDL was detected as a diffuse band whereas LDL_1 and LDL_2 were detected as single bands. When Sudan black B prestain and 3.0% main gel were employed, the spreading of VLDL was somewhat less than that observed with amido black 10B or oil red O stain. At all main gel concentrations used in this study (3.75, 3.0, 2.5, and

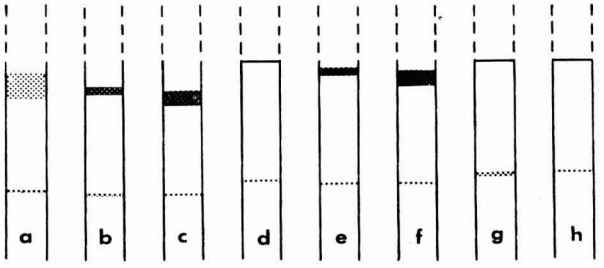

FIG. 2. Disc electrophoretic patterns using standard spacer gel and amido black 10B stain. Tubes a, b, c, VLDL, LDL_1, and LDL_2, respectively, using 3.0% main gel. Tubes d, e, f, VLDL, LDL_1, and LDL_2, respectively, using 3.75% main gel. Tubes g, h, VLDL and LDL_1, respectively, using 5.0% main gel.

1.9%), LDL_1 and LDL_2 migrated as single bands with the former migrating somewhat less than the latter.

The relative migration distances in 3.0% gel of VLDL, LDL_1, and LDL_2 were confirmed by subjecting appropriate mixtures of these fractions to disc electrophoresis (Fig. 3). For instance, two bands were observed with a mixture of LDL_1 and LDL_2 and at least two bands were observed with a mixture of VLDL and LDL_1 (Fig. 3g,h). Using Sudan black B stain and 2.5% gel, three incompletely resolved diffuse zones appeared to be present in the VLDL fraction. Furthermore, it appeared that one of these zones merged with the chylomicron component in the main gel and the other occupied approximately the same position as the LDL_1 component. An intermidiate component between the fast and slow VLDL components was possibly also present. With 1.9% gel, the VLDL, LDL_1, and LDL_2 components penetrated farther into the main gel than in the case of other gels but the resolution was not enhanced.

The present results in 3.0% gel demonstrated that the presence of S_f 10–20 species (LDL_1) and S_f 20–400 species (VLDL) could be detected as separate bands behind the usual β-lipoprotein band (LDL_2, S_f 3–8 species). Furthermore, the S_f 20–400 species was further resolved, although incompletely, into two to three components. By paper electrophoresis, the glyceride rich S_f 20–400 species have been detected as a fast moving pre-β-lipoprotein band, whereas the S_f 10–20 species have not been resolved from the β-lipoprotein band *(11)*. Using agar gel, Gustafson *et al. (2)* observed that the S_f 20–50 and S_f 50–100 species

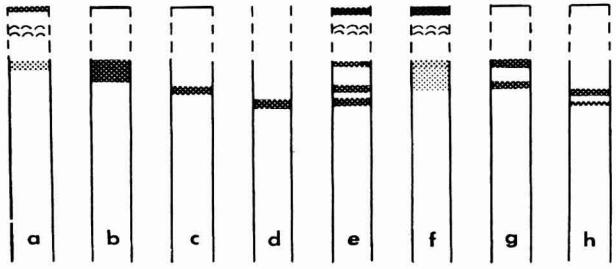

FIG. 3. Disc electrophoretic patterns using standard spacer gel, a main gel of 3.0% and Sudan black B prestain. Tubes a, b, c, d, chylomicrons, VLDL, LDL_1, and LDL_2, respectively. Tube e, a mixture of chylomicrons, VLDL, LDL_1, and LDL_2; Tube f, a mixture of chylomicrons and VLDL; Tube g, a mixture of VLDL and LDL_1; Tube h, a mixture of LDL_1 and LDL_2.

migrated to a pre-β-lipoprotein position, whereas the S_f 100–400 species was incompletely resolved and that the $S_f > 400$ species remained at the origin. The reason why part of the VLDL species did not migrate to the pre-β-lipoprotein in 3.75% acrylamide gel was perhaps due to the pore size of the gel which retarded the penetration of these large macromolecules. When larger pore size gels were used, part of the VLDL species migrated to the LDL_1 position.

The present results, which indicated that the bulk of isolated human serum chylomicrons [particle diameter from 70 to over 400 mμ, Ref. *(1)*] did not penetrate the standard spacer gel, are in agreement with previous work from this laboratory using rat serum chylomicrons *(8)*. On the basis of the present work, it was clear that the VLDL species S_f 20–400 [particle diameter from 27 to about 90 mμ, Ref. *(2)*] are sufficiently small when compared to the pore size of the spacer gel. In the purification of the VLDL fraction, it was, however, possible that a part of the larger VLDL macromolecules may have been removed along with the chylomicron impurity. Therefore, the limiting size of a macromolecule for complete penetration into the standard spacer gel cannot be accurately estimated from the present data.

In the case of either paper or agar gel electrophoresis, the quantities of samples used are generally much more than that required for the present method. Furthermore, the resolution obtained in this investigation using 3.0% acrylamide gel was superior to that reported using other electrophoretic methods. Hence, the techniques described here may perhaps find application in clinical investigations involving serum lipoproteins.

SUMMARY

Serum chylomicrons, very low density lipoproteins ($d < 1.006$) and two classes of low density lipoproteins ($1.006 < d < 1.019$ and $1.019 < d < 1.050$) were isolated from freshly collected normal human blood. Disc electrophoresis was performed with microquantities of these fractions using the standard spacer gel and with main gel concentrations of 1.9, 2.5, 3.0, 3.75, and 5.0%. Three different stains, namely, amido black 10B, Sudan black B and oil red O were employed for detecting the lipoprotein components in gels of 3.0, 3.75, and 5.0% concentration and sudan black B prestain was used for gels of 2.5 and 1.9% concentration. The results demonstrated that all the lipoproteins, except the chylomicrons, penetrated the standard spacer gel. In 3.0% gel, the very low density lipoproteins and the larger low density lipoproteins ($1.006 < d < 1.019$) were detected as separate bands behind the β-lipoprotein band. The relative migration distances in 3.0% gel

of these lipoproteins were confirmed by investigating the electrophoretic behavior of synthetic mixtures of lipoproteins. The resolution obtained was superior to that previously reported with other electrophoretic methods and may find application in clinical investigations involving serum lipoproteins.

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Barium Titration of Sulfate with Chlorophosphonazo III as Indicator

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INTRODUCTION

Recently a comparison was made of six color indicators for the precipitation titration of sulfate with barium perchlorate *(4)*. Dimethysulfonazo III, i.e., 3, 6-bis (*p*-methyl-*o*-sulfophenylazo)-4, 5-dihydroxy-2, 7-naphthalenedisulfonic acid, was found as most suitable indicator. Chlorophosphonazo III, i.e., 3, 6-bis(*p*-chloro-*o*-phosphonophenylazo)-4, 5-dihydroxy-2, 7-naphthalenedisulfonic acid, is known as the most sensitive metallochromic reagent for the spectrophotometric determination of barium *(3, 5)*. The barium complex of Chlorophosphonazo III is of higher stability than barium complex of Dimethylsulfonazo III so that its use as an indicator for sulfate titration with barium in aqueous solution gives only very poor results *(4)*. However the sensitivity of barium sulfate precipitation may be increased by the use of an acetone medium. In this case Chlorophosphonazo III is an excellent indicator and comparable with Dimethylsulfonazo III.

MATERIALS AND METHODS

Apparatus

All photometric measurements were made with a recording spectrophotometer Cary 14, Applied Physics Corporation, Monrovia, California, with 1.00-cm quartz cells. The organic sulfur was determined in a 250-ml Schöniger flask.

Reagents

Aqueous 0.01 *M* solutions of barium chloride, potassium sulfate, potassium hydrogen phosphate, sodium hydrogen arsenate, the disodium salt of EDTA and sodium chloride were prepared in the usual manner. Other metal ions were used as 0.10 *M* aqueous chloride or nitrate solu-

tions. Chlorophosphonazo III and Dimethylsulfonazo III were used as 0.1% (w/v) aqueous solutions. Both reagents were prepared as given in the patent literature (*2*), and were obtained as their sodium salts. Since the free acids are the best form for elemental analyses, the sodium salts were converted by passing their aqueous solutions through a column of a strongly acidic cation exchanger, e.g., Dowex 50WX2. The results obtained for sulfur and nitrogen are collected in Table 1. The purity of the indicators was also checked by paper chromatography on Whatman no. 1 paper with aqueous 2 *M* ammonia saturated with isobutanol as eluant. Both indicators used were chromatographically pure; corresponding R_F values are also given Table 1.

All chemicals used were the "Analar" products of BDH (Canada).

TABLE 1

QUANTITATIVE ANALYSIS OF REAGENTS

Reagent	R_F	Mol. wt.	N (%)		S (%)	
			Found	Calc	Found	Calc
Dimethylsulfonazo III	0.38	716.7	7.71	7.82	17.75	17.90
Chlorophosphonazo III	0.65	757.4	7.32	7.40	8.33	8.47

Determination of Sulfate

A sample containing 0.01–0.10 mmole of sulfate was dissolved in 5 ml of water; 20 ml of acetone and 3 drops of the indicator solution were added and the solution was titrated with 0.01 *M* barium chloride. The color change at the end point was from wine red to blue. 1.00 ml of 0.01 *M* barium chloride corresponds to 0.32066 mg of sulfur.

The results obtained are given in Table 2. The volume of acetone may vary from 5 to 30 ml but the volume given above appears as most suitable. The use of alcohols, such as methyl alcohol, ethyl alcohol, propyl alcohol, and iso-propyl alcohol, or of dioxane and methylethyl ketone leads to a poorer sharpness of color change at the equivalence point. Barium perchlorate or nitrate may be used instead of barium chloride without any effect on accuracy of sulfate determination.

TABLE 2

DETERMINATION OF SULFUR IN SULFATES

Sample	S (%)		SD (%)
	Found [a]	Calc	
Lithium sulfate	29.14	29.17	±0.04
Sodium sulfate [b]	22.53	22.57	±0.02
Potassium sulfate	18.38	18.40	±0.03
Ammonium sulfate	24.24	24.27	±0.03

[a] Average values of 10 determinations.
[b] Anhydrous.

Photometric comparison of Chlorophosphonazo III and Dimethylsulfonazo III as indicators of a sulfate titration is given in Fig. 1. A greater difference between spectra 1 and 2 appears in the case of

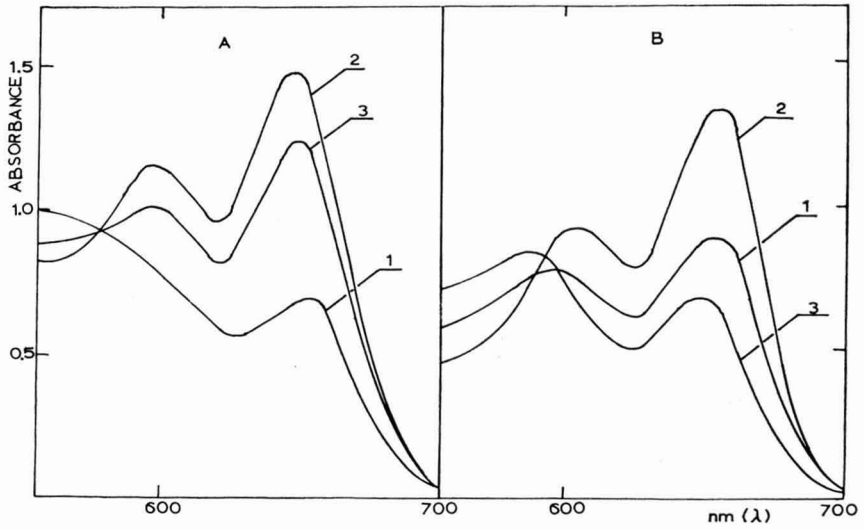

FIG. 1. Variations of spectra near of equivalent point of a sulfate titration; (A) Chlorophosphonazo III indicator, (B) Dimethylsulfonazo III indicator. (1) indicator alone, $C_L = 2.00 \times 10^{-5}$ M in 25 ml of 80% of aqueous acetone; (2) the same solution as (1) after addition of 0.04 ml of 0.01 M barium chloride; (3) the same solution as 2 after addition of 0.08 ml of 0.01 M potassium sulfate.

Chlorophosphonazo III because of its higher sensitivity for barium. In the case of Dimethylsulfonazo III, however, the greater difference between spectra 2 and 3 means a more quantitative decomposition of Dimethylsulfonazo III–barium complex because of a barium sulfate precipitation.

Effect of Foreign Ions on the Sulfate Determination

The effect of 21 cations and 10 anions on the sulfate determination was investigated. The limiting value of the concentration of a foreign ion was taken as that which caused an error of $\pm 0.3\%$ of sulfur determination. The results obtained are summarized in Table 3.

If any uranyl, yttrium, lanthanum, zirconium, hafnium, and thorium ions are present, the sulfate determination with Chlorophosphonazo III is impossible because of a blocking of the indicator. The molar absorptivities for complexes of all these elements are in the range 20,000 to 70,000 $mmole^{-1}cm^2$ and complexes with a molar ratio 1:1 or 1:2 (metal:ligand) are usually formed [see (*1*)].

Determination of Organic Sulfur

Samples (4–7 mg) were burned by Schöniger's flask method (6) with a 250–ml flask, the combustion products being absorbed in 4 ml of water and 0.15 ml of 30% hydrogen peroxide solution. The flask was shaken for 15 minutes to obtain complete absorption 20 ml of acetone and 3 drops of Chlorophosphonazo III solution were added and the solution was titrated with 0.01 *M* barium chloride. Some typical results are shown in Table 4.

DISCUSSION

In comparison with Dimethylsulfonazo III, Chlorophosphonazo III shows several advantages such as a higher sensitivity for barium, a possibility of titration in acidic solution up to a concentration of 0.1 N of strong mineral acid (perchloric, nitric, or hydrochloric acid) and a possibility of EDTA masking. Both acidic medium and EDTA masking increase remarkably the selectivity of sulfate titration. Unfortunately Chlorophosphonazo III forms complexes of extremely high stability with yttrium, lanthanum, zirconium, hafnium, thorium, and uranium(IV, VI) so that no masking and no acidity adjustation is effective for suppression of their intereference.

TABLE 3

DETERMINATION OF SULFUR IN SULFATE[a] IN PRESENCE OF OTHER IONS

Added (mmole)	S (%) Found	Added (mmole)	S (%) Found
—	18.38	0.10 $CoCl_2$ [c]	18.56
2.50 $HClO_4$	18.55	0.15	—[b]
5.00	19.14	0.10 $NiCl_2$[c]	18.53
2.50 HCl	18.52	0.15	—[b]
5.00	19.07	0.10 $CdCl_2$[c]	18.56
2.50 HNO_3	18.56	0.15	—[b]
5.00	19.09	0.10 $MgCl_2$[c]	18.42
2×10^{-5} $FeCl_3$	18.51	0.15	—[b]
1×10^{-4}	—[b]	0.10 $Hg(NO_3)_2$[c]	18.40
0.06 $ZnCl_2$	18.58	0.20	18.43
0.10	18.75	0.50	—[b]
2×10^{-5} $CuCl_2$	18.45	0.10 $CuCl_2$[c]	18.42
1×10^{-4}	—[b]	0.15	—[b]
1×10^{-5} $UO_2(NO_3)_2$	18.44	0.05 $Y(NO_3)_3$[c]	—[b]
1×10^{-4}	—[b]	0.05 $La(NO_3)_3$[c]	—[b]
1×10^{-5} $La(NO_3)_3$	18.45	0.10 $AlCl_3$[c]	18.58
1×10^{-4}	—[b]	0.15	—[b]
1×10^{-5} $Zr(NO_3)_4$	18.61	0.05 $Zr(NO_3)_4$[c]	—[b]
1×10^{-4}	—[b]	0.05 $Th(NO_3)_4$[c]	—[b]
1×10^{-5} $Hf(NO_3)_4$	18.58	0.05 $UO_2(NO_3)_2$[c]	—[b]
1×10^{-4}	—[b]	0.10 NH_4VO_3[c]	18.41
1×10^{-5} $Th(NO_3)_4$	18.54	0.10 $MnCl_2$[c]	18.61
1×10^{-4}	—[b]	0.15	—[b]
0.01 KH_2PO_4	23.55	0.10 $BeCl_2$[c]	18.65
0.001	18.45	0.15	—[b]
0.01 Na_2HAsO_4	23.46	0.10 $FeCl_3$[c]	18.62
0.001	18.42	0.15	—[b]
0.01 EDTA	18.41	0.01 NH_4F	18.40
0.10	18.55	0.10	18.65
0.30	18.75	0.20	19.10
0.01 $FeCl_3$[d]	18.25	1.00 $HClO_4$[d]	18.42
0.10	—[b]	2.50	19.20[e]
0.10 $FeCl_3$ + 0.10 KH_2PO_4[d]	18.52	1.00 HCl[d]	18.39
0.50 $ZnCl_2$	—[b]	2.50	19.30[e]
0.10 $Th(NO_3)_4$[d]	—[b]	1.00 HNO_3[d]	18.40
0.10 EDTA	18.80	2.50	19.35[e]

[a] 0.10-mmole (= 17.43 mg) sample of potassium sulfate was used in every case.

[b] Titration was impossible because of blocking of indicator.

[c] A 0.10-mmole sample of EDTA was added.

[d] Dimethylsulfonazo III was used as indicator.

[e] Poor color change in equivalent point.

TABLE 4

DETERMINATION OF ORGANIC SULFUR (Chlorophosphonazo III as indicator)

Sample	S (%)		SD (%)
	Calc	Found [a]	
l-Cysteine	26.47	26.50	±0.15
Thiosemicarbazide	35.19	35.26	±0.18
Taurine	25.62	25.55	±0.15
Chromotropic acid (dihydrate)	18.00	17.88	±0.14
Thiourea	42.13	41.95	±0.21
Dimethylsulfonazo III	17.90	17.65	±0.20
Sulfanilic acid	18.52	18.50	±0.13
Thiamine	9.51	9.53	±0.10

[a] Average of 5 determinations

The reaction of barium with Dimethylsulfonazo III is less sensitive but more selective than its reaction with Chlorophosphonazo III. For such reasons, the titration of sulfate indicated by Dimethylsulfonazo III can be performed in the presence of magnesium, cadmium, aluminum, managnese(II), uranium(VI) and chromium(VI) without any masking, which is not the case with Chlorophosphonazo III.

SUMMARY

Chlorophosphonazo III may be used for visual indication of a sulfate precipitation titration with barium chloride, perchlorate or nitrate. Titration in acidic medium up to 0.1 *N* of strong mineral acid and application of EDTA masking are possible. The determination of organic sulfur by the combustion method is also possible with use of this indicator.

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Microcrystalloptic Test for Some Minor Alkaloids of *Peumus boldus*

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Leaves of the Chilean shrub *Peumus boldus* Molina (Monimiaceae), Boldo leaves, are used in pharmacy and contain a number of alkaloids, mostly aporphines. Boldo leaves are mainly used as cholagogues. The major alkaloids, boldine, isocorydine, *N*-methyllaurotetanine and norisocorydine have been isolated by Rüegger *(27)*. Recently, some minor alkaloids have been detected in the same plant: reticuline, isoboldine, laurotetanine, laurolitsine, and isocorydine-*N*-oxide *(18–20)*. These alkaloids are not restricted to *P. boldus* but are also found in a number of other species in various plant families, i.e.: reticuline in *Cocculus laurifolius (24)* (Menispermaceae), *Anona reticulata (17), Xylopia papuana (23)* (Annonaceae), *Argemone hispida, A. munita* subsp. *routundata (29), Romneyi coulteri (30)* (Papaveraceae) and *Hernandia ovigera (14)* (Hernandiaceae); isoboldine in *Nandina domestica (28)* (Berberidaceae), *Cassytha pubescens (21), Beilschmiedia elliptica (11)* (Lauraceae) and *Laurelia novae-zelandiae (4)* (Monimiaceae); laurotetanine in *Litsea chrysocoma, L. citrata, L. cubeba, Actinodaphne procera (25), Neolitsea sericea (26)* (Lauraceae), *Illigera pulchra (25)* (Combretaceae), and *Palmeria fengeriana (22)* (Monimiaceae); laurolitsine in *N. sericea (26), L. japonica (28)* (Lauraceae), *X. papuana (23)* (Annonaceae) and *L. novae-zelandiae (4)* (Monimiaceae). Reticuline and isoboldine have also been found in opium *(7, 8)*. Reticuline, a benzyltetrahydroisoquinoline, has a key position in the biogenesis of various alkaloidal groups since it was shown that it can serve not only as a precursor for aporphine alkaloids *(5)* but also of alkaloids of the protoberberine *(6)*, berberine, protopine *(2)*, and morphine *(1, 3)* type.

It appears likely that the minor alkaloids of *P. boldus* will probably be detected in many other plant species in the future since only a small

percentage of all plants have been chemically investigated, many screening programs for alkaloids *(13)* are currently undertaken and research work in biosynthesis and chemotaxonomy of alkaloids *(31)* is steadily increasing.

While microchemical tests for boldine and the other major alkaloids of *P. boldus* have been reported *(10, 16)*, no such procedure for the minor alkaloids has appeared in the literature.

METHODS

The hanging drop technique of Clarke and Williams *(9)* and microoptical measurements as outlined earlier *(15)* were applied. Crystal forms were described according to the classification scheme published earlier *(12)*. For the microoptical measurements a Spencer polarizing microscope (model 37A), equipped with a mechanical, graduated circular rotatory stage and a first order red retardation plate was used. The

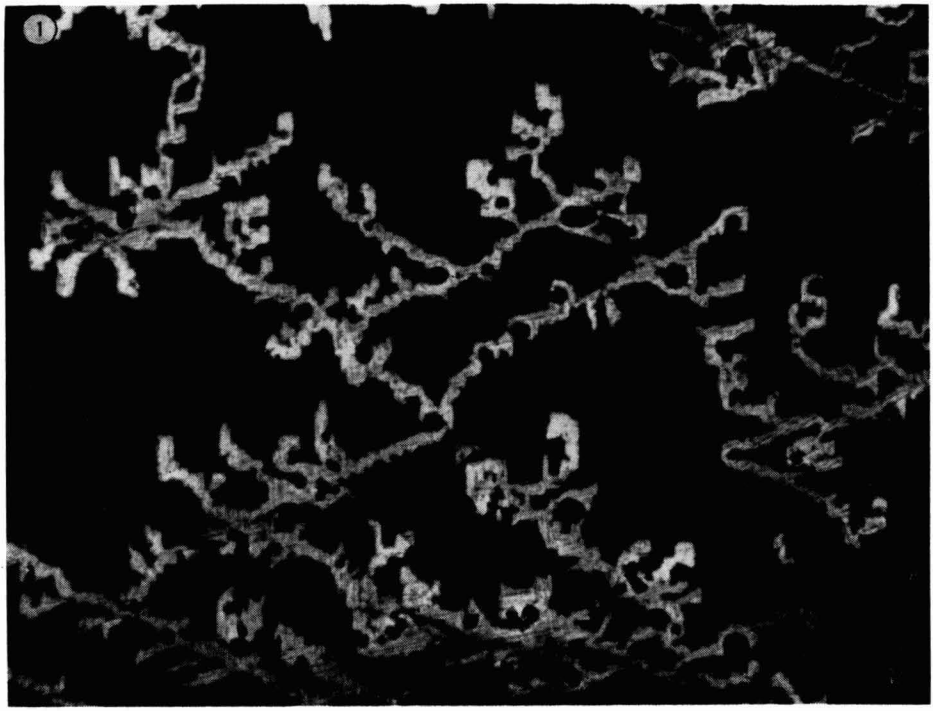

FIG. 1. Microcrystals of reticuline with gold cyanide in polarized light; 95×.

microphotographs were taken with a Carl Zeiss photomicroscope with polarizing equipment. The vibration direction of the plane polarized light in this microscope is in the north–south direction.

Solutions of (+)-reticuline, isoboldine, laurotetanine, laurolitsine, and isocorydine-*N*-oxide in 1% acetic acid (1mg/ml) were used. Microdrops produced with a microrod (diameter 1 mm) of the alkaloid and reagent solutions were applied to a cover glass, mixed and, after inversion of the cover glass, placed on a microscope slide. Several crystals of the same slide were examined. The sensitivity of the tests in between 0.025 and 1 μg *(9, 10)*. Reagent solutions are mostly taken from Clarke *(9, 10)*; they are 5% aqueous solutions, unless otherwise indicated.

RESULTS

Reticuline

Reagents. Gold cyanide (5 g of gold chloride dissolved in 100 ml water, solid sodium cyanide is added until the precipitate redissolves).

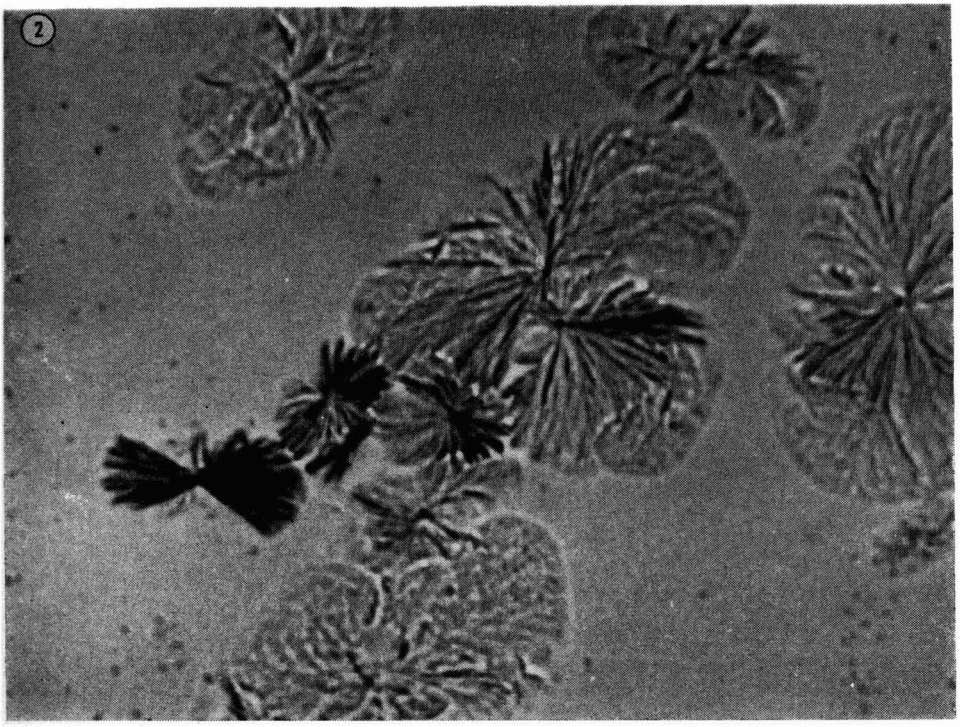

FIG. 2. Microcrystals of reticuline with lead iodide; 120×.

Form of crystals: rods, irregular "stair cases," Class 5 (Fig. 1); moderate birefringence (first order); inclined extinction; angle of extinction: 19°; negative sign of elongation.

Lead iodide (a 30% solution of lead acetate is adjusted to pH 6 with acetic acid and saturated with lead iodide). Form of crystals: sheaves, Class 4b (Figs. 2 and 3); moderate birefringence (first order); parallel extinction; positive sign of elongation.

Isoboldine

Reagents. Potassium chromate (5% in water). Form of crystals: rods, in small clusters, "railroad tracks", Class 5 (Figs. 4 and 5); no birefringence.

Potassium chromate (1% in water). Form of crystals: rosettes of rods, Class 9c (Fig. 6); no birefringence.

FIG. 3. Microcrystals of reticuline with lead iodide in polarized light; 120×.

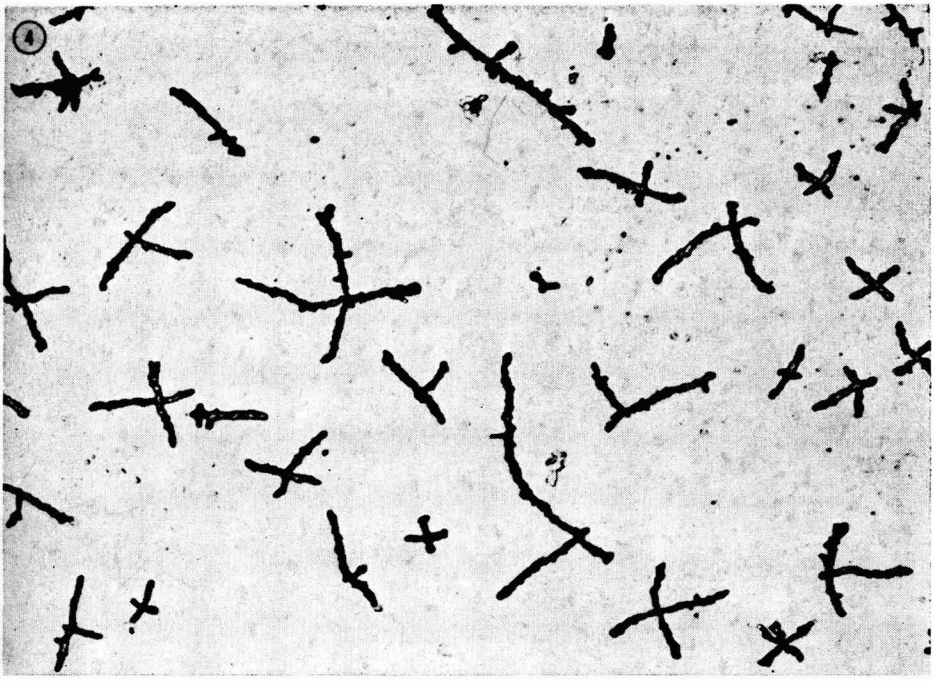

FIG. 4. Microcrystals of isoboldine with potassium chromate (5%); 120×.

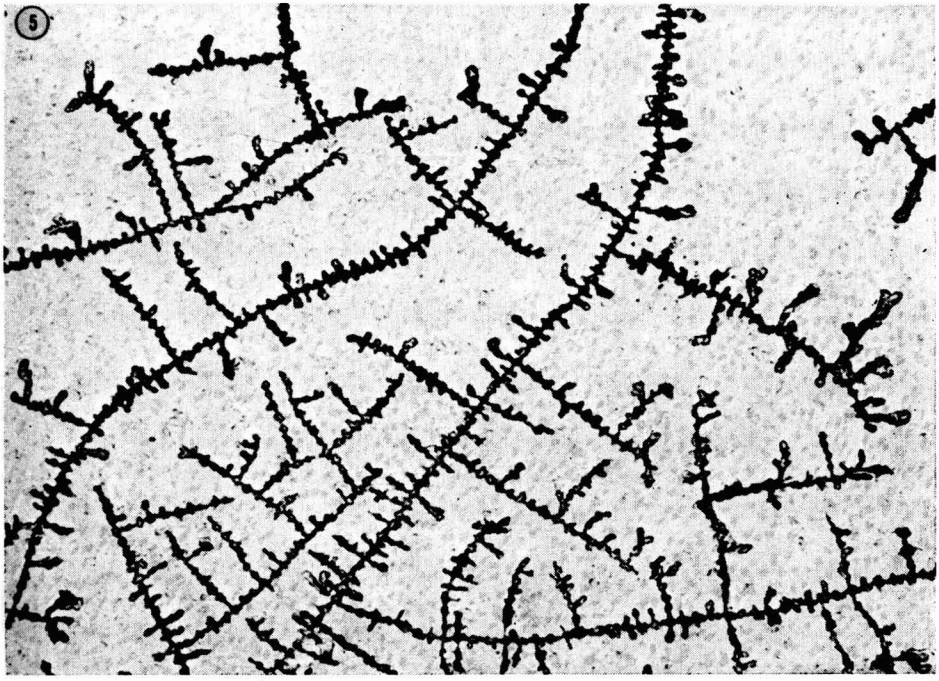

FIG. 5. Microcrystals of isoboldine with potassium chromate (5%); 120×.

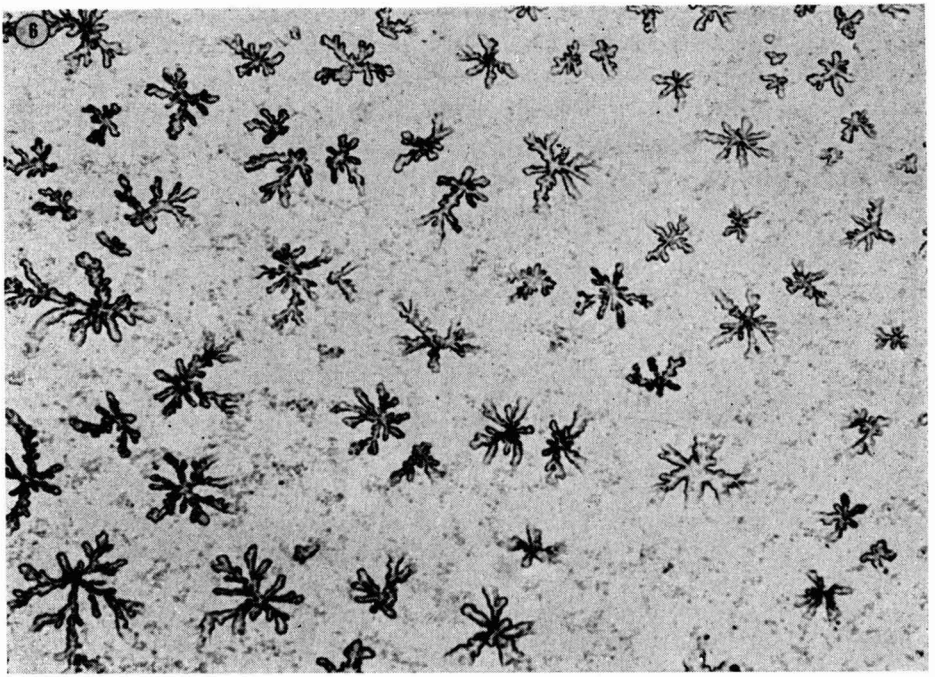

FIG. 6. Microcrystals of isoboldine with potassium chromate (1%); 120×.

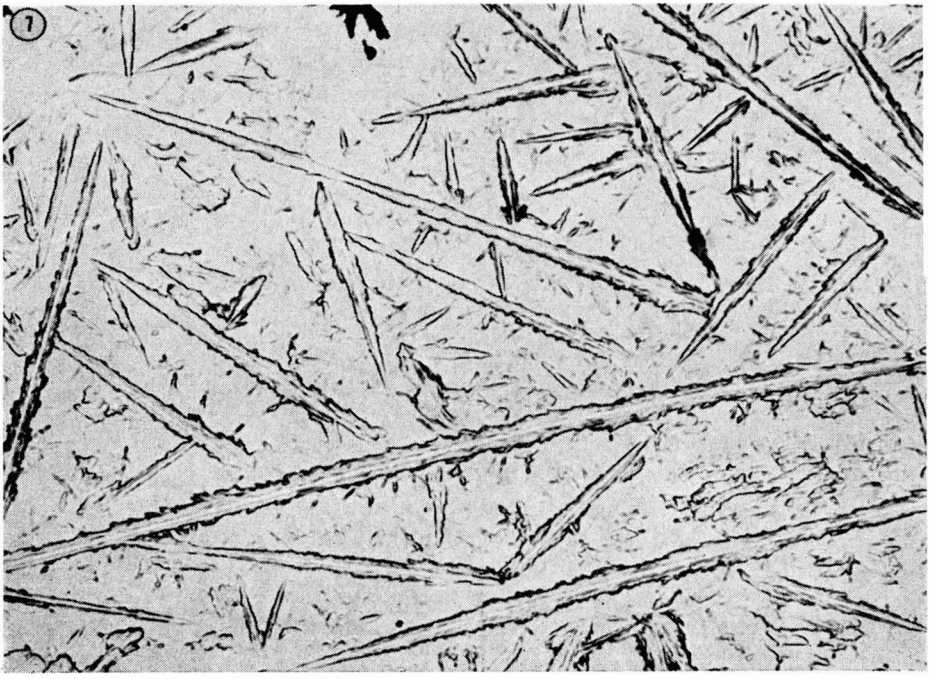

FIG. 7. Microcrystals of isoboldine with sodium carbonate; 120×.

Sodium carbonate. Form of crystals: blades, serrated edges, Class 6 (Fig. 7); dim birefringence (first order); parallel extinction; positive sign of elongation.

Ammonium thiocyanate. Form of crystals: plates in clusters, Class 7a (Fig. 8); moderate birefringence (first order); parallel extinction; positive sign of elongation.

Laurotetanine

Reagent. Picric acid. Form of crystals: rosettes of needles, Class 9c (Fig. 9); moderate birefringence (second order); parallel extinction.

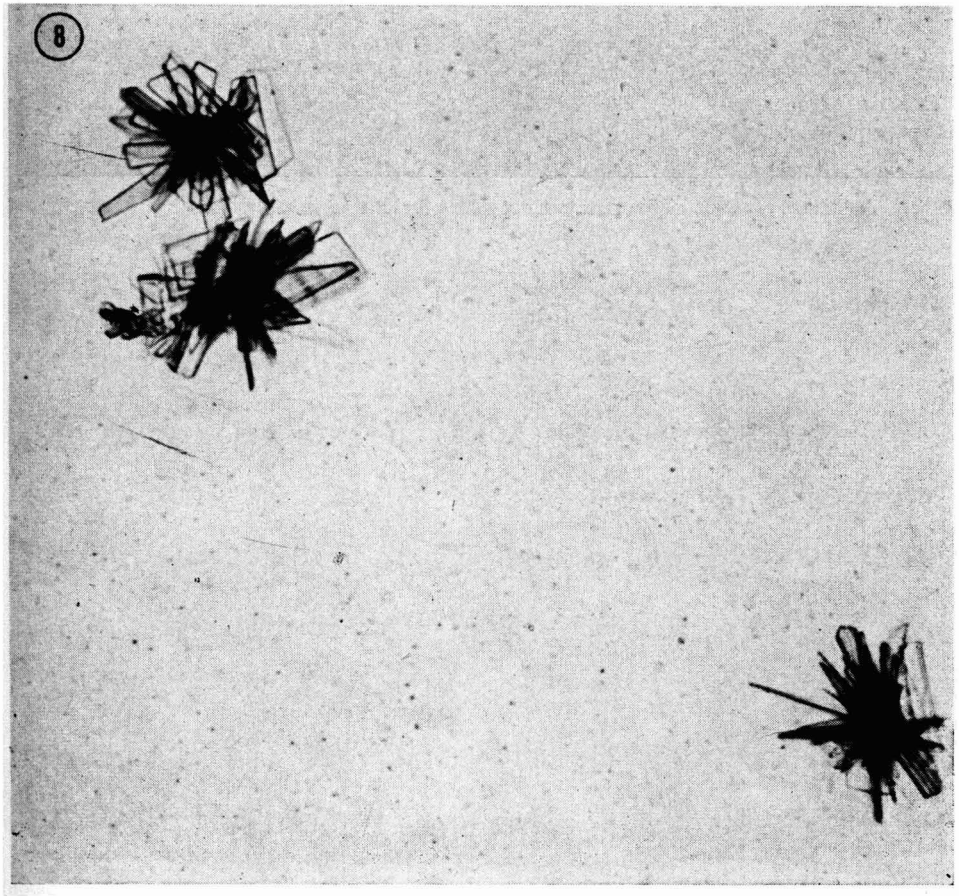

FIG. 8. Microcrystals of isoboldine with ammonium thiocyanate; 120×.

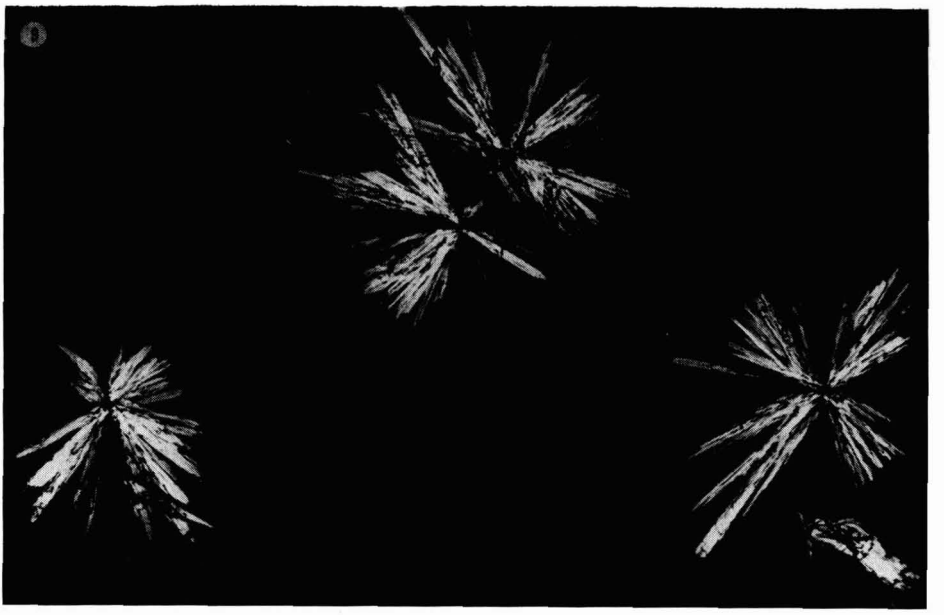

FIG. 9. Microcrystals of laurotetanine with picric acid in polarized light; 120×.

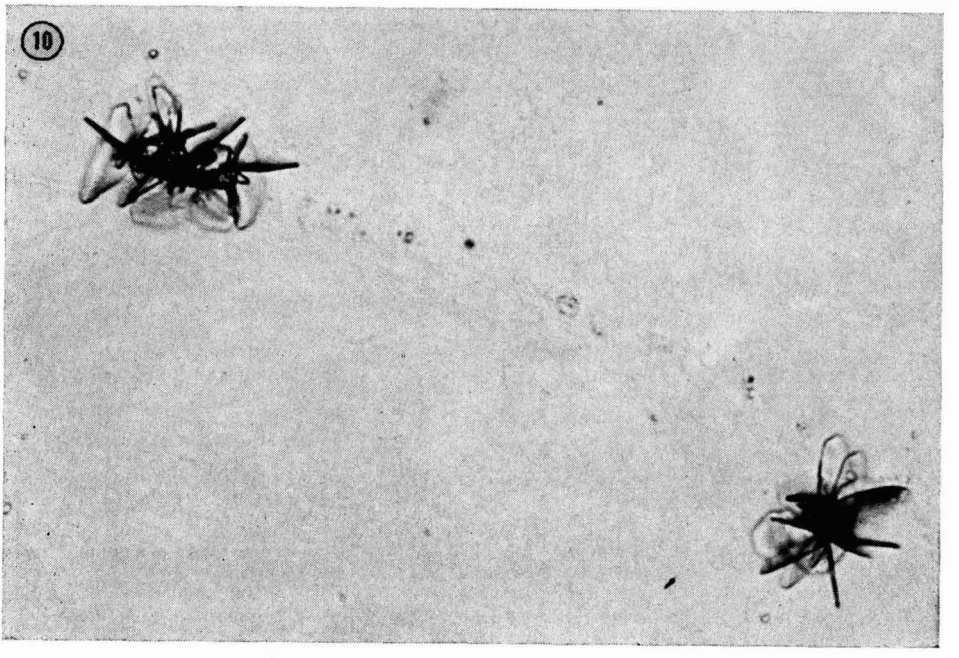

FIG. 10. Microcrystals of laurolitsine with mercuric chloride; 300×.

Laurolitsine

Reagents. Mercuric chloride. Form of crystals: very small and dense branching aggregates of plates, Class 9g (Fig. 10); dim birefringence (second order); parallel extinction; negative sign of elongation.

Picrolonic acid (saturated solution in water). Form of crystals: curved hairs in sheaves and tufts, Class 4 (Fig. 11); dim birefringence (first order); inclined extinction; angle of extinction 22.5°; indifferent sign of elongation.

Isocorydine-N-oxide

Reagent. Potassium cyanide. Form of crystals: rods, branched, "antlers", Class 5 (Fig. 12); dim birefringence (second order); parallel extinction; indifferent sign of elongation.

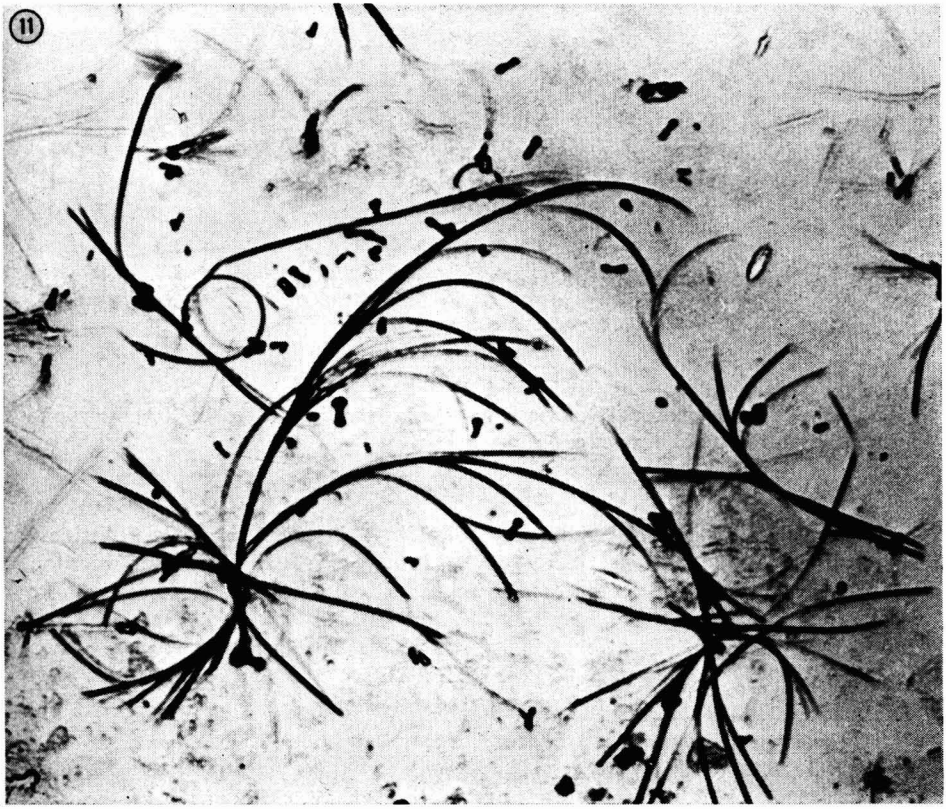

FIG. 11. Microcrystals of laurolitsine with picrolonic acid; 240×.

FIG. 12. Microcrystals of isocorydine-*N*-oxide with potassium cyanide; 120×.

ACKNOWLEDGMENT

The advice and professional assistance of Mr. B. V. Korda on microphotography is gratefully acknowledged.

SUMMARY

Microcrystal tests for reticuline, isoboldine, laurotetanine, laurolitsine, and isocorydine-*N*-oxide are described. In addition to crystal habit, other microoptical data, such as birefringence, extinction, angle of extinction, signs of elongation and absorption, and pleochroism were measured.

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A Spectrophotometric Study of the Chelates of Chromotrope 2R with Praseodymium, Neodymium, Samarium, and Europium

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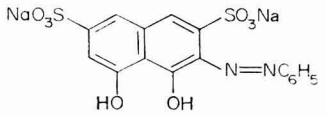
Within the last 2 decades a large number of chromogenic reagents have been developed for the microdetermination of the metal ions, but still many reagents for the photometric determination of rare earths are not available. In search of some sensitive and selective chromogenic reagents for lanthanoids, Chromotrope 2R was recently studied in these laboratories. The reagent is designated by the following structure.



In an earlier publication *(3)* the formation of colored chelates for rare earths with Chromotrope 2R (CTR) were reported. In the present communication the results on the composition, stability and other characteristics of the chelates formed between CTR and Praseodymium, Neodymium, Samarium, and Europium are reported.

EXPERIMENTAL METHODS

Instruments. The measurement of the absorbances were carried out with a Beckman spectrophotometer, model B using glass cells (1 cm) supplied with the instrument. Below the wavelength of 600 mμ blue sensitive photocell was used for the measurements, and for the absorbances above 600 mμ red sensitive photocell was used. All absorbances were measured against distilled water blank.

For measuring the hydrogen ion concentration, a Toshniwal (Type CL41) pH meter was used which was standardized with the pH solutions supplied with the instrument. The electrodes used were combined glass calomel type.

Reagents. Standard solutions of praseodymium, neodymium, samarium and europium were prepared by dissolving the oxides of Pr, Nd,

and Sm in hydrochloric acid and $EuCl_3$ in water containing dilute hydrochloric acid. These were standardized by the usual methods and then were diluted suitably to obtain solutions of different concentrations.

A standard solution of CTR was prepared by dissolving a spot test reagent in distilled water. All solutions were freshly prepared.

Conditions of study. All experiments were carried out at room temperature ranging from 28–30°C. The pH of the solutions was adjusted by addition of HCl and NaOH, keeping the total volume 25 ml in each case.

RESULTS AND DISCUSSION

Absorption spectra of the chelates. The method of Vosburgh and Cooper *(4)* was employed to determine the nature of complexes formed in solution. Mixtures containing 0:1, 2:1, 1:1, 1:2, 1:3, and 1:4 ratios of metal to CTR were prepared keeping the total volume 25 ml in each case. Absorbance measurements were carried out between 400 and 600 mμ. The observations show that in all cases only one complex is formed with CTR under the conditions of study. The λ_{max} of the different chelates are given in Table 1 (Figs. 1 and 2).

Variation of λ_{max} with pH. The absorbance of a number of mixtures containing 0.5 x 10^{-4} *M* each of reagent and the metal were noted at various pH values from 400 to 600 mμ. The variation in λ_{max} of the chelates with pH is shown in Table 2 (Figs. 3 and 4).

Stability of color at room temperature. The absorbance of the mixtures containing 0.5 x 10^{-4} *M* of metal and 0.5 x 10^{-4} *M* CTR was taken after different intervals at respective wavelength maxima of the chelate at pH 6.0; the absorbance remains constant even after 24 hours as shown in Table 3.

Composition of the chelates. The compositions of the chelates were derived spectrophotometrically using two different methods, i.e., (i) method of continuous variation *(2)*, and the (ii) mole ratio method *(5)*.

In both the methods the absorbances were noted at 590 and 600 mμ because the difference in the absorbances of CTR and chelates at the λ_{max} are very small. The results of both the methods show that the stoichiometric ratios of the metal and CTR are 1:1 in all cases. (Figs. 5, 6, 7, and 8).

TABLE 1

WAVELENGTH OF MAXIMUM ABSORBANCE OF CTR CHELATES

Metal chelate	pH	λ_{max} of CTR (mμ)	λ_{max} of chelate (mμ)
Pr–CTR	6.0	530	550
Nd–CTR	6.0	530	550
Sm–CTR	6.0	530	550
Eu–CTR	6.0	530	550

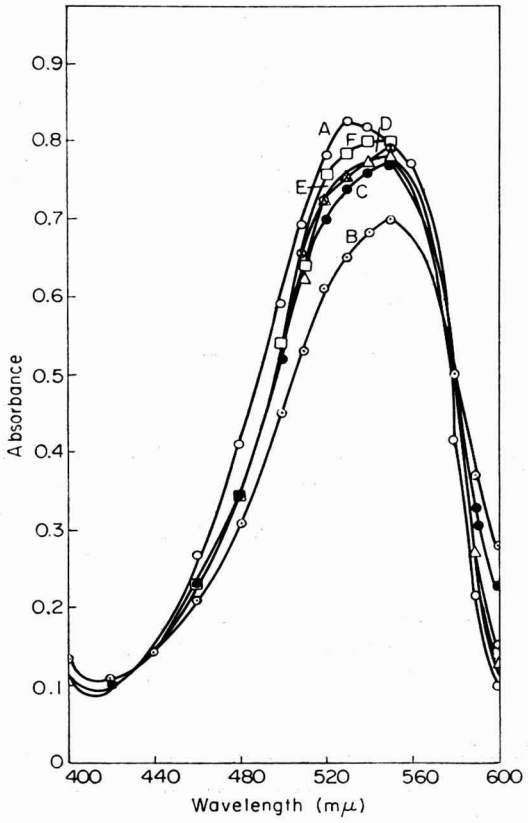


FIG. 1. Absorption spectra of Pr–CTR chelate at pH 6.0; concentration of the reagents (CTR, 0.5 $\times$ 10^{-4} M; $PrCl_3$, $\times 10^{-4}$ M): Curve A, CTR; Curve B, CTR and $PrCl_3$, 1.0; Curve C, CTR and $PrCl_3$, 0.5; Curve D, CTR and $PrCl_3$, 0.25; Curve E, CTR and $PrCl_3$, 0.167; and Curve F, CTR and $PrCl_3$, 0.125.

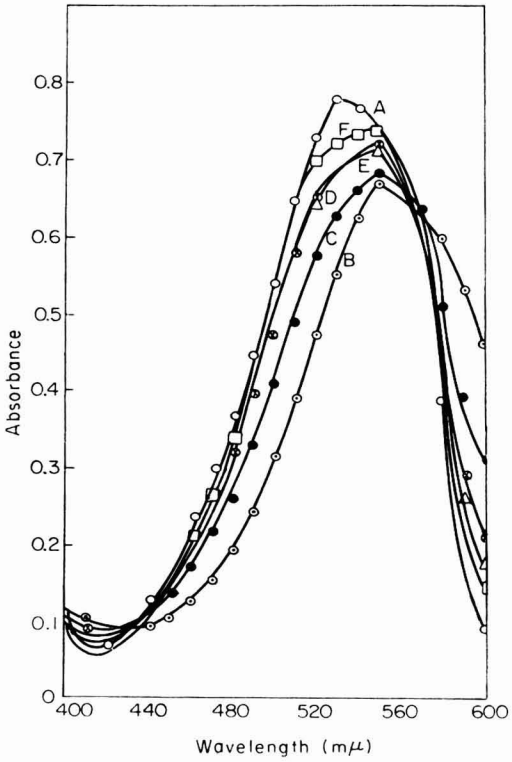


FIG. 2. Absorption spectra of Eu–CTR chelate at pH 6.0; concentration of the reagents (CTR, 0.5 × 10^{-4} *M*; $EuCl_3$, ×10^{-4} *M*): Curve A, CTR; Curve B, CTR and $EuCl_3$, 1.0; Curve C, CTR and $EuCl_3$, 0.5; Curve D, CTR and $EuCl_3$, 0.25; Curve E, CTR and $EuCl_3$, 0.167; and Curve F, CTR and $EuCl_3$, 0.125.

Evaluation of stability constants. The apparent stability constants of the chelates formed were calculated from the absorbance data by two methods: (i) method of Mukherji and Dey *(1),* and (ii) mole ratio method.

The results of both methods are shown in Table 4. The free energy change of formation of the chelates were also calculated with the help of the following expression:

$$\Delta G^0 = -RT \ln K,$$

the terms having their usual importance.

TABLE 2

VARIATION IN MAX OF THE CTR CHELATES WITH pH

Chelate	pH	λ_{max} (mμ)
Pr–CTR	1.0–4.0	530
	4.0–7.6	550
	7.6–12.0	530
Nd–CTR	1.0–5.1	530
	5.1–6.9	550
	6.9–12.0	530
Sm–CTR	1.0–6.0	530
	6.0–9.0	550
	9.0–12.0	530
Eu–CTR	1.0–4.8	530
	4.8–6.9	550
	6.9–12.0	530

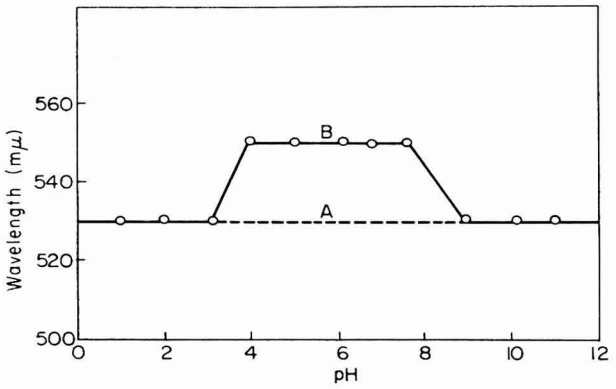


FIG. 3. Variation of λ_{max} of Pr chelate with pH; final concentration of reagents (0.5×10^{-4} *M*): Curve A, CTR; Curve B, Pr–CTR chelates.

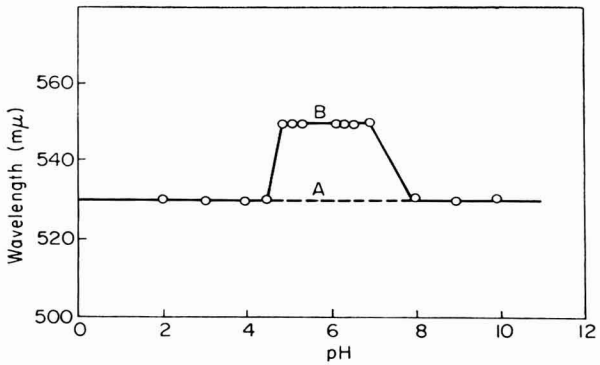


FIG. 4. Variation of λ_{max} of Eu–CTR chelate with pH. Final concentration of reagents (0.5×10^{-4} *M*): Curve A, CTR; Curve B, Eu–CTR chelate.

TABLE 3

STABILITY OF THE COLOR OF THE CHELATES OF RARE EARTHS WITH TIME

Time (hours)	Pr–CTR	Nd–CTR	Sm–CTR	Eu–CTR
1	850	790	790	770
2	850	790	790	770
4	845	790	790	770
6	845	790	790	770
8	845	790	780	770
10	845	790	780	770
12	845	790	780	770
24	820	780	780	750

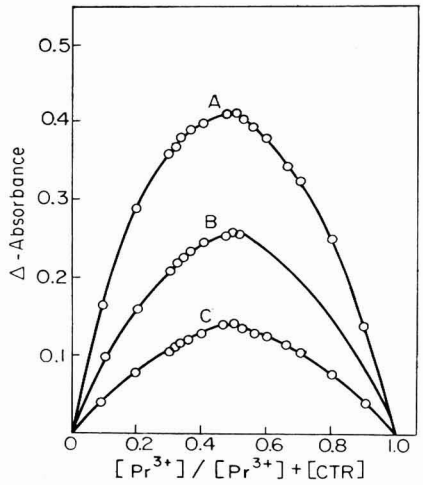


FIG. 5. Composition of Pr–CTR chelate by the method of continuous variation at pH 6.0 and 600 mμ; concentration of the reagents ($\times 10^{-4}$ *M*): Curve A, 1.33; Curve B, 1.00; and Curve C, 0.66.

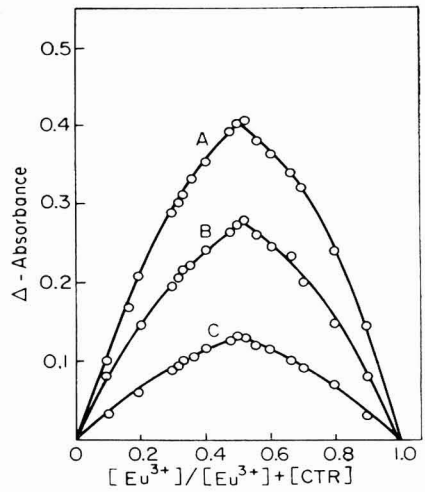


FIG. 6. Composition of Eu–CTR chelate by the method of continuous variation at pH 6.0 and 600 mμ; concentration of the reagents ($\times 10^{-4}$ *M*): Curve A, 1.33; Curve B, 1.00; and Curve C, 0.66.

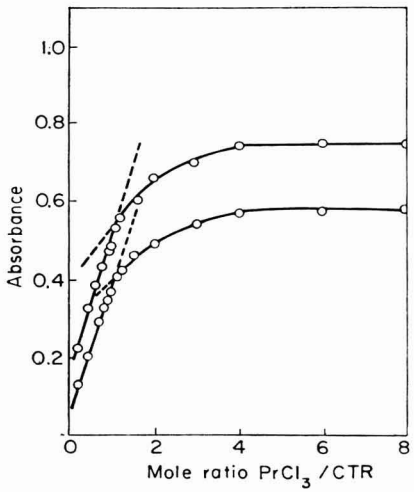


FIG. 7. Determination of the composition of Pr–CTR chelate by mole ratio method at pH 6.0 and 600 mμ; final concentration of CTR ($\times 10^{-4}$ *M*): Curve A, 0.66; and Curve B, 0.50.

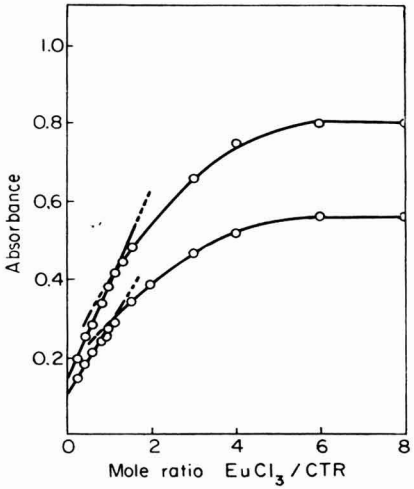


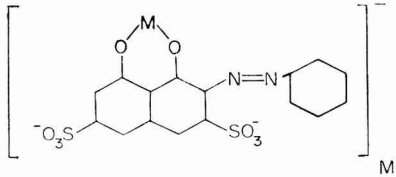
FIG. 8. Determination of the composition of Eu–CTR chelate by mole ratio method at pH 6.0 and 600 mμ; final concentration of CTR ($\times 10^{-4}$ *M*): Curve A, 0.66; and Curve B, 0.50.

TABLE 4

STABILITY CONSTANT AND FREE ENERGY CHANGES OF VARIOUS CHELATES OF CTR

Chelate	Method	log K at 28°C	ΔG^0 at 28°C (kcals)
Pr–CTR	(i)	3.98 ± 0.0	−5.492
	(ii)	5.1 ± 0.2	−7.037
Nd–CTR	(i)	4.22 ± 0.0	−5.822
	(ii)	5.3 ± 0.2	−7.313
Sm–CTR	(i)	5.2 ± 0.1	−7.174
	(ii)	4.5 ± 0.1	−6.209
Eu–CTR	(i)	3.9 ± 0.0	−5.381
	(ii)	4.7 ± 0.2	−6.485

Tentative suggestions on the structure of the chelates. The study of the chelates with ion exchange resins show that the chelates are anionic in character and thus are absorbed completely by the anion exchange resin. On this basis the position of the chelation in the dye can be shown by the following structure:



M = Pr, Nd, Sm or Eu

SUMMARY

The formation of the violet colored complexes of 4,5-dihydroxy-3-phenylazo-2,7-naphthalenedisulfonic acid, disodium salt (Chromotrope 2R) and praseodymium, neodymium, samarium, and europium was reported. The studies include the determination of composition, stability, and other characteristics of the chelates formed. The absorbance curves of the complexes show the wavelength maxima of all the chelates at 550 mμ. The composition of the chelates was established by two different methods, i.e., the method of continuous variation and mole ratio method and the stoichiometric ratio came to be 1:1(metal:ligand). The apparent stability constants of the chelates are determined by the mole ratio method and Mukherji and Dey method at pH 6.0, and 28°C. The chelates are stable for indefinite time.

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MICROCHEMICAL JOURNAL 14, 271–274 (1969)

N-Bromosuccinimide as a Direct Titrant for Thiourea and Thioacetamide Using Bordeaux Red as an Indicator

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I. INTRODUCTION

Methods for the determination of thiourea and thioacetamide utilizing hypobromite, hypoiodite and chloramine-T have been reported (1–4). The great disadvantage of using these reagents is that none can be used as a primary standard. The methods, therefore, are time consuming, since they require standardization of the titrant and the reagents are not very stable. In this laboratory *N*-bromosuccinimide was used for the determination of various sulfur amino acids *(7)* and also for the indirect determination of thiourea and thioacetamide *(6)*. An excess of *N*-bromosuccinimide was added to the test solution and the residual amount was back titrated with sodium thiosulfate. Eight equivalents of the oxidant were used for the complete oxidation, but the spread of error for an individual result was large when working with smaller quantities. A need was felt to devise a direct method for thiourea and thioacetamide which is more precise and more accurate. In this investigation bordeaux red was a suitable indicator in the direct titration of thiourea and thioacetamide with *N*-bromosuccinimide. The big advantage of employing *N*-bromosuccinimide is that it can serve as a primary standard *(7)*. In the present method *N*-bromosuccinimide is the only standard solution required. The end point with bordeaux red is very sharp (it changes from a rose red color to distinct yellow). *N*-Bromosuccinimide is stable *(7)* for several days when protected from light and kept refrigerated.

II. EXPERIMENTAL METHODS

Reagents

Thiourea and thioacetamide solutions were prepared from analytical grade reagents and standardized *(6)* to check the concentrations.

N-Bromosuccinimide was prepared by dissolving 0.124 g of recrystallized reagent in deionized water and diluting to 100 ml.

Sodium bicarbonate solution, 10% in water.

Bordeaux red solution, 0.05% in water.

All other reagents used were of analytical grade.

Procedure

An accurately measured volume (1 ml) of the test solution was taken in a 50-ml Erlenmeyer flask. To this solution, 10 ml of 10% sodium bicarbonate solution and two drops of bordeaux red were added. The solution was then titrated with a standard solution of *N*-bromosuccinimide, added dropwise from a microburette graduated at 0.02 ml intervals. The end point was reached when the rose red color of bordeaux red was changed to distinct yellow by the slightest excess of *N*-bromosuccinimide. In another flask, the same number of drops of bordeaux red were added in 10 ml of sodium bicarbonate solution and titrated with *N*-bromosuccinimide, and this reading was subtracted from the titer before calculation.

Calculations

Amount of thiourea or thioacetamide in mg $= N \times E \times V$; where N = normality of *N*-bromosuccinimide; V = volume of *N*-bromosuccinimide used for titer; and E = equivalent weight of the test substance.

TABLE 1

DETERMINATION OF THIOUREA AND THIOACETAMIDE

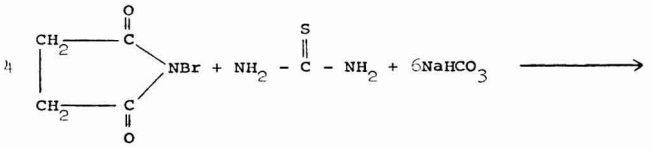
Compound	Sample (mg)	Found (mg; av of 5 results)	SD (mg)
Thiourea	4.834	4.818	0.003
	2.563	2.554	0.007
	1.282	1.282	0.005
	0.641	0.636	0.008
	0.321	0.321	0.004
Thioacetamide	5.241	5.229	0.004
	4.637	4.621	0.005
	3.296	3.289	0.005
	1.648	1.637	0.006
	0.824	0.817	0.006

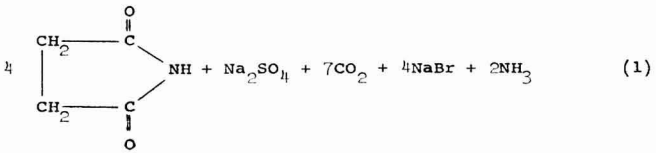
III. RESULTS

Table 1 shows the oxidation of thiourea and thioacetamide by *N*-bromosuccinimide.

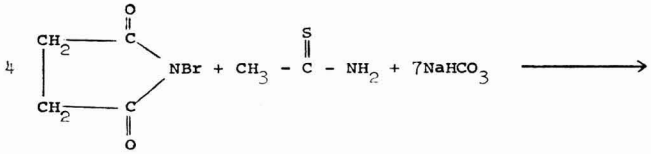
IV. DISCUSSION

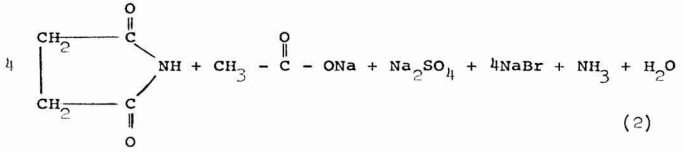
In aqueous solutions, *N*-bromosuccinimide hydrolyzes to form succinimide and hypobromous acid *(5)* which is thought to be responsible for the oxidizing properties of this reagent. The reactions using *N*-bromosuccinimide in the determination of thiourea and thioacetamide as shown in Table 1 are quantitative. At a pH of 8 the following reactions take place:





(1)





(2)

These are in agreement with oxidation by chloramine-T *(1, 3)*.

In Eq. (1) and (2) it can be seen that the sulfur in thiourea and thioacetamide is in the oxidation state of -2, which on reaction with *N*-bromosuccinimide increases to $+6$ oxidation state. There is a loss of a total of 8 electrons. In *N*-bromosuccinimide, the bromine changes from an oxidation state of $+1$ to -1 with a gain of 2 electrons. When

calculating the equivalent weights of the respective substances these changes of oxidation state were taken under consideration. The reactions were not quantitative at neutral or acid pH levels, unlike the case at pH 8 as shown in Table 1.

The error is less than $\pm 1\%$ and in most cases it is even less than $\pm 0.5\%$. The method is quite rapid, precise, and accurate. Small quantities of thiourea and thioacetamide can be readily standardized. *N*-Bromosuccinimide was stable for at least 3 weeks or more depending on the concentration, when refrigerated and protected from light *(7)*.

SUMMARY

A rapid, precise, and accurate method for the determination of small amounts of thiourea and thioacetamide is described. Bordeaux red was a suitable indicator when *N*-bromosuccinimide was used as direct titrant. From 5–0.3 mg of thiourea and thioacetamide were analyzed with an average standard deviation of about 0.005 mg.

ACKNOWLEDGMENT

The financial support of the National Research Council of Canada is gratefully acknowledged.

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MICROCHEMICAL JOURNAL 14, 275–278 (1969)

New Titrimetric Microdetermination of Acetoin (2-Butanone-3-hydroxy) and Tartronic Acid (Hydroxymalonic Acid)

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INTRODUCTION

Various methods have been described for the determination of acetoin (2-butanone-3-hydroxy) and tartronic acid (hydroxymalonic acid). Acetoin has been determined by oxidation with ferric chloride *(3)*, potassium permanganate *(8)* and iodometrically *(4)*. Tartronic acid has been determined by oxidation with hydrogen peroxide, *(5)* periodic acid *(2,7)* c hromic acid *(9)* and chromatographically *(6)*.

The present method deals the microdetermination of acetoin and tartronic acid with Cu(III). Known volume of acetoin is mixed with the excess volume of Cu(III) solution. On heating acetoin oxidized to CO_2 and H_2O stage. The stoichiometry of the reaction is as follows:

$$CH_3CHOHCH_2CHO + 100 \rightarrow 4CO_2 \text{ and } 4H_2O.$$

In case of tartronic acid an aliquot (see table) of the solution is mixed with the excess of Cu(III) solution. After heating the reaction mixture, tartronic acid oxidized to CO_2 and water stage. Immediate consumption of less Cu(III) shows that tartronic acid oxidized to formic acid at room temperature which after heating oxidised to CO_2 and water stage. The reaction probably follows the following two steps:

$$C_3H_4O_5 + 2O \rightarrow HCOOH + 2CO_2 + H_2O,$$

and

$$HCOOH + O \rightarrow CO_2 + H_2O.$$

Acetoin and tartronic acid have determined on micro scale with above ideas.

EXPERIMENTAL METHODS

Reagents

Copper sulfate, sulfuric acid, sodium bicarbonate, and potassium hydroxide used were ANALAR B.D.H. grade. Acetoin (extra pure) and tartronic acid (Purkis and Williams product) were used.

Preparation of Cu(III). Method of preparation and standardization of Cu(III) has been described earlier *(1)* in which on heating Cu(II) was oxidized to Cu(III) by potassium persulfate, and Cu(III) is stabilized by suitable anion tellurate in alkaline medium.

Procedure

For the determination of acetoin and tartronic acid, an aliquot (see Tables 1 and 2) of the solution was mixed with an excess solution of Cu(III). The mixture is treated in the same way as described in Tables 1and 2. After completion of the reaction the unconsumed Cu(III) was

TABLE 1

DETERMINATION OF ACETOIN

Taken (ml)			
0.002 *M* acetoin	0.035 *M* of Cu(III) soln	Cu(III) consumed (equivalents/mole of acetoin)	Error (%)
Cu(III)–acetoin mixture had stood for 0 min			
2.0	8.0	6.42	−67.90
Cu(III)–acetoin mixture had been boiled and cooled			
2.0	8.0	19.95	−0.25
3.0	8.0	19.90	−0.50
	9.0	20.04	+0.20
	10.0	20.06	+0.30
4.0	10.0	20.08	+0.40
5.0	10.0	20.07	+0.35
6.0	10.0	20.03	+0.15

determined with the addition of excess arseneous oxide solution and the remaining arseneous oxide solution is determined iodometrically in bicarbonate media using starch as an indicator. The equivalents in terms of Cu(III) for 1 g mole of the compound were calculated.

RESULTS

It was observed that for acetoin and tartronic acid 20 and 6 equivalents of Cu(III) are required, respectively, hence the calculations were made accordingly.

TABLE 2

DETERMINATION OF TARTRONIC ACID

Taken (ml)		Cu(III) consumed (equivalents/mole of tartronic acid)	Error (%)
0.003 *M* tartronic acid	0.035 *M* of Cu(III)		
Cu(III)–tartronic acid mixture had stood for 0 min			
2.0	3.0	3.84	−36.00
Cu(III)–tartronic acid mixture had been boiled and cooled			
2 0	3.0	5.98	−0.33
3.0	3.0	6.00	0.00
	5.0	6.00	0.00
	6.0	6.03	+0.50
5.0	6.0	5.98	−0.33
6.0	6.0	5.99	−0.16
	8.0	6.02	+0.33

DISCUSSION

From Tables 1 and 2 it is clear that acetoin and tartronic acid have oxidized to the CO_2 and H_2O stage by heating with an excess of Cu(III). Less consumption of Cu(III) by these compounds at room temperature indicates that their oxidation at ordinary temperature is partial.

SUMMARY

A new method for the microdetermination of acetoin and tartronic acid was described. This was done by an oxidation–reduction reaction. Oxidation of acetoin and tartronic acid were effected by means of an excess of Cu(III) solution on heating, the remaining excess of Cu(III) was titrated back by acidifying and adding a known excess of arseneous oxide solution against a standard iodine solution with the use of starch as an indicator.

ACKNOWLEDGMENT

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MICROCHEMICAL JOURNAL 14, 279–288 (1969)

General Method for Microdetermination of Mercury in Organic Compounds

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INTRODUCTION

The determination of organomercury compounds involves preliminary decomposition followed by an estimation of the resulting mercury(II) solution. The preliminary decomposition is affected by strong oxidizing agents *(1, 3–5, 8, 9, 12)* and the soluble mercury is commonly determined by Volhard titration with potassium thiocyanate *(13)*. The Volhard procedure is simple and there are few metal interferences. Halide ions, however, do interfere *(2, 6, 11)* and the visual Volhard end point is not well defined when dilute potassium thiocyanate solution (say 0.01 *M*) is used in a microdetermination. In addition, there are literature reports of loss of mercury due to the presumed volatilization of mercury of mercury halides *(2, 6, 11)*.

In order to establish a reliable method for the microdetermination of mercury in organic compounds, including those which also contain halogen, we carried out a systematic investigation of various strongly acidic oxidizing media. Potassium permanganate in a mixture of concentrated nitric and sulfuric acids achieved both, quantitative decomposition and the volatilization of any halogen either as hydrogen halide or as the free element. No loss of mercury by volatilization occurs under these conditions. The use of an automatic second-derivative spectrophotometric titrator *(7)* coupled to an automatic buret leads to reproducible and accurate Volhard endpoints even with very dilute solutions. Thus, our investigation contributes a simple and accurate method for the microdetermination of all types of organomercury compounds.

EXPERIMENTAL METHODS

Apparatus

Automatic second derivative spectrophotometric titrator and automatic buret assembly (10). The basic components for automatic

spectrophotometric titrations and an automatic buret are illustrated in Fig. 1.

Automatic buret. Scientific Instruments Laboratory Apparatus BG–7546 with 24/40 stopcock, 10-ml capacity, subdivisions $\frac{1}{20}$, 1000-ml bottle capacity.

Kjeldahl digestion flask 30 ml.

Kjeldahl digestion rack with fume duct. Digestion rack which is either a circular type or a straight type can be used.

Glass capillary. 5 × 0.5 mm.

Amber tubing. $\frac{1}{8} \times \frac{1}{32}$-inch latex.

Reagents

Concentrated sulfuric acid 95.5%, specific gravity 1.84.

Concentrated nitric acid 70–71%, specific gravity 1.42.

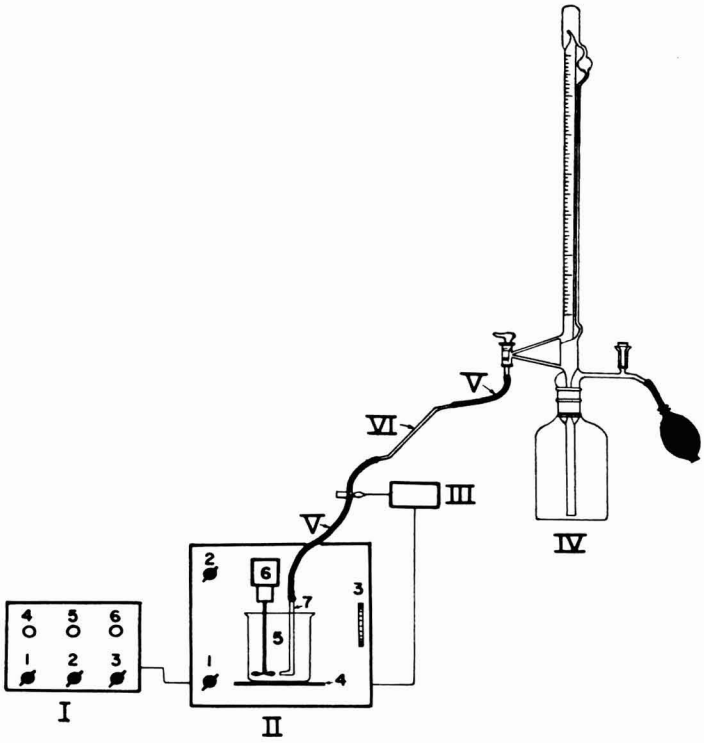

FIG. 1. Automatic second-derivative spectrophotometric titrator and automatic buret assembly: (I) controler; (II) spectrophotometric titrator; (III) solenoid valve; (IV) automatic buret; (V) amber tubing $\frac{1}{8} \times \frac{1}{32}$-inch latex; and (VI) glass capillary 5 × 0.5 mm.

Hydrogen peroxide 6 and 30%.
Perchloric acid 71%.
Potassium sulfate.
Potassium thiocyanate, 0.01 M.
Triple distilled mercury.
Indicator. Ferric ammonium sulfate • $12H_2O$; 40–50 g of ferric ammonium sulfate dissolved in 100 ml of distilled water; few drops of concentrated nitric acid are added and filtered.

Preparation of Sample

The sample (4–13 mg yields titers of 0.01 *M* potassium thiocyanate in the range 2–8 ml) is introduced into a 30-ml Kjeldahl flask in methyl cellulose capsules. Potassium permanganate (0.4 g) and concentrated nitric acid (1 ml) are added. The mixture is allowed to stand for about 10 min at room temperature, and then concentrated sulfuric acid (3 ml) is introduced. (This procedure is necessary if subsequent bumping and, possibly, explosions are to be avoided). The flask is first heated with a small flame (HOOD!) for 5 minutes and then with a stronger flame for 35 minutes, or until the solution is clear. If there is any excess potassium permanganate in the neck of the flask, it is decomposed by adding a few drops of hydrogen peroxide (6%) and boiling the mixture for 3 minutes. The contents of the flask are allowed to cool and then are transferred to a 100-ml tall beaker, 40–45 ml of water being used for washing. Ferric ammonium sulfate (20 drops) is added and the total volume made up to 50 ml. The solution is now ready for automatic titration analyses.

Standardization of potassium thiocyanate solution. Triple distilled mercury (1.003 g) is dissolved in 50 ml of 50% (by volume) nitric acid in a Kjeldahl flask. After the oxides of nitrogen, are removed by boiling, the mixture is cooled and diluted to 1 liter. A calibration curve is determined by titrating 1.0, 2.0, 3.0, and 10 ml of 0.005 *M* mercuric nitrate with the unknown normality potassium thiocyanate. The curve is linear from 2 to 8 ml after allowing for the blank (usually about 0.04–0.06 ml in our case).

RESULTS AND DISCUSSION

1. Effect of acidity and temperature on automatic Volhard titration. The results obtained for the titration of 2–8 ml of standard mercuric nitrate solution in 48–42 ml of 1.0, 1.5, and 3.0 *M* nitric acid at both 15

and 25°C are illustrated in Table 1. Kolthoff *(6)* recommended that the titration be carried out at 15°C but this proves to be unnecessary here.

2. *A systematic investigation of various oxidizing media.* A systematic investigation of various oxidizing media for the destruction of mercury compounds prior to determination of mercury were carried out in several samples. Medium No. 1 was composed of 0.4 g of $KMnO_4$, 1 ml of concentrated nitric acid, and 3 ml of concentrated sulfuric acid. Medium No. 2 was composed of 0.4 g $KMnO_4$ and 4 ml of concentrated sulfuric acid. Medium No. 3 was composed of 0.4 g $KMnO_4$, 0.2 g of potassium sulfate and 3 ml of concentrated sulfuric acid. Medium No. 4 was composed of 0.4 g of potassium sulfate, 3 ml of sulfuric acid, and 1 ml of hydrogen peroxide (30%). Medium No. 5 was composed of 2 ml of concentrated nitric and 2 ml of concentrated sulfuric acids. Medium 6 was composed of 2 ml of sulfuric, 1 ml of concentrated nitric, and 1 ml of perchloric acids. The results are summarized in Table 2: Based on our systematic studies, Medium No. 1 was the best choice for digesting all types of organomercury compounds.

3. *Loss of mercury by volatilization during digestion.* Loss of mercury during wet oxidation has been widely reported and attributed to the volatilization both of mercury itself and many of its compounds *(2, 6, 11)*. The presence of halide ions in the sample has been claimed to increase such loss of mercury *(2, 6, 11)*, and the presence of halide ions after digestion precludes Volhard titration.

To test these factors we treated several mercury compounds with various oxidizing digestion media for various lengths of time. The data (Table 2) showed that 40 minutes were sufficient for complete digestion and no loss of mercury was detected after 2 hours from a sample size of 4–13 mg. The digestion mixture of potassium permanganate in concentrated sulfuric and nitric acids also removed completely any halide which may be present and hence led to trouble-free Volhard titrations. The accuracy and precision of the method has been established over a period of 5 years in the analysis of more than 100 organomercury compounds of all types. Typical results are shown in Table 3. They are within the absolute error of $\pm 0.30\%$ which is acceptable for microanalysis.

SUMMARY

A method was developed for the analysis of all types of organomercury compounds. The use of a strong acidic and powerful oxidizing digestion medium con-

TABLE 1

EFFECT OF ACIDITY AND TEMPERATURE ON THE TITRATION OF STANDARD $Hg(NO_3)_2$ SOLUTION

0.005 *M* $Hg(NO_3)_2$ (ml)	Nitric acid (ml)			0.01 *M* SCN Solution (ml)	
	1.0 *M*	1.5 *M*	3.0 *M*	25°C	15°C
2.0	48			2.05	2.05
4.0	46			4.06	4.05
6.0	44			6.04	6.06
8.0	42			8.06	8.06
2.0		48		2.06	2.05
4.0		46		4.05	4.05
6.0		44		6.05	6.06
8.0		42		8.06	8.06
2.0			48	2.05	2.06
4.0			46	4.06	4.06
6.0			44	6.06	6.05
8.0			42	8.06	8.06

sisting of potassium permanganate, concentrated nitric, and concentrated sulfuric acids afforded rapid conversion to Hg(II) without loss of mercury by volatilization and with complete removal of halogen. By means of a second-derivative spectrophotometric titrator coupled to an automatic buret even dilute Hg(II) solutions obtained from micro-samples can be analyzed accurately by the Volhard method. Low temperature titration was unnecessary and reasonable variations of acidity from 1 to 3 *M* proved unimportant. Analyses of all types of mercury compounds were presented and their accuracy was within an absolute error of $\pm 0.3\%$.

ACKNOWLEDGEMENTS

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TABLE 2

MERCURY ANALYSES WITH VARIOUS OXIDIZING DIGESTION MEDIA FOR VARIOUS LENGTHS OF TIME

Compound	Sample wt (mg)	Digestion time (min)	Hg (%) Theoretical values	Found: Medium No. 1 $KMnO_4$–HNO_3 H_2SO_4	Medium No. 2 $KMnO_4$ H_2SO_4	Medium No. 3 $KMnO_4$–K_2SO_4 H_2SO_4	Medium No. 4 K_2SO_4–H_2O_2 H_2SO_4	Medium No. 5 HNO_3 H_2SO_4	Medium No. 6 HNO_3–$HClO_4$ H_2SO_4
Mercuric acetate	4.216	40	62.95	62.99	62.88	62.51	62.49	62.70	61.99
				62.79	62.75	62.34	62.33	62.69	61.09
	8.922	40		62.81	62.89	62.43	62.31	62.69	61.79
				62.77	62.71	62.03	62.40	62.71	61.33
	4.692	60		63.01	63.00	62.29	62.47	63.01	61.99
				63.11	62.99	62.49	62.17	62.99	62.30
	9.034	60		62.93	62.79	62.45	62.11	63.11	62.01
				62.90	62.83	62.32	62.03	62.89	61.89
	4.393	120		63.01	62.72	62.33	62.51	63.00	62.00
				63.11	63.02	62.50	62.49	62.99	62.30
	9.013	120		63.09	62.70	62.43	62.39	62.78	62.01
				62.89	62.69	62.14	62.19	62.69	61.95

Diphenyl mercury	5.222	40	56.35	56.57	55.75	53.83	54.12	55.44	53.94
				56.29	55.35	53.45	53.69	55.61	53.09
	12.344	40		56.60	55.39	53.71	53.70	55.09	54.00
				56.49	55.42	53.99	53.66	55.33	53.78
	5.134	60		56.57	55.83	54.23	53.92	55.70	53.71
				56.58	55.62	54.00	53.68	55.39	53.66
	13.139	60		56.35	55.44	54.43	53.74	55.75	53.95
				56.29	55.29	54.29	53.48	55.55	53.69
	5.923	120		56.44	55.70	54.77	53.99	55.79	54.09
				56.51	55.49	54.49	54.29	55.50	53.84
	12.099	120		56.09	55.89	54.42	54.00	55.88	53.99
				56.31	55.72	54.13	54.27	55.70	53.70
Phenyl mercuric chloride	4.001	40	64.09	63.88	63.48	63.17	59.74	62.00	60.35
				63.90	63.60	63.00	59.19	62.10	60.00
	9.234	40		64.01	63.28	62.92	60.00	62.00	60.03
				63.79	63.01	62.76	59.77	61.69	59.62
	4.312	60		63.92	63.78	63.00	59.93	62.31	60.15
				64.20	63.61	63.36	60.11	62.11	60.39
	9.699	60		63.81	63.51	63.22	60.17	62.01	59.87
				63.79	63.44	63.00	60.33	62.30	59.72
	4.034	120		64.09	63.59	63.00	60.34	62.09	60.01
				64.19	63.63	62.78	60.41	62.30	60.00
	10.001	120		64.00	63.49	62.99	59.73	62.00	60.00
				63.90	63.20	63.16	59.44	61.78	59.79

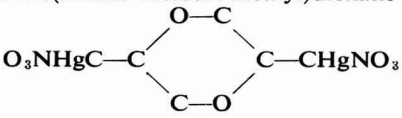
TABLE 3

RESULTS OBTAINED BY PRESENT METHOD FOR MICRODETERMINATION OF MERCURY

Compound	Hg (%)	
	Calc	Found
Inorganic Mercury Salts		
Mercuric acetate $Hg(O_2CCH_3)_2$	62.95	62.99, 63.21
trifloroacetate $Hg(O_2CCF_3)$	47.02	47.25, 47.10
salicylate $HgC_7H_4O_3$	59.57	59.40, 59.62
sulfate $HgSO_4$	67.62	67.70, 67.55
Organic mercury salts Phenyl mercurials		
Diphenyl mercury $(C_6H_5)_2Hg$	56.35	56.06, 56.29
Phenylmercuric acetate $C_6H_5HgO_2CCH_3$	59.60	59.48, 59.60
benzoate $C_6H_5HgO_2CC_6H_5$	50.30	50.12, 50.06
borate $C_6H_5HgH_2BO_3 \cdot C_6H_5HgOH$	63.40	63.31, 63.12
chloride C_6H_5HgCl	64.10	64.00, 64.18
hydroxide C_6H_5HgOH	68.10	67.98, 67.89
8-hydroxyquinolinate $C_6H_5HgOC_9H_6N$	47.3	47.03, 47.00
ortho benzoic sulfimide $C_6H_5HgNSO_3H_4C_7$	43.6	43.32, 43.59
salicylate $C_6H_5HgO_2CC_6H_4OH$	48.4	48.29, 48.60

TABLE 3 (*Continued*)

RESULTS OBTAINED BY PRESENT METHOD FOR MICRODETERMINATION OF MERCURY

Compound	Hg (%)	
	Calc	Found
Ethyl mercurials		
ethylmercuric acetate $C_2H_5HgO_2CCH_3$	69.50	69.29, 69.33
bromide C_2H_5HgBr	64.80	64.52, 64.63
chloride C_2H_5HgCl	75.90	75.60, 75.59
hydroxide	81.20	81.00, 81.30
Others		
3,6-Bis(nitrato-mercuri-methyl)dioxane 	62.70	62.99, 62.79
Thimerosal C_2H_5–Hg–S–C_6H_4–COONa	49.55	49.68, 49.38
Methylmercuric ethylxanthate C_2H_5O–CS–$SHgCH_3$	59.56	59.77, 59.86
p-Tolylmercuric ethylxanthate C_2H_5O–CS–$SHgC_6H_4CH_3(p)$	48.62	48.42, 48.71

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MICROCHEMICAL JOURNAL 14, 289–292 (1969)

New Titrimetric Microdetermination of Formic and Acetic Acids in Their Mixture

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INTRODUCTION

Literature concerning the microdetermination of formic and acetic acids in presence of each other, does not claim to have a relevant method. Although some methods have been developed such as chromatography *(4)*, catalytic pyrolysis *(3)*, cerimetry *(1, 6)* and by permanganate *(5)* in the relating topic.

Present work deals with microdetermination of formic and acetic acids separately in mixture. Formic acid is determined by oxidation with ceric sulfate using chromium ion as catalyst. The remaining ceric sulfate was determined by standard ferrous ammonium sulfate using *n*-phenyl anthranilic acid as an indicator *(7)*. In another portion of the mixture a known excess of standard Cu(III) solution is added. It is then boiled for 0.5 hour and cooled. The remaining Cu(III) solution is determined as has been described *(2)*. In the case of oxidation of formic acid, probably the following reaction takes place:

$$HCOOH + O \rightarrow H_2O + CO_2.$$

Acetic acid is unaffected in the case of oxidation with ceric sulfate.

In the final step both formic acid and acetic acid react with Cu(III) solution and following reaction appears to take place:

$$HCOOH + CH_3COOH + 50 \rightarrow 3CO_2 + 3H_2O.$$

From the above equation it may be concluded that 2 equivalents is consumed for oxidation of formic acid and 8 equivalents are consumed for oxidation of acetic acid. Interference has been observed if other organic compounds are present.

EXPERIMENTAL METHODS

Reagents used. Formic acid, acetic acid, copper sulfate, potassium hydroxide, sodium bicarbonate, starch, sulfuric acid, and ferrous ammonium sulfate used were of ANALAR B.D.H. grade. Potassium tellurite (B.D.H. grade), potassium persulfate (B.D.H. grade), *N*-phenylanthranilic acid (B.D.H. grade), ceric sulfate (Technical B.D.H. grade) and chrome alum (extra pure) were used.

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

The Cu(III) solution is prepared by oxiding cupric sulfate with potassium persulfate and stabilizing it with potassium tellurate in alkaline medium ($\sim$ 6 *M*). It is standardized by addition of known excess of sodium arsenite solution, and the remainder of it is determined iodometrically in bicarbonate media *(2)*.

PROCEDURE

Different sets are carried out by taking known volume of standard formic and acetic acids (see Table 1) raising the solution in 50 ml flasks. 5 ml of mixture is added to 20 ml of ceric sulfate solution in the presence of two or three drops of chrome alum along with the addition of sulfuric acid. It is then refluxed for 90 minutes (see Table 2). The remaining excess of ceric sulfate solution is titrated with standard ferrous ammonium sulfate using *N*-phenyl anthranilic acid as indicator.

TABLE I

OBSERVATIONS WITH FORMIC AND ACETIC ACID

Mixture no.	Vol taken (ml)		Total vol of mixture prepared
	2.50×10^{-2} *M* HCOOH	3.00×10^{-2} *M* CH_3COOH	
1	5	20	50
2	10	15	50
3	12	13	50
4	15	10	50
5	20	5	50

TABLE 2

FORMIC ACID DETERMINATION

Mixture no.	Vol (ml)				Formic acid in mixture (g)	
	Mixture solution taken	1.00×10^{-2} *N* ceric sulfate added	Conc H_2SO_4 added	Ceric sulfate used[a]	Found	Present
1	5	20	30	2.50	0.000575	0.000575
2	5	20	30	5.01	0.001154	0.001150
3	5	20	30	6.02	0.001384	0.001380
4	5	20	30	7.52	0.001729	0.001725
5	5	20	30	10.16	0.002236	0.002230

[a] Remaining ceric sulfate is determined after refluxing it with the mixture for about 90 minutes.

In the second and final step the same volume is added to excess of Cu(III) solution (see Table 3). The mixture is boiled for about 0.5 hour and then cooled. The unconsumed Cu(III) solution is determined as has been described earlier *(2)*.

RESULTS AND DISCUSSION

Different sets for preparation of mixture are given in Table 1. Results for estimation of formic and acetic acids are given in Table 2 and 3. The

TABLE 3

ACETIC ACID DETERMINATION

Mixture no.	Vol of soln (ml)			Total formic and acetic acids found in mixture (g)	Acetic acid in mixture (g)	
	Mixture taken	3.50×10^{-2} *M* Cu(III) taken	Cu(III) consumed[a]		Found	Present
1	5	20	14.44	0.003854	0.003600	0.003600
2	5	20	11.72	0.004175	0.002700	0.002700
3	5	20	10.63	0.003724	0.002340	0.002340
4	5	20	9.01	0.003529	0.001800	0.001800
5	5	20	6.30	0.003229	0.000893	0.000900

[a] Remaining Cu(III) is determined after the mixture is boiled about 30 minutes.

final products are CO_2 and H_2O. The amount of formic acid present is determined from ceric sulfate and the total amount of formic acid and acetic acid present in the mixture is determined by Cu(III) solution. Thus the amount of formic and acetic acids in the mixture was determined separately up to micro scale.

SUMMARY

Titrimetric microdetermination of formic and acetic acids is described. Formic acid required two equivalents of the oxidant for complete oxidation, whereas, acetic acid required eight equivalents of the oxidant. Ceric sulfate oxidized only formic acid but Cu(III) oxidized formic acid as well as acetic acid. Interference was observed if other organic compounds were present.

ACKNOWLEDGMENTS

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MICROCHEMICAL JOURNAL 14, 293–297 (1969)

Rapid Microdetermination of Hemoglobin Iron: Standardization of Hemoglobin[1]

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Procedures employed for the standardization of hemoglobin by means of heme iron content include partial or complete destruction of the organic portion of hemoglobin followed by the determination of iron in a filtrate or residue *(1, 2)*. However, techniques used for complete destruction are somewhat tedious and iron may be lost either through volatilization at high temperature *(1)* or conversion to partially unreactive oxides which yield low values in the absorptiometric step. The present paper describes a simple and rapid method for the determination of hemoglobin iron in which micro quantities of red cell hemolysates are completely destroyed in 3 minutes.

MATERIALS AND METHODS

Reagents

Bathophenanthroline-buffer-thioglycolic acid (BBT). Dissolve 5.6 g of anhydrous sodium acetate and 60.0 mg of sulfonated bathophenanthroline in iron-free water. Add 3.0 ml of glacial acetic acid and 1.0 ml of thioglycolic acid to the solution and dilute it to 200 ml with iron-free water.

Iron stock standard (FeSS). Dissolve 100 mg of analytical grade iron wire in a minimum amount of concentrated HCl and dilute to 100 ml with water. Commercially available atomic absorption standard can be used also.

[1] Aided by NIH general support grant No. 05641, the Detroit General Hospital Research Corporation and the Michigan Heart Association.

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Phosphorus stock solution (PSS). Dissolve 438.1 mg of potassium dihydrogen phosphate in water and dilute to 100 ml with water.

Iron working standard (FeWS). Dilute 4.0 ml of FeSS and 4.0 ml of PSS to 100 ml with water.

Digestion mixture (NAC). Dissolve 30 mg of anhydrous calcium carbonate in a liter of concentrated nitric acid. Calcium is added to the acid used for digestion in order to prevent the loss or rearrangement of phosphate. This mixture permits the analysis of phosphate in the residue, if desired *(3).*

Preparation of Red Cell Hemolysate

Centrifuge heparinized whole blood at 3000 rpm for 3 minutes. Aspirate and discard plasma and buffy coat. Wash the cells twice with saline, centrifuging the mixture and discarding the wash solution each time. Add an equal amount of distilled water and a drop of 2% aqueous saponin solution to the packed cells and shake the suspension vigorously to accomplish complete hemolysis.

PROCEDURE

Dilute 0.1 ml of the red cell hemolysate to 2.5 ml with distilled water, then pipet 0.1 ml of this solution into a 25 × 150-mm borosilicate glass tube. Add 2.0 ml of NAC and 2 boiling stones to the hemolysate. Heat the tube on a bunsen burner until the acid evaporates and yellow fumes of nitrogen oxides appear. Continue heating until no more fumes are present and then allow the tube to cool. Prepare a standard by pipetting 0.1 ml of FeWS and 2.0 ml of NAC into a 25 × 150-mm tube and treating it in the same fashion as the sample. Process a reagent blank which contains only the NAC mixture in a similar manner. Add 2.0 ml of BBT to each tube, mix the contents well and determine the absorbances of sample and standard at 535 nm against the reagent blank.

RESULTS AND DISCUSSION

Because the red cell hemolysate was digested and the released iron was analyzed, it was necessary to determine whether a standard iron solution could be processed in the same manner as the sample. It was observed that when the iron standards contained in concentrated nitric acid alone were evaporated to dryness, the amounts of iron recovered were always lower (Fig. 1, solid circles) and the values were erratic

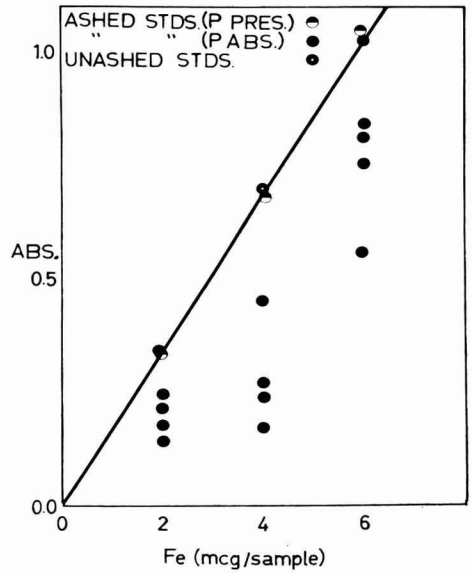

FIG. 1. The effect of phosphate on iron recovery in digested standards.

compared to those obtained for standards when the heating and evaporating step was omitted (Fig. 1, half circles). The erratic losses were eliminated by the incorporation of phosphate into the mixture prior to the ashing step (Fig. 1, hollow circles). It was postulated that phosphate present in the digestion mixture complexed iron and prevented volatilization or iron oxide formation. In contrast to standards, when red cell hemolysates were ashed as described, the concentrations of iron found were consistent and replicate recoveries were not erratic. This precision was assumed to be due to the phosphate normally present in red cells which served as a natural protective agent during the ashing process. To test this hypothesis iron was added to a number of hemolysates prior to the ashing step. The results shown in Table 1 indicate that in all cases satisfactory recovery of added iron was obtained and by inference that hemolysate iron recovery was complete. One can conclude from both base value studies and iron addition recoveries that hemolysates contain sufficient amounts of phosphate to assure quantitative recovery of iron. However, it is essential to incorporate phosphate in the ashing solutions when applying the method to purified preparations including standards or to iron-containing compounds which do not contain phosphate.

TABLE 1

RECOVERY OF IRON ADDED TO RED CELL HEMOLYSATES

Present [a]	Added	Total	Found
2.3	2.0	4.3	4.2
2.3	2.0	4.3	4.2
2.4	1.9	4.3	4.3
2.2	1.9	4.1	4.0
2.3	2.0	4.3	4.2
2.4	2.0	4.4	4.1
2.2	1.9	4.1	4.1
2.3	1.9	4.2	4.2
2.2	2.0	4.2	4.2
2.4	2.0	4.4	4.3
2.4	2.0	4.4	4.3
2.4	2.0	4.4	4.2

[a] Micrograms of iron.

Table 2 compares the results of hemoglobin iron determinations carried out on 15 different red cell hemolysates. In one case the results were checked by the cyanmethemoglobin method and its results were based on the value of a hemoglobin standard available commercially and standardized by both atomic absorption and spectrophotometric iron analysis *(4)*. In the other case the results were obtained by the method described in this paper. In the ashing technique, the hemoglobin values were calculated from the iron content of the sample using the conversion factor of 3.47.

SUMMARY

A simple method for the determination of iron in hemoglobin is described. It includes a rapid (3-minute) digestion of red cell hemolysate by nitric acid and subsequent analysis of the iron in the residue. Inclusion of phosphate in the nitric acid prevented apparent loss of standard iron by volatilization or formation of unreactive iron oxides. Accuracy is proven by recovery of standard iron, comparative analysis of hemoglobin iron, and iron additions to redetermined base values.

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TABLE 2

DETERMINATION OF HEMOGLOBIN

Sample	Present [a]	Found [b]
1	9.2	9.7
2	9.7	10.1
3	12.8	12.8
4	12.6	12.5
5	8.6	8.7
6	10.1	9.9
7	10.6	10.5
8	9.7	9.7
9	12.6	12.6
10	12.8	12.6
11	10.1	10.1
12	10.8	10.8
13	9.7	9.5
14	10.1	9.7
15	12.5	12.8

[a] Tested by cyanmethemoglobin method and commercially available standard which has been standardized by atomic absorption and spectrophotometry.

[b] Calculated from hemoglobin iron found in g/100 ml.

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Microdetermination of Thorium

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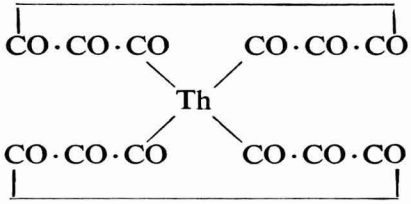
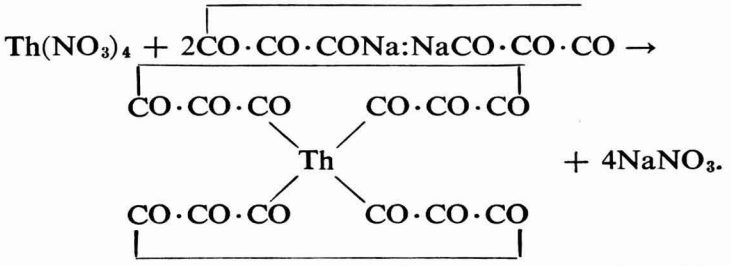
From the literature it appears that a large number of scientists have contributed a lot, with different techniques, towards the determination of thorium. However, generally it is determined titrimetrically (*1–8*); colorimetrically (*9, 10*); potentiometrically (*11*); by activation analysis (*12*); *and spectrophotometrically* (*13, 14*).

The present work deals with the quantitative determination of thorium in micro amounts by titrimetric method. A dark violet colored complex results



when solutions of thorium nitrate and sodium rhodizonate are boiled together. Potentiometric data and results of analysis confirmed that the reaction between thorium nitrate and sodium rhodizonate takes place in the ratio of 1:2. Probably the following reaction takes place:



The violet colored complex, thus resulted, is dissolved in dilute sulfuric acid and then titrated against a standard solution of ceric sulfate using *N*-phenyl anthranilic acid as indicator.

It is observed that Pb^{2+}, Co^{2+}, Zn^{2+}, Mn^{2+}, and Be^{2+} do not interfere, but Ag^{+}, Ni^{2+}, Cd^{2+}, Mg^{2+}, Ca^{2+}, Ba^{2+}, Hg^{2+}, Tl^{+}, UO_2^{2+}, Nd^{+3}, Sm^{+3}, La^{+3}, Gd^{+3}, Zr^{+4} do.

EXPERIMENTAL METHODS

Reagents employed. Thorium nitrate (B.D.H. grade); sodium rhodizonate (B.D.H. grade); ceric sulfate (Technical B.D.H. grade); sulfuric acid (ANALAR B.D.H. grade); and ferrous ammonium sulfate (ANALAR B.D.H. grade).

The 0.0021 *M* ceric sulfate (in 4 *N* H_2SO_4) solution was standardized by titration against a standard solution of ferrous ammonium sulfate (in 1 *N* H_2SO_4) using *N*-phenylanthranilic acid as indicator.

Thorium nitrate solution was prepared by dissolving the exactly weighed amount in distilled water. This solution was further standardized against a standard solution of EDTA (*4, 8*).

Apparatus used. Micro pipette and burette used had LC = 0.01.

PROCEDURE

A known volume of a known standard thorium nitrate solution is taken by a micropipette in a beaker and then diluted with distilled water to 15 ml. A concentrated solution of sodium rhodizonate is then added slowly in small portions with constant stirring to the solution in the beaker until the whole solution assumes a deep orange red color. The solution mixture is boiled, on a heater, for about 5 minutes and then cooled at room temperature. Filter and wash the violet colored precipitate with distilled water till the filter paper is again white, i.e., free from yellow color that adheres. The filtrate is discarded. The precipitate on the filter paper is treated with 4 *N* H_2SO_4 which dissolves it completely. Wash the filter paper again with distilled water. Collect the washings and the dissolved complex, and tritrate against a standard solution of ceric sulfate (in 4 *N* H_2SO_4), from a microburette, using *N*-phenylanthranilic acid as indicator. At the end point a reddish brown color appears sharply.

RESULTS AND DISCUSSION

Results are given in Table 1. The range in which thorium was estimated varies from 11.2663×10^{-4} to 45.0752×10^{-4} mg/liter. Results in Table 1 show that in these determinations the maximum

TABLE 1

MICRODETERMINATION OF THORIUM

$Th(NO_3)_4$ 0.0098 *M* (ml)	$Ce(SO_4)_2$ 0.0021 *M* (ml)	Amount of thorium ($\times 10^4$ mg/liter)		Error (%)
		Taken	Found	
0.5	4.62	11.3704	11.2663	0.9
1.0	9.26	22.7408	22.5620	0.8
1.5	13.88	34.1112	33.8186	0.8
2.0	18.50	45.4816	45.0752	0.9

error is only 0.9%. It is observed that below 0.5 ml of 0.01 *M* $Th(NO_3)_4$, it is not possible to get concurrent results but above 2.0 ml there is every possibility of good results. Since the complex is formed in the ratio og 1:2, calculations were done by dividing the observed calculated value by 2. The present method is an accurate and simple method provided (i) sodium rhodizonate is not used in solid form, and (ii) the precipitate is very carefully washed thoroughly.

SUMMARY

Thorium was determined titrimetrically by dissolving the complex, formed by boiling thorium nitrate and sodium rhodizonate together, in dilute H_2SO_4. The dissolved complex in acid solution was titrated against standard ceric sulfate solution using *N*-phenylanthranilic acid as indicator. Complex is formed in the ratio of 1:2. Maximum error is 0.9%. The range in which thorium was estimated varied from 11.2663×10^{-4} to 45.0752×10^{-4} mg/liter. Interference by certain metal ions was observed.

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The Microdetermination of Mercuric Mercury

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INTRODUCTION

Previous common methods for the determination of mercuric mercury include titrimetric or gravimetric methods *(1)*. Of the titrimetric methods, the ammonium thiocyanate method (British Pharmacopoeia 1963; USP 1960) is the most generally accepted method for the determination of mercuric mercury. This titrimetric method recommends the assay on at least 0.4 g of mercury or 0.3 g of mercuric chloride; otherwise the error increases with dilution of the mercuric solution.

The present work involves the determination of quantities as low as 100 μg of mercuric mercury by the use of L-ascorbic acid.

MATERIALS AND METHODS

Equipment and Reagents

1. Microburette of 5-ml capacity graduated to 0.01 ml.
2. Graduated pipettes of 1-, 2-, 5-, and 10-ml capacity.
3. Volumetric flasks of 100-ml capacity.
4. Erlenmeyer flasks of 50- and 100-ml capacity.
5. Centrifuge and graduated centrifuge tubes of 10-, 15-, and 25-ml capacity.
6. Standard L-ascorbic acid, e.g., 0.01 or 0.005 *N*, freshly prepared aqueous solution.
7. Standard *N*-bromosuccinimide (NBS), freshly prepared aqueous solution, e.g., 0.01 or 0.005 *N* solution.
8. Methyl red indicator 0.04% (w/v), alcoholic solution.
9. Dilute hydrochloric acid 10% (v/v).
10. Potassium iodide 4%, aqueous solution.
11. Starch solution 1% (w/v), freshly prepared aqueous solution.
12. Dilute acetic acid 5% (v/v).

Action of L-Ascorbic Acid on Mercuric Mercury

A 0.88-g portion of L-ascorbic acid (1 mole) in aqueous solution (30 ml) was added gradually with shaking to an aqueous solution (70 ml) containing 2.716 g of mercuric chloride (2 moles). The mixture was allowed to stand for 5 minutes at rom temperature (20°C) when a white precipitate was deposited. The mixture was filtered off giving solid (A) and filtrate (B).

Solid (A) proved to be mercurous chloride by two recognized tests *(2)* as follows:

1. Ammonia solution produced a black precipitate consisting of a mercuric amino salt and finely divided mercury.

2. Potassium iodide solution gave a yellowish-green precipitate of mercurous iodide Hg_2I_2, which yielded soluble potassium mercuric iodide $K_2[HgI_4]$ and black finely divided mercury with excess of the reagent.

The presence of HCl in filtrate (B) was proved by adding 2 ml of 10% lead acetate solution to a 10-ml portion when a white precipitate of lead chloride was deposited and which dissolved in hot water.

Filtrate (B) was treated with an excess of phenylhydrazine hydrochloride (1.445 g, 2 moles) and sodium acetate (1.64 g, 2 moles). The mixture was boiled and concentrated on the electric plate for 0.5 hour and then allowed to cool. Red crystals were deposited, filtered, and recrystallized from ethyl alcohol. The red crystals melted at 218°C (yield 1.5 g) and proved to be dehydro-L-ascorbic acid osazone by melting-point and mixed melting-point determination with an authentic sample.

Validity of the Reaction for Quantitative Determination

Before applying the reaction between L-ascorbic acid and mercuric mercury for the quantitative determination it was decided to verify the validity of the reaction from a quantitative point of view. An accurately measured volume, e.g., 5 ml of an aqueous solution containing 0.2716 g of mercuric chloride (1 mmole)/100 ml was placed in a 15-ml centrifuge tube. Then an equal volume of an aqueous solution containing 0.176 g of L-ascorbic acid (1 mmole)/100 ml was added. The mixture was thoroughly shaken and allowed to stand at room temperature for 5 minutes and then centrifuged at 3000 rpm for 5 minutes. The clear supernatent solution was quantitatively transferred to a 50-ml Erlenmeyer flask and then 5 ml of dilute hydrochloric acid (10%) and 2 drops of methyl red indicator were added. The excess of standard L-ascorbic

acid was determined by back titration with a standard *N*-bromosuccinimide solution containing 0.178 g (1 mmole)/100 ml.

The standard *N*-bromosuccinimide solution was added dropwise from a microburette with continuous shaking after each addition until the red color of the indicator just disappeared and the volume of the titer was noted. The volume of standard L-ascorbic acid solution equivalent to the volume of mercuric chloride solution was easily calculated by subtracting the volume of the N-bromosuccinimide from the volume of L-ascorbic acid added. The reaction was stoichiometric at room temperature and the results were as follows:

Volume of mercuric chloride solution (1 mmole/100 ml) taken (ml):					
10	5	4	3	2	1
Volume of L-ascorbic acid solution (1 mmole/100 ml) added [ml, (A)]:					
10	5	4	3	2	1
Titer of *N*-bromosuccinimide solution (1 mmole/100 ml) used [ml, (B)]:					
5.00	2.55	2.00	1.51	1.00	0.52
Volume of L-ascorbic acid solution (1 mmole/100 ml) taken [ml, (A − B)]:					
5.00	2.45	2.00	1.49	1.00	0.48

A similar series of experiments was done using a known volume of mercuric chloride solution containing double the number of molecules of solute (2 mmoles)/100 ml. A double volume of L-ascorbic acid solution (1 mmole)/100 ml was added. It was found that the reaction was stoichiometric at room temperature. The results were:

Volume of mercuric chloride solution (2 mmole/100 ml) taken, (ml):					
10	5	4	3	2	1
Volume of L-ascorbic acid solution (1 mmole/100 ml) added, [ml, (A)]:					
20	10	8	6	4	2
Titer of *N*-bromosuccinimide solution (1 mmole/100) used, [ml, (B)]:					
9.90	5.00	4.00	3.00	2.00	1.00

Volume of L-ascorbic acid solution (1 mmole/100 ml) taken [ml, (A − B)]:

10.10	5.00	4.00	3.00	2.00	1.00

Procedure

To an accurately measured volume, e.g., 5 ml of an unknown aqueous mercuric chloride solution in a centrifuge tube of 15-ml capacity, add an equal volume of a standard aqueous solution of L-ascorbic acid. The standard ascorbic acid solution added must be in excess. Shake thoroughly and allow the mixture to stand for 5 minutes at room temperature. Centrifuge for 5 minutes at 3000 rpm and transfer quantitatively the supernatent fluid to a 50-ml stoppered Erlenmeyer flask. Then add 5 ml of dilute hydrochloric acid (10%) and 2 drops of methyl red indicator. Titrate back the excess of standard L-ascorbic acid with standard *N*-bromosuccinimide solution of the same normality added dropwise from a microburette with shaking after each addition until the red color just disappears. A blank experiment is simultaneously done and the reading is subtracted from the titer before calculation. Calculate the mercuric mercury content or the mercuric chloride content of the unknown solution from the expression:

$$\text{mercuric mercury content (mg or } \mu\text{g)} = V \times C \times \frac{200.6 \times 2}{176},$$

$$\text{mercuric chloride content (mg or } \mu\text{g)} = V \times C \times \frac{271.6 \times 2}{176},$$

where V = volume of standard L-ascorbic acid solution taken in the reaction,

and C = concentration of L-ascorbic acid in mg or μg per 1 ml of solution.

RESULTS

Microdetermination of Mercuric Mercury

A stock aqueous solution containing 0.1 g/100 ml of mercuric mercury was prepared by dissolving 0.1354 g of mercuric chloride in warm distilled water and the volume was completed with distilled water to 100 ml in a volumetric flask.

The mercuric mercury content was determined by the proposed method in various volumes of the stock solution as if they were unknowns us-

ing an excess of 0.01 *N* L-ascorbic acid solution for reduction of mercuric mercury and 0.01 *N* *N*-bromosuccinimide solution for back titration of excess L-ascorbic acid. The results are shown in Table 1.

The 0.1 g/100 ml mercuric mercury stock solution was diluted 10 times with distilled water so that each milliliter of the diluted solution contained 100 μg of mercuric mercury.

The mercuric content of this diluted solution was then determined in various volumes by the proposed method as if they were unknowns by adding a known volume of 0.005 *N* L-ascorbic acid which should be in excess for reduction of mercuric mercury and using 0.005 *N* *N*-bromosuccinimide solution for back titration of excess standard L-ascorbic acid. The results are recorded in Table 2.

Comparative Analysis of Mercuric Mercury by the Proposed Method and the Thiocyanate Method

A 0.1 g/100 ml of mercuric mercury solution was determined as if it was an unknown solution by the proposed method adding 0.01 *N* L-ascorbic acid solution in excess and using 0.01 *N* *N*-bromosuccinimide solution for back titration of excess L-ascorbic acid. Simultaneously the determination was done by the generally accepted ammonium thiocya-

TABLE 1

RECOVERY OF MERCURIC MERCURY (mg) BY THE PROPOSED METHOD

Original Hg^{2}† content (mg)	0.01 *N* L-ascorbic acid soln. added (A) (ml)	Titer of 0.01 *N* NBS [a] solution used (B) (ml)	0.01 *N* L-ascorbic acid taken (A − B) (ml)	Hg^{2}† found (mg)	Error (%)
10	10	5.05	4.95	9.93	0.70
9	9	4.45	4.55	9.13	1.44
8	8	3.95	4.05	8.12	1.50
7	7	3.50	3.50	7.02	0.29
6	6	3.05	2.95	5.92	1.33
5	5	2.50	2.50	5.02	0.40
4	4	2.00	2.00	4.01	0.25
3	3	1.50	1.50	3.01	0.33
2	2	1.00	1.00	2.01	0.50
1	1	0.50	0.50	1.00	—

[a] NBS = *N*-bromosuccinimide.

TABLE 2

RECOVERY OF MERCURIC MERCURY (μg) BY THE PROPOSED METHOD

Original Hg^{2+} content (μg)	0.005 *N* L-ascorbic acid solution added (A) (ml)	Titer of 0.005 *N* NBS solution used (B) (ml)	0.005 *N* L-ascorbic acid solution taken (A − B) (ml)	Hg^{2+} found (μg)	Error (%)
1000	10	9.00	1.00	1003	0.30
900	9	8.11	0.89	893	0.78
800	8	7.20	0.80	802	0.25
700	7	6.31	0.69	692	1.14
600	6	5.39	0.61	612	2.00
500	5	4.51	0.49	491	1.80
400	4	3.60	0.40	401	0.25
300	3	2.70	0.30	301	0.33
200	2	1.80	0.20	201	0.50
100	1	0.90	0.10	100	—

nate method using 0.1 *N* ammonium thiocyanate solution. The results are shown in Table 3.

Interfering Substances

Typical interfering ions are silver, ferrric, and cupric ions. Silver ions are removed in the form of insoluble silver chloride by addition of 10% hydrochloric acid followed by filtration. Sodium fluoride masks ferric ions and diphenylamine masks cupric ions *(3)*. Hydrazine sulfate may also mask cupric ions (unpublished observation).

Experimental Error

The experimental error of the proposed method does not exceed ±2% in determination of amounts ranging from 10 mg to 100 μg of mercuric mercury (Tables 1, 2). In the determination of corrosive sublimate in such biological fluids as urine or milk the error rises to as high as 10% when amounts range from 5 to 1 mg (Tables 5, 6).

Application of the Proposed Method

A. Assay of liquor hydrargyri perchloridi (USP 1960). This pharmaceutical preparation containing 1 g of mercuric chloride/liter (1 ml ≡ 1 mg of $HgCl_2$) was determined by the proposed method as if it was an

TABLE 3

COMPARATIVE ANALYSIS OF MERCURIC MERCURY BY THE PROPOSED METHOD AND AMMONIUM THIOCYANATE METHOD

	Proposed method					Ammonium thiocyanate method		
Original Hg^{2}† content (mg)	0.01 *N* L-Ascorbic acid solution added (A) (ml)	Titer of 0.01 *N* NBS used (B) (ml)	0.01 *N* L-Ascorbic acid taken (A − B) (ml)	Hg^{2}† found (mg)	Error (%)	Titer of 0.1 *N* ammonium thiocyanate (ml)	Hg^{2}† found (mg)	Error (%)
10	10	5.05	4.95	9.93	0.70	1.03	10.33	3.30
8	8	3.95	4.05	8.12	1.50	0.77	7.72	3.50
5	5	2.50	2.50	5.02	0.40	0.48	4.81	3.80
4	4	2.00	2.00	4.01	0.25	0.38	3.81	4.75
2	2	1.00	1.00	2.01	0.50	0.19	1.90	5.00

unknown using various volumes of the preparation. The results are recorded in Table 4.

B. Determination of corrosive sublimate in human urine. A 0.1-g aliquot of mercuric chloride was dissolved in normal human urine and completed with the same urine to 100 ml in a volumetric flask. Various volumes of this urine were determined by the proposed method as if they were unknowns following the same procedure, but using dilute acetic acid, 5%; potassium iodide solution, 4%; and starch solution, 1%; to detect the end point (*6*). A blank experiment was simultaneously done on the original sample of urine and the reading was subtracted from the titer of standard *N*-bromosuccinimide solution before calculation. The results are shown in Table 5.

C. Determination of corrosive sublimate in buffalo milk. A 0.1-g aliquot of mercuric chloride was dissolved in 5 ml of warm distilled water and buffalo milk was added to the mark in a 100-ml volumetric flask. Various volumes of this milk were analyzed by the proposed method as if they were unknowns. An accurately measured volume of the milk sample, e.g., 5 ml, was placed in a 15-ml centrifuge tube and treated with 1 ml of acetic acid (5%). The mixture was allowed to stand at room temperature for 10 minutes until precipitation of protein was complete; then centrifuged at 3000 rpm for 5 minutes. The supernatent clear fluid was transferred quantitatively to similar centrifuge tube and the same procedure was followed, but the end point was detected as mentioned above (*6*). The results are recorded in Table 6.

DISCUSSION

In aqueous solution, L-ascorbic acid readily reduces mercuric chloride to mercurous chloride; itself being oxidized to dehydro-L-ascorbic acid. This finding is supported by a previous report on determination of mercury by ascorbinometry (*4*).

The reaction proceeds quantitatively at room temperature in the proportion of two molecules of mercuric chloride to one molecule of L-ascorbic acid as is shown below.

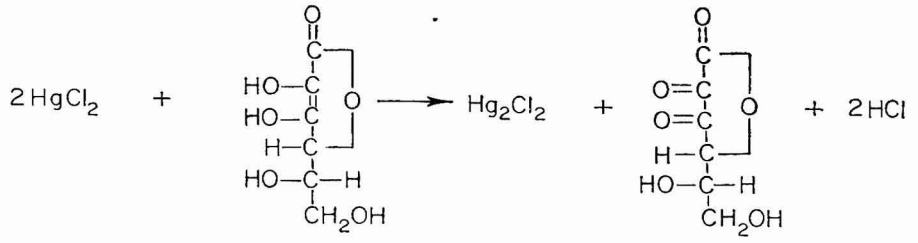

TABLE 4

ASSAY OF LIQUOR HYDRARGYRI PERCHLORIDI BY THE PROPOSED METHOD

Volume of solution taken (ml)	Original mercuric chloride content (mg)	0.01 *N* L-ascorbic acid solution added (A) (ml)	Titer of 0.01 *N* NBS solution used (B) (ml)	0.01 *N* L-ascorbic acid solution taken (A − B) (ml)	Mercuric chloride found (mg)	Error (%)
10	10	10	6.35	3.65	9.91	0.90
9	9	9	5.62	3.38	9.18	2.00
8	8	8	5.12	2.88	7.82	2.25
7	7	7	4.40	2.60	7.06	0.86
6	6	6	3.78	2.22	6.03	0.50
5	5	5	3.15	1.85	5.02	0.40
4	4	4	2.50	1.50	4.07	1.75
3	3	3	1.90	1.10	2.99	0.33
2	2	2	1.25	0.75	2.04	2.00
1	1	1	0.64	0.36	0.98	2.00

TABLE 5

RECOVERY OF CORROSIVE SUBLIMATE IN URINE BY THE PROPOSED METHOD

Volume of urine taken (ml)	Original corrosive sublimate content (mg)	0.01 *N* L-ascorbic acid solution added (A) (ml)	Titer of 0.01 *N* NBS used (B) (ml)	0.01 *N* L-ascorbic acid solution taken (A − B) (ml)	Corrosive sublimate found (mg)	Error (%)
5	5	5	3.25	1.75	4.75	5.00
4	4	4	2.42	1.58	4.29	7.25
3	3	3	1.80	1.20	3.26	8.67
2	2	2	1.20	0.80	2.17	8.50
1	1	1	0.60	0.40	1.09	9.00

TABLE 6

RECOVERY OF CORROSIVE SUBLIMATE IN MILK BY THE PROPOSED METHOD

Volume of milk taken (ml)	Original corrosive sublimate content (mg)	0.01 *N* L-ascorbic acid solution added (A) (ml)	Titer of 0.01 *N* NBS solution used (B) (ml)	0.01 *N* L-ascorbic acid solution taken (A − B) (ml)	Corrosive sublimate found (mg)	Error (%)
5	5	5	3.25	1.75	4.75	5.00
4	4	4	2.40	1.60	4.35	8.75
3	3	3	1.80	1.20	3.26	8.67
2	2	2	1.20	0.80	2.17	8.50
1	1	1	0.60	0.40	1.09	9.00

The mechanism of the reaction was formulated on the basis of the data gained. The formation of mercurous chloride was established by two recognized tests. Dehydro-L-ascorbic acid was isolated in the form of its osazone. The production of hydrogen chloride was also confirmed. The reaction between L-ascorbic acid and mercuric chloride is not unexpected because it is now settled that L-ascorbic acid possesses strong reducing properties *(5)*.

The proposed method for the microdetermination of mercuric mercury is based on the reducing capacity of L-ascorbic acid as well as the ability of the latter to react readily with *N*-bromosuccinimide at room temperature *(6)*. As a result, addition of an excess of standard L-ascorbic acid solution to an unknown solution of mercuric chloride will cause precipitation of mercurous chloride due to reduction, whereas an equivalent amount of L-ascorbic acid will be oxidized to dehydro-L-ascorbic acid. The excess of standard L-ascorbic acid can be determined by back titration with standard *N*-bromosuccinimide solution of the same normality. Thus by subtraction the volume of standard L-ascorbic acid equivalent to the mercuric mercury in the unknown solution is obtained. The use of methyl red indicator in titrations with *N*-bromosuccinimide is previously known *(7)*.

The advanced method is simple, rapid, and sufficiently sensitive to determine quantities as low as 100 μg of mercuric mercury. The experimental error does not exceed $\pm 2\%$ (Tables 1, 2). Comparative analysis shows that the ammonium thiocyanate method is not suitable to determine low concentrations since the experimental error rises to as high as 5% as the concentration decreases to as low as 2 mg of mercuric mercury in the solution (Table 3).

The proposed method was used for the assay of pharmaceutical preparations, e.g., liquor hydrargyri perchloridi (USP 1960) giving reproducible results (Table 4). Moreover, the determination of corrosive sublimate in such biological fluids as urine and milk shows an experimental error not exceeding $\pm 10\%$ (Tables 5, 6).

SUMMARY

A new titrimetric method for the microdetermination of mercuric mercury is described. The proposed method is based on the fact that aqueous L-ascorbic acid solution reduces mercuric mercury to mercurous mercury; itself being oxidized to dehydro-L-ascorbic acid. The mechanism of the reaction is discussed. The proposed method is simple, rapid, and sufficiently accurate to determine

concentrations as low as 100 μg of mercuric mercury. The experimental error does not exceed $\pm 2\%$. The method was also used for the determination of corrosive sublimate in such biological fluids as urine or milk with an error not exceeding $\pm 10\%$.

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Use of Microcosmic Salt as a New Titrant for the Microdetermination of Benzoic, Salicyclic, and Phthalic Acids

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Carboxylic acids are most simply determined by titration with standard alkali *(1)*. When the samples are soluble in water, titrations are carried out in aqueous medium *(2)* itself. Otherwise titrations are done by alcoholic caustic soda solutions in some nonaqueous medium, such as 1:1 ethylene glycol–isopropanol *(3)*, benzene–methanol *(4)*, dimethyl formamide *(5)*, butylamine *(6)*.

In an earlier communication *(7)* we reported the use of microcosmic salt as a new titrant for the determination of aspartic and glutamic acids. In the present paper, its use in the determination of benzoic, salicylic, and phthalic acids is described. In the case of benzoic and salicylic acids, the end point is obtained at one equivalent, but with phthalic acid it comes at two equivalents.

EXPERIMENTAL METHODS

Reagents used. Benzoic Acid (M.A.S.H.W. England), salicylic acid (AnalaR), phthalic acid (AnalaR), microcosmic salt (E-Merck), and bromcresol purple (B.D.H.).

Stock solutions of salicylic and phthalic acids were prepared in water and standardized by standard methods. Benzoic acid was dissolved in a 1:1 water–alcohol mixture and standardized as above. The solutions were suitably diluted to required strength.

PROCEDURE

To a given volume of a solution of any of these acids, add some distilled water to raise its volume to about 20 ml, followed by one or two drops of 0.1% solution of bromcresol purple indicator. The solution is yellow at this point. Now titrate it with a standard *(8)* microcosmic salt solution till the yellow color is completely discharged and the solution acquires a faint pinkish color, indicating its end point.

RESULTS

The results are given in Tables 1–3; these acids were estimated over a concentration range of 0.0001–0.002 *M*. The results are concordant and precise.

SUMMARY

Benzoic, salicylic, and phthalic acids were determined in micro quantities with a new titrant, i.e., microcosmic salt solution, using bromcresol purple as indicator. Estimations were made in the range 0.126–1.257 mg of benzoic acid, 0.075–0.746 mg of salicylic acid, and 0.083–0.831 mg of phthalic acid with maximum error of ±0.004 mg.

TABLE 1

MICRODETERMINATION OF BENZOIC ACID

Sample no.	Vol of soln (ml): Benzoic acid taken (0.00206 *M*)	Vol of soln (ml): Microcosmic salt used (0.001 *M*)	Amount of benzoic acid (mg): Found	Amount of benzoic acid (mg): Theoretical value	Amount of benzoic acid (mg): Error
1	5.0	10.30	1.257	1.257	0.000
2	2.5	5.12	0.625	0.628	0.003
3	2.0	4.12	0.503	0.503	0.000
4	1.0	2.08	0.254	0.251	0.003
5	0.5	1.02	0.124	0.126	0.002

TABLE 2

MICRODETERMINATION OF SALICYLIC ACID

Sample no.	Vol of soln (ml): Salicylic acid taken (0.00108 *M*)	Vol of soln (ml): Microcosmic salt (0.001 *M*)	Amount of salicylic acid (mg): Found	Amount of salicylic acid (mg): Theoretical value	Amount of salicylic acid (mg): Error
1	5.0	5.40	0.746	0.746	0.000
2	2.5	2.72	0.376	0.373	0.003
3	2.0	2.16	0.298	0.298	0.000
4	1.0	1.08	0.149	0.149	0.000
5	0.5	0.52	0.072	0.075	0.003

TABLE 3

MICRODETERMINATION OF PHTHALIC ACID

Sample no.	Vol of soln (ml)		Amount of phthalic acid (mg)		
	Phthalic acid taken (0.001 *M*)	Microcosmic salt used (0.001 *M*)	Found	Theoretical value	Error
1	5.0	10.06	0.835	0.831	0.004
2	2.5	5.00	0.415	0.415	0.000
3	2.0	3.98	0.331	0.332	0.001
4	1.0	2.00	0.166	0.166	0.000
5	0.5	0.98	0.081	0.083	0.002

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The Extraction and Photometric Determination of Zinc in the Presence of Large Amounts of Cadmium Using 1-(2-Pyridylazo)-2-naphthol (PAN) and Employing Iodide Masking

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INTRODUCTION

Since its introduction as an analytical agent by Cheng and Bray *(1)* 1-(2-pyridylazo)-2-naphthol (PAN) has been used extensively as the extractive photometric reagent in the determination of many metals *(6)*. While in general it is quite low in selectivity, it shows some differences in its reactions with zinc and cadmium. As was reported by Shibata *(5)* and by Püschel *(4)*, at a pH of about 6, zinc reacts completely with PAN and the complex formed can be fully extracted into chloroform. Under the same conditions only a negligible amount of cadmium moves into the organic layer. Thus PAN can be used for a selective determination of zinc in the presence of cadmium but per se the approach is successful only if the two metals are present in small and comparable amounts. With large amounts of cadmium difficulties are unavoidable. It seemed, however, that a considerable improvement in selectivity could be obtained by operating in the presence of iodide which, as was shown by Flaschka and Butcher *(3)*, is an effective masking agent for cadmium. Exploratory experiments gave very encouraging results and upon further study a method was developed that permits the rapid and simple determination of traces of zinc in cadmium metal and cadmium compounds.

EXPERIMENTAL METHODS

Apparatus

The analytical measurements were made with a Bausch and Lomb 505 or Spectronic 20 spectrophotometer. The former instrument was also employed to record the absorbance curves.

Reagents

Zinc solutions. A 0.01 *F* stock solution was prepared by dissolving "Baker Analyzed" reagent zinc nitrate hexahydrate. Solutions 10^{-3} and $10^{-4}F$ were prepared by appropriate dilution of the stock solution. The exact zinc concentrations in the solutions were established by EDTA titrations.

Cadmium solutions. "Baker Analyzed" reagent cadmium nitrate tetrahydrate, lot no. 21252, was used to prepare solutions 0.1, 1, and 4 *M* in cadmium.

Potassium iodide. "Baker Analyzed" reagent, Baker USP and Curtin USP grade potassium iodide were used interchangably. No differences were noted.

Maleate buffer (pH 6.8). An approximately 1 *F* solution of maleic acid was prepared and neutralized with 50% sodium hydroxide solution. After cooling the pH was adjusted to about 7. Metal impurities were removed by dithizone extraction and then the solution was diluted to a concentration of about 0.5 *F* in maleate. Hydrochloric acid or sodium hydroxide was added to bring the pH to 6.8. The buffer solution is usable for a few days up to a few weeks depending on the experimental conditions of its use, temperature, storage, etc. Attempts to increase the useful life of the buffers by adding thymol as a bactericide were unsuccessful. The problems associated with the use of aged buffers, and the remedies are discussed below.

Phosphate buffer (pH 7.2). The buffer was prepared by adding 0.08 *F* phosphoric acid to 0.08 *F* tripotassium phosphate under a pH meter until a pH of about 7 was reached. After cooling, either acid or salt solution was added to adjust the pH to 7.2. Extraneous cations were then removed by a dithizone (in chloroform) extraction.

Tartrate solution (pH 6.8). "Baker Analyzed" reagent *d*-tartaric acid was employed to prepare a 1 *F* solution which was then brought to pH 6.8 with sodium hydroxide.

Backwash solution. The solution was prepared by placing 50 g of potassium iodide, 18 ml of maleate buffer, 5 ml of tartrate solution, pH 6.8, 5 ml of 1 *F* sodium thiosulfate and some water in a beaker. After dissolution was complete water was added to bring the volume to about 100 ml.

PAN solution. Fisher Certified PAN (0.62 g) was dissolved in 95% ethanol (250 ml) to yield a 0.01 *F* solution.

Chloroform. NF chloroform was used after extractive washing with dilute aqueous hydrochloric acid to remove any metal traces.

Water. Doubly de-ionized water was used exclusively.

All other reagents were of the highest available grade and used without further purification.

Procedure 1

Limitations for the application of the procedure are as follows: The cadmium content of the sample must not exceed about 1 g and the zinc content must not exceed about 50 μg. The molar cadmium:zinc ratio must not exceed 100,000:1 (corresponding weight ratio 170,000:1). For samples with a molar ratio up to 500,000:1 minor procedural changes are necessary as described in Note 8.

Interferences can be handled according to type and tolerance limit as shown in Table 5. Procedure 2 should be applied if the amounts of nickel or cobalt or their combination exceed the values specified in Table 5 or if nickel or cobalt or both are present together with large amounts of one or more of the following metals: copper, silver, and mercury.

Simplifications are possible if metals (other than zinc and cadmium) that react with PAN are present only in negligible amounts or are absent. Then the addition of tartrate solution of pH 6.8 in step ii and the addition of thiosulfate in step iv can be omitted. Both of these solutions are also omitted in preparing the backwash solution. Furthermore, the phosphate buffer may be substituted for the maleate buffer on an equal volume basis in step iv and in the preparation of the backwash solution.

Procedure

(i) Place the sample in a volumetric flask and dissolve it in water or the minimum amount of hydrochloric or nitric acid. Bring to volume with water. Transfer an aliquot of appropriate size to a 50-ml beaker (Note 1).

(ii) Add 1 ml of tartrate solution of pH 6.8, 20 g of potassium iodide (Note 2), and sufficient water to dissolve all solids.

(iii) Immerse the electrodes of a pH meter in the solution and add sodium hydroxide or hydrochloric or nitric acid until the meter reads about pH 7 (Note 3).

(iv) Add 2 ml of 1 *F* sodium thiosulfate solution and a spatula tip (2–4 mg) of ascorbic acid. Add 1 drop of 1 *F* potassium cyanide solu-

tion and 5 ml of maleate buffer of pH 6.8 (Note 4). Now proceed without undue delay until step viii is completed.

(v) Transfer the solution to a 60 or 125-ml separatory funnel equipped with a Teflon stopcock.

(vi) Add 0.7 ml of 0.01 *F* PAN in five increments: four of 0.1 ml and a final one of 0.3 ml. Shake the funnel for 2–5 seconds after each 0.1-ml increment and for 45 seconds after the final increment.

(vii) Add 6–10 ml of chloroform and shake the funnel for 20 seconds. Add water to almost fill the separatory funnel (Note 5). Shake it briefly and allow the phases to separate.

(viii) Drain the organic layer into a second separatory funnel, the stem and stopcock bore of which must be free of water. Extract the aqueous layer in the first separatory funnel with five 3–4-ml portions of chloroform (Note 6) and collect these extracts in the second funnel too.

(ix) To the combined extracts add 10 ml of the backwash solution and a spatula tip (2–4 mg) of ascorbic acid and shake the funnel for 20 seconds. Add some water, shake briefly and allow the phases to separate.

(x) Drain the organic layer into a dry 50-ml volumetric flask. Wash the aqueous phase with two 3–4-ml portions of chloroform and collect the washings in the volumetric flask.

(xi) Place 10 ml of ethanol in the volumetric flask, mix, allow to reach room temperature and then bring to volume with chloroform. Measure the absorbance of the solution at 556 mμ against water as reference and obtain the amount of zinc present from a calibration curve (Note 7, Note 8).

Notes

(1) If the composition of the sample and its mode of dissolution are such that it is unnecessary to carry out the neutralization as directed in step iii, the solution may be transferred directly to the separatory funnel.

(2) The 20-g portion of potassium iodide specified includes a reasonable safety factor even for samples containing 1 g of cadmium. If the method is applied routinely to samples containing less than 1 g of cadmium economic considerations may justify a study to determine the minimum potassium iodide:cadmium ratio required to mask cadmium adequately.

(3) The system cools considerably upon dissolution of potassium iodide; however, it is not necessary to wait until the solution warms before continuing.

(4) If the electrodes are immersed in the cold solution on hand at this point the meter should read "pH" 7.1–7.3. The discrepancy between this value and the nominal pH value of the buffer, 6.8, stems from grossly changed conditions (lower temperature and much greater ionic strength). Under these conditions reference is clearly and appropriately made to "meter readings" instead of pH value.

(5) Without added water the two phases have approximately equal densities and the layers fail to separate.

(6) Less than five portions may be used if the chloroform layer is colorless at an earlier extraction.

(7) Prepare a calibration curve from data obtained using solutions containing known quantities of zinc. Carry the standards through the procedure with the following modifications. In step ii add only 10 g of potassium iodide. In step viii drain the organic layer and all the extracts directly into the 50-ml volumetric flask thus omitting step ix (the backwashing operations) and a portion of step x.

(8) Increased sensitivity can be obtained by using cells with a longer path length or slightly reducing the amount of chloroform used in the extraction and after the backwashing so that a 25-ml volumetric flask can be employed in step x or a combination of these approaches. A further increase in sensitivity is possible if a 10-ml volumetric flask is used; however, the following procedural modification is then required. Carry out steps i through viii. Clamp the separatory funnel in a stand above a hot plate. Allow the warm air to evaporate the extract down to 2–4 ml. (The Teflon stopcock serves as a boiling chip and prevents bumping). Cool to room temperature, occasionally increasing the tension on the stopcock to prevent leakage. Carry out step ix. Drain into a 10-ml volumetric flask. Wash the aqueous phase with two 1–2-ml portions of chloroform. Add 2 ml of ethanol and make to volume with chloroform. Complete the determination as described above.

Procedure 2

This procedure must be applied if the sample contains (a) nickel or cobalt or both in significant amounts or (b) nickel or cobalt in any amounts plus significant amounts of one or more of the following met-

als: copper, silver, or mercury. Procedure 2 has the same limitations as Procedure 1 and differs in essence only by including the additional operations described in (A) through (D) below.

(i) Place the sample in a volumetric flask and dissolve it in water or the minimum amount of hydrochloric or nitric acid. If the sample material is free of cobalt add 1 drop of 0.01 *F* cobalt nitrate. Bring to volume with water. Transfer two aliquots of appropriate volume to two separate beakers.

(ii) through (iv) Treat both aliquots as directed in the corresponding steps of Procedure 1 but in step iv omit the addition of cyanide. Reserve one of the treated aliquots for the zinc determination.

(A) Transfer the other treated aliquot to a 50-ml graduate cylinder and dilute with water to 40 ml. The resulting solution is referred to as the working solution.

(B) Place a 4-ml portion of the working solution in a test tube. Then, in the order stated, add 1 drop of 1 *F* potassium cyanide, shake the tube, add a few drops of 0.01 *F* PAN and shake the tube again. Observe the solution after not less than 30 seconds and not more than 5 minutes. If the solution is red or orange proceed to (C). If the solution is green or brown repeat the foregoing with another 4-ml portion of the working solution but add several drops of 1 *F* potassium cyanide. Repeat the foregoing with fresh 4-ml portions of the working solution and with the addition of differing amounts of 1 *F* potassium cyanide as many times as may be necessary to establish the minimum number of drops of the cyanide solution required to prevent the solution from turning green or brown within the specified time interval. (A bracketing approach is the most efficient way of obtaining the necessary information). Then proceed to (C).

(C) Proceed as in (B) but use 0.1 *F* potassium cyanide instead of 1 *F* posassium cyanide. From (B) the amount of 0.1 *F* cyanide required to prevent the appearance of the green or brown color is known to the nearest 10 drops. Now establish to $\pm 10\%$ the number of drops of 0.1 *F* potassium cyanide that is required.

(D) Add 12 times [1] the amount of cyanide established in (C) to the reserved treated aliquot. Addition may be made in drops of 0.1 *F* cya-

[1] Theoretically a 10-fold amount should be required. Practice, however, has shown that the recommended factor of 12 provides optimum conditions.

nide or as an equivalent amount of a more concentrated solution providing the 10% tolerance established in (C) is not to be exceeded.

(v) through (xi) Proceed as in Procedure 1.

DISCUSSION AND RESULTS

Influence of pH. The pH of the solution has a profound influence on both the effectiveness of the iodide masking and the efficiency of the extraction of the zinc–PAN complexes. The iodide masking of cadmium becomes increasingly effective when the pH is lowered, but at the same time the efficiency of the zinc extraction decreases sharply, and a larger number of extractions becomes necessary to fully remove the zinc from the aqueous phase. Consequently, a certain pH value is required to assure speedy and complete extraction of the zinc and at the same time to effect adequate masking of the cadmium. Preliminary experiments established that pH to be around 7. It is important to notice that at the specified pH the iodide masking of cadmium is not fully effective and that the cadmium because it is only partially masked, can still compete with the zinc for complexing with PAN.

Influence of kinetic factors. PAN itself as well as its zinc and cadminum complexes are insoluble in water. Upon addition of the alcoholic PAN solution to the aqueous test solution several processes proceed simultaneously and at different rates. PAN forms the zinc and cadmium complexes and the reagent itself and the complexes precipitate. Because cadmium is only partially masked it consumes a certain amount of PAN. Although the PAN complexes of zinc are of a much higher apparent stability in the medium at hand, the PAN combined with the cadium remains blocked for the zinc because of the very low rate at which the precipitated cadmium–PAN complex exchanges its cadmium for zinc. Consequently, if the cadmium:zinc ratio is large insufficient PAN is available for the zinc and low results are obtained. The situation is not significantly altered by increasing the amount of PAN added. Raising the quantity of iodide added is helpful up to a certain amount but increases above that level are of no further value and thus a practical limit is set for the amount of cadmium that can be tolerated. Most effective in improving the situation is the fractional addition of reagent solution as specified in step vi of the procedure. But even then a significant amount of cadmium is coextracted and the zinc determination is affected. The elimination of this problem is also based on kinetic effects. The PAN

complexes of zinc and cadmium as well as any free PAN (precipitated before it could react with a metal) are extracted into chloroform extremely rapidly and effectively. However, upon longer shaking portions of the metals move back into the aqueous phase with the cadmium fortunately returning much faster than the zinc. Thus within the short shaking time prescribed in step vii of the procedure essentially no zinc is backtransferred to the aqueous phase whereas a significant amount of cadmium leaves the organic phase. Any cadmium still remaining in the organic phase is then removed by the backwashing in step ix. The situation as described prevails only when chloroform is used as the organic solvent. With other solvents, the equilibrium conditions or kinetics or both seem to be completely different; e.g., neither amyl alcohol nor ethyl acetate can be employed. When significant amount of ethanol or methanol are present in the solution to be extracted with chloroform unsatisfactory results are also obtained. However, the small amounts introduced by the alcoholic PAN solution are tolerable.

The peculiar extraction kinetics observed in the course of this investigation are similar to those reported and explained by Zolotov and co-workers (*7*) for the indium-PAN system. This behavior of metal-PAN systems is responsible for the fact that the quantitative extraction of the metals as PAN complexes cannot be effected by shaking the aqueous phase with a solution of the reagent in an organic solvent. Rather the reagent must be added to the aqueous solution which is then shaken with the solvent.

The maleate buffer. Despite a very complicated situation with regard to equilibrium and kinetics the method is very rugged and can tolerate changes in the prescribed condition within astonishingly broad intervals. The maleate buffer, however, requires special attention, since its quality has a pronounced influence. If the buffer is not fresh, low and markedly erratic results are obtained for the zinc. Whenever this behavior occurred it was observed that some insoluble material accumulated at the water–chloroform interface upon phase separation in the extraction step. The greater the quantity of this material, the worse the results. Attempts to identify the material failed. Its formation did not depend on the provenience or degree of purity of the maleic acid employed and could not be prevented by stabilizing the buffer against microbic attacks by addition of thymol. The material appears in various forms and colors; wispy, gelatinous, almost colorless, whitish, or reddish; its amount depends on

the concentration of cadmium and iodide or both and particularly on the age of the maleate buffer. The material absorbs PAN, zinc, and cadmium and hinders a clear phase separation because it has a honeycomb structure and traps water as well as chloroform in its cells. The extent of the honeycomb formation depends on the time interval between addition of the buffer and extraction of the PAN complexes; consequently step iv of the procedure prescribes proceeding rapidly after addition of the buffer. When studying interferences and their elimination potassium cyanide was added to some solutions; these solutions showed a marked decrease in the formation of the material. Thus cyanide should be added to solutions even when not required for masking purposes; considerably older buffer solutions can then be used. With these remedies applied it is not difficult to control the symptoms and avoid difficulties.

The phosphate buffer. According to studies of anion interferences the tolerance limit of phosphate is 50 mg of (PO_4) which corresponds to about 10 ml of a 0.05 *F* phosphate solution. Even less than this amount would suffice for adequate buffering. There are certain advantages in using a phosphate buffer. The reagents are more readily available, the buffer capacity at pH 7 is larger and no formation of interfering material occurs. However, the tolerance limits for metallic interferences are greatly decreased as shown in Table 5.

Optical Properties of the system. PAN and zinc form two complexes, Zn(PAN) and $Zn(PAN)_2$. The amount of PAN prescribed in the procedure provides an excess thus assuring the almost exclusive formation of the 1:2 complex. When operating at the absorbance maximum of that species, 556 mμ, it is necessary then to account for the nonnegligible absorbance of the free PAN present. Compensation, however, is readily achieved by employing the same fixed amount of PAN in every analysis and when establishing the calibration curve. The molar absorptivity of the $Zn(PAN)_2$ complex was found to be 5.8×10^4 liter/mole-cm in good agreement with the value 5.55×10^4 reported by Püschel (*4*). In the chloroform–ethanol medium employed Beer's Law is obeyed up to concentrations of at least 1 μg/ml of zinc. The absorbance remains constant for at least several hours. Stopcock grease has a very detrimental effect and must be absent. If it is present the absorbance readings are too low and continuously decrease upon standing. Thus the use of separatory funnels equipped with Teflon stopcocks is almost mandatory.

Results with solutions containing only zinc and cadmium. Some artificial "unknowns" were prepared by dissolving known amounts of cadmium nitrate and adding measured quantities of zinc. Some results obtained from application of procedure 1 are shown in Table 1. For "unknowns" with larger cadmium:zinc ratios the procedure modified according to Note 8 was applied. The results then obtained are shown in Table 2. The zinc content in the cadmium salt employed was quite small; nevertheless it had to be taken into account at least for test samples of a high cadmium:zinc ratio. Best fit of the results was obtained by assuming the cadmium salt contained 4×10^{-5} % zinc. This value was essentially identical with the one obtained independently by flame absorption photometric analysis.[2] Table 1 shows the explicit separation of

TABLE 1

REPRESENTATIVE RESULTS OF THE DETERMINATION OF ZINC IN CADMIUM USING PROCEDURE 1

	Zinc (μg)				
	Taken				
Cadmium taken (g)	As dilute standard zinc nitrate solution	As an impurity in the cadmium	Found	Error	Cadmium:zinc molar ratios
0.22	12.6	0.2	12.8	0	10,000
0.22	25.2	0.2	24.9	−0.5	5000
0.22	37.8	0.2	37.4	−0.6	3300
0.45	12.6	0.5	12.8	−0.3	20,000
0.45	25.2	0.5	25.6	−0.1	10,000
0.45	37.8	0.5	37.7	−0.6	5000
0.45	50.4	0.5	50.1	−0.8	2500
0.90	3.2	1.0	4.2	0	120,000
0.90	6.3	1.0	7.1	−0.2	70,000
0.90	12.6	1.0	12.8	−0.8	40,000
0.90	25.2	1.0	26.3	+0.1	20,000
0.90	37.8	1.0	37.9	−0.9	13,000
0.90	50.4	1.0	50.5	−0.9	10,000

[2] W. Wolfram, Baker Chemical Co., Phillipsburg, N.J. performed this analysis. His aid is gratefully acknowledged.

TABLE 2

REPRESENTATIVE RESULTS OF THE DETERMINATION OF ZINC IN CADMIUM USING PROCEDURE 1 MODIFIED TO OBTAIN INCREASED SENSITIVITY [a]

Zinc (μg)			Cadmium:zinc molar ratios
Taken	Found	Error	
7.3	6.9	−0.4	70,000
7.3	6.7	−0.6	70,000
7.3	7.2	−0.1	70,000
4.2	3.6	−0.6	120,000
4.2	3.5	−0.7	120,000
2.6	2.5	−0.1	200,000
2.6	2.6	0	200,000
2.3	1.8	−0.5	230,000
1.6	2.3	+0.7	330,000
1.0	1.3	+0.3	520,000
1.0	1.3	+0.3	520,000

[a] Each sample contained 0.90 g of cadmium.

"zinc taken" into the portion added as a zinc salt and that present as an impurity in the cadmium salt.

Increasingly large negative errors are encountered when more than 1 g of cadmium is taken. A reasonable explanation is that the potassium iodide, even at a molar ratio of 12:1 with respect to cadmium, fails to mask the cadmium adequately against reaction with PAN. Much of the PAN added is then bound to cadmium, thereby kinetically blocked and no longer available for reaction with zinc; low results for that metal are the consequence. Neither increasing the iodide:cadmium ratio to 20:1 nor doubling the quantity of PAN employed gives a significant improvement. Thus there is an upper limit of about 1 g of cadmium imposed on the method.

The data in Tables 1 and 2 show the method to yield results that are reliable within some tenths of a microgram of zinc; on the average about 0.5 μg. Considering a relative error of 10% as acceptable the procedure requires a minimum of 5 μg zinc to be present. Of course, it is possible to operate with smaller amounts if the various means to increase sensitivity are employed.

Interference studies. A number of anions were investigated. The anions chosen for study were those likely to be present as counter ions

when analyzing cadmium compounds, or added to the system for masking or buffering purposes. At least 1g of the following can be tolerated: acetate, chloride, nitrate, sulfate; at least decigram amounts of carbonate, fluoride, tartrate, and thiosulfate; at least miligram amounts of hexacyanoferrate(II), hexacyanoferrate(III), and thiocyanate can be present. No attempts were made to determine the tolerance limits for these anions and actually far greater amounts than mentioned above may well be permissible.

It is of interest to note that sizable amounts of tartrate can be tolerated; but even small amounts of citrate cause complete masking of the zinc against reaction with PAN. Up to 50 mg of phosphate can be present without causing detrimental effects.

Special attention was paid to cyanide ion because it was to be used as masking agent for some of the cationic interference expected to be present in high purity cadmium. In solutions containing zinc and no cadmium, slightly less than 2 mg of cyanide could be tolerated. In systems containing cadmium the tolerance limit is higher because the cadmium acts as a cyanide "sink."

Several metallic interferences were studied. The cations selected for study were those expected to be found in cadmium and its compounds. Some results selected from an extensive number of experiments are presented in Tables 3 and 4. The majority of interferences cause a positive error simply because the respective metal ions form complexes with PAN that are coextracted and absorb at the operating wavelength. A few metals cause negative errors. With a maleate buffered solution, tin(IV) is the only one so acting; but in a phosphate-containing medium, several others exhibit the same behavior. Thallium(I) interferes due to the formation of a precipitate. Phase separation is thereby made difficult and losses of zinc and zinc–PAN complex seem to occur owing to occlusion and adsorption.

For practical purposes it is of value to establish tolerance limits or interference thresholds. Since the error increases with increasing amount of interference, it is necessary to define the minimum error permissible. For the present purpose the interference threshold is arbitrarily defined as the amount of interfering substance that causes an error equivalent to 1 μg of zinc. The approximate tolerance limits for some interferences are listed in Table 5. The results show clearly the superiority of maleate buffer over phosphate buffer for systems containing significant amounts

TABLE 3

REPRESENTATIVE RESULTS FOR THE DETERMINATION OF ZINC IN CADMIUM IN SAMPLES CONTAINING CATION INTERFERENCES USING PROCEDURE 1 [a]

Interfacing metal (*M*)	Quantity of M present (mg)	M:zinc molar ratio	Zinc (μg)	
			Found	Error
Pb, Hg, Cu, Ag	42, 41, 13, 21	500	26.8	+0.6
Al	2.2	200		
Pb, Hg, Cu	42, 41, 13	500	26.3	+0.1
Al	2.8	250		
Ni	1.2	50	26.2	0
Co	0.24	10	26.9	+0.7
Bi	4.3	50	27.1	+0.9
Sb	0.60	12	26.8	+0.6
Fe(II)	0.15	7	27.1	+0.9
Fe(III) [b]	0.32	15	26.9	+0.7
Mn	0.022	1	27.5	+1.3
In	0.094	2	26.1	−0.1
Ga	0.26	9	26.4	+0.2
Tl(I)	0.71	9	26.5	+0.3
Sn(II)	0.49	10	25.7	−0.5
Sn(IV)	0.049	1	24.6	−1.6

[a] Each sample contained 26.2 μg of zinc and 0.90 g of cadmium (cadmium:zinc molar ratio, 20,000:1).

[b] The addition of ascorbic acid was omitted (step iv).

of cationic impurities. It should be emphasized that the figures given are understated to allow a safety factor; furthermore, they are approximate and will vary with changes in the zinc and cadmium content or both.

For lead, copper, mercury, and silver, no attempt was made to establish the actual interference thresholds. These metal ions are effectively masked by the iodide and can be tolerated in amounts that cannot be present when analyzing cadmium of even only moderate purity. As is commonly the case the tolerance limits are not additive. This has been

TABLE 4

REPRESENTATIVE RESULTS FOR THE DETERMINATION OF ZINC IN CADMIUM IN SAMPLES CONTAINING CATION INTERFERENCES USING PROCEDURE 2 [a]

Interfering metal (M)	Quantity of M present (mg)	M:zinc molar ratio	Zinc (μg)	
			Found	Error
Pb	42	500	26.4	+0.2
Hg	41	500		
Cu	13	500		
Al	2.2	200		
Ni	2.4	100		
Co	1.2	50		
Pb	42	500	26.9	+0.7
Hg	41	500		
Cu	5.7	220		
Al	2.2	200		
Ni	2.4	100		
Co	2.2	90		
Pb	42	500	25.1	−1.1
Hg	41	500		
Cu	13	500		
Ag	21	500		
Al	2.2	200		
Ni	2.4	100		
Co	1.9	80		
Pb	42	500	25.5	−0.7
Hg	41	500		
Cu	8.6	330		
Al	2.2	200		
Ni	2.4	100		
Co	2.2	90		

[a] Each sample contained 26.2 μg of zinc and 0.90 g of cadmium (cadmium to zinc molar ratio: 20,000:1).

demonstrated specifically for two combinations: iron–manganese and bismuth–antimony.

Of special interest is some elaboration on cyanide as masking agent. Both iron(II) and iron(III) when transferred to the respective hexacyanoferrates are completely masked against PAN. Unfortunately the

TABLE 5

TOLERANCE LIMITS FOR VARIOUS SPECIES IN THE DETERMINATION OF ZINC IN 0.90-g CADMIUM SAMPLES EMPLOYING PROCEDURE 1 USING DIFFERENT BUFFERS

Interference	Tolerance limit (μg; exceptions marked)	
	Maleate buffer	Phosphate buffers
Hg(II)	>>40 mg	>40 mg
Pb	>>40 mg	>40 mg
Cu	>>10 mg	>10 mg
Ag	>>20 mg	>20 mg
Al	2.5 mg	<<10 mg [d]
Fe(III)	130	70 [d]
Fe(III) [a]	300	100 [d]
Ni	1.2 mg [b]	Not determined [c]
Co	200 [b]	Not determined [c]
Sb	600	50
Bi	4 mg	50
Ga	300	60 [d]
In	100	<<20 [d]
Ti(I)	700	1.5 mg
Sn(II)	500 [d]	<<50 [d]
Sn(IV)	<<50 [d]	<<50 [d]
Mn	<20	<20

[a] The addition of ascorbic acid was omitted (step iv).

[b] When using Procedure 2 the tolerance limits of nickel and cobalt is increased to about 2.5 and 2 mg, respectively.

[c] A positive error was observed when Procedure 2 was used with a test solution containing 1.8 mg of Ni and 1.6 mg of Co. This positive error persisted even when the amount of cyanide was increased by 20%.

[d] The presence of excessive amounts of these species introduces a negative error.

transfer to the complex cyanides is quite slow and proceeds satisfactorily only in the presence of an excess of cyanide. Such an excess, however, is not permitted here because it would result in an attack on the zinc. The possibility exists of adding a quantity of cyanide sufficient for rapid masking of the iron and then on destroying any excess by addition of formaldehyde. But no experiments were performed in this direction. Attempts to mask iron(II) with *o*-phenanthroline or salicylhydroxamic acid failed. So did attempts to mask iron(III) with thiocyanate, sulfosalicylate, phosphate, malonate, and Tiron. However, the amount of iron

tolerable is larger than would be expected in a high purity cadmium sample and thus the failure to find an adequate masking procedure is of no grave practical consequence.

Copper, as stated above, is masked by iodide with the thiosulfate serving to reduce the iodine formed in the reaction between the copper-(II) and the iodide. But still, copper must be considered in connection with cyanide masking. The copper(I)–dicyano complex is more stable than the corresponding iodo complex and thus cyanide is consumed by the copper. The behavior of mercury and silver with respect to iodide masking and cyanide consumption is essentially analogous except that no reduction step is included in the masking process.

The cyanide masking of nickel takes place without complications. The nickel reacts rapidly with the cyanide to form a complex far more stable than the cyano complexes of zinc and cadmium.

Cobalt may cause difficulties. In order to convert it efficiently to the cyano complex excess cyanide is necessary. With insufficient amounts of cyanide present the green cobalt(III)–PAN complex is formed. This species is produced slowly but once established is remarkably resistant to decomposition and persists even in strongly acidic solutions and in the presence of excess cyanide. Its absorptivity at 556 mμ is sufficient to interfere with the photometric determination of the zinc. The medium employed in the present work is reducing due to the presence of ascorbic acid and thiosulfate and the addition of large amounts of iodide. Yet even under these conditions cobalt(II) in the presence of PAN is oxidized to cobalt(III). If there is present much cobalt that is not complexed by cyanide, in addition to the formation of the green cobalt-(III)–PAN complex, a dark precipitate is produced and in such quantities as to make it impossible to complete the determination. When operating with 1 g of cadmium of higher purity the one drop of 1 *F* potassium cyanide to be added according to Procedure 1 is more than sufficient to take care of all cyanide-consuming impurities possibly present. However in exceptional cases the amount of cyanide consumed may be high and the cobalt may not be fully masked. Fortunately it is not necessary to anticipate this undesirable situation because its occurrence manifests itself quite clearly. Hence it is not required to have foreknowledge of the amounts of cyanide consumed in the sample in order to add a suitable additional quantity of potassium cyanide. That amount is readily found by following Procedure 2, where the formation of the in-

tensely colored green complex is used for purpose of indication. If large amounts of nickel are suspected and no cobalt is present the adequacy of cyanide masking can be readily tested by adding a drop of 0.01 *F* cobalt nitrate solution to see whether the green complex is formed or not. As shown in Table 4, the results obtained by use of Procedure 2 show somewhat greater fluctuation than those by Procedure 1 but are still quite acceptable.

ACKNOWLEDGMENT

Financial assistance to one of us (RW) by the National Science Foundation, Washington, D.C., is gratefully acknowledged.

SUMMARY

The zinc in high purity cadmium is determined photometrically as the PAN complex after extraction into chloroform. Massive amounts of potassium iodide are added to the sample solution to mask cadmium. Copper, mercury, lead, and silver are also masked by the iodide. Some cyanide is added which effects masking of nickel and cobalt. Tolerance limits for these and several other interferences are given. The results are reliable to several tenths of a microgram of zinc. Samples containing up to about 1 g of cadmium can be handled. Zinc can be determined even at a cadmium:zinc molar ratio of 500,000:1; although additional operations are required at ratios above 100,000:1.

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MICROCHEMICAL JOURNAL 14, 335–343 (1969)

Human and Rat Serum Proteins, Lipoproteins, Ammonium Persulfate, Gel Concentration, and Disc Electrophoresis

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Investigations from this laboratory on the disc electrophoretic separations of serum lipoproteins of several species using polyacrylamide gels, demonstrated a high degree of resolution of lipoproteins that was not obtainable with ultracentrifugal or other electrophoretic methods *(6–10)*. Several investigators have reported that the use of ammonium persulfate as a catalyst in the preparation of polyacrylamide gels for use in electrophoresis of proteins may, in some instances, lead to the formation of artifacts *(1, 3, 5)*. However, no studies have been previously reported on the effect of ammonium persulfate on serum protein and lipoprotein patterns in polyacrylamide gels. Since lipoproteins are well known for their lability, it seemed important to determine whether the improved resolution observed by the disc electrophoretic method was merely fortuitous as a result of interaction of lipoproteins with a strong oxidizing agent such as ammonium persulfate.

Therefore, the following experiments were performed to determine the effect of ammonium persulfate on the human and rat serum protein and lipoprotein patterns in polyacrylamide gel: (a) substitution of riboflavin and light as a catalyst for ammonium persulfate at four different gel concentrations; (b) removal of ammonium persulfate by preliminary electrophoresis; (c) incubation of serum proteins and isolated lipoproteins with ammonium persulfate prior to electrophoresis.

MATERIALS AND METHODS

Unless otherwise stated the electrophoretic method and the staining procedures used were as described previously *(8–10)*. Human sera were obtained from Carle Hospital Clinic (Urbana, Ill.) and rat sera were obtained from rats maintained on a semisynthetic diet *(8)*. The lipoprotein fractions were ultracentrifugally isolated as described before *(9)*. The sample quantities were generally 10 μl of serum for lipoprotein patterns

and 1 μl of serum for protein patterns. In the case of lipoprotein fractions, between 100–200 μg of samples were used. Ammonium persulfate catalyzed gels (AP-gels) of various concentrations were prepared as before *(8)*. Riboflavin catalyzed gels (RF-gels) were prepared according to suitable modification of the formula commonly used for photogel solutions except that solution A containing *N, N, N′, N′*-tetramethylethylenediamine (temed) (0.46 ml/100 ml solution A), solution C and water were substituted for solution B, solution D and solution F, respectively *(2)*. These gel solutions were polymerized by illuminating with light for 50 minutes. When it was desired to remove the ammonium persulfate from the polymerized main gels, a preliminary electrophoresis was performed for 2 hours at 5 mA/tube using a Tris–HCl buffer pH 8.9 of the same final composition as used in the preparation of the main gel solutions. After this preliminary electrophoresis, disc electrophoresis was performed according to standard conditions. In order to determine the effect of ammonium persulfate on proteins and lipoproteins, both rat and human serum as well as their low density and high density lipoproteins were incubated at room temperature for 1.5 hours with freshly prepared ammonium persulfate solution 1.40 mg/ml) at a volume ratio of either 1:1 or 1:2. After incubation, the control and the incubated samples were run simultaneously on disc electrophoresis and were stained with amido black 10B (a protein stain).

RESULTS AND DISCUSSION

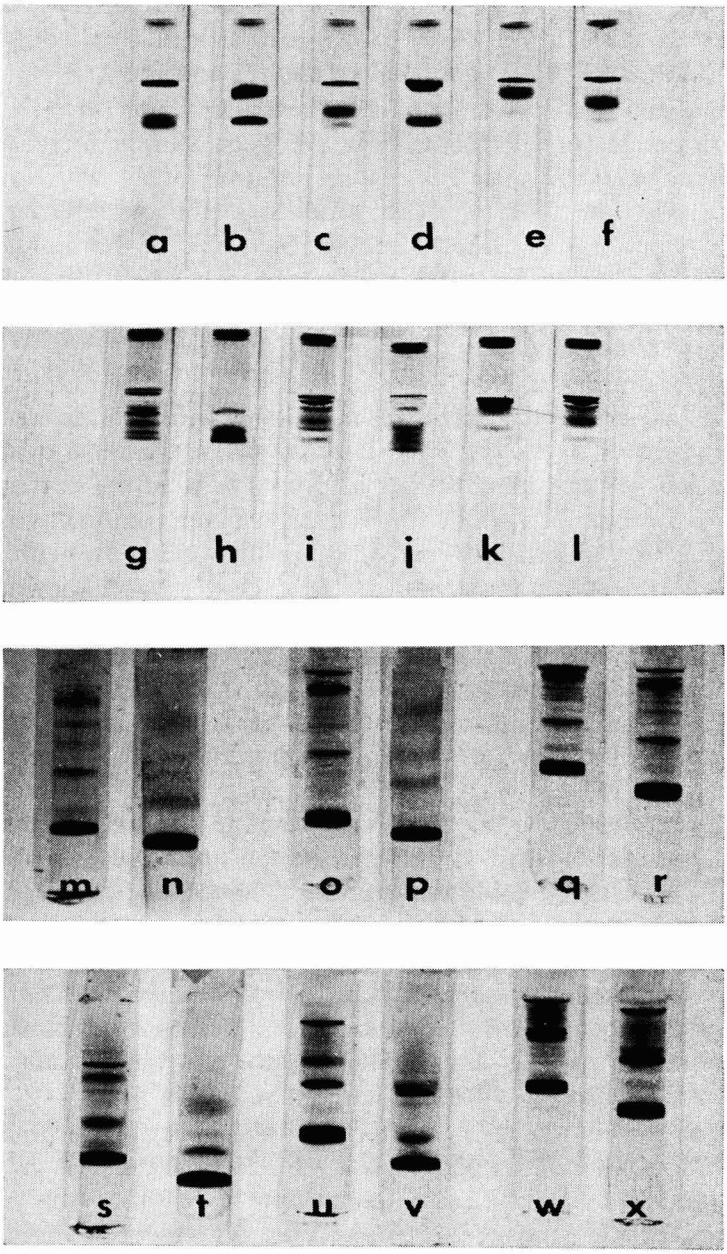
The results indicated that there was increased penetration of lipoproteins into RF-gels as compared with AP-gels of the same concentration (Fig. 1a–l). This difference was particularly noticeable in 3.75% gels with prestained rat serum (Fig. 1g, h). The RF-gels of 3.75% concentration were very soft and were similar in physical characteristics to the 2.0% AP-gel reported earlier *(6)*. The lipoprotein patterns observed in 5.0% FR-gel were somewhat similar to those observed in 3.75% AP-gel (Fig. 1d and a; j and g) and suggested that the pore sizes of these two gels were approximately the same. Furthermore, all the bands seen in 3.75% AP-gel were noticeable in 5.0% RF-gel (Fig. 1g and j) and indicated that the bands routinely observed in 3.75% AP-gel with rat serum lipoproteins were not artifacts. Although in the case of 7.5% gels, the patterns obtained with either catalyst were qualitatively the same, differences in the extent of pentration of the lead bands could be clearly

seen (Fig. 1e, f, k, l). On the basis of the lipoprotein patterns, it may be concluded that the 7.5% RF-gel had a pore size that was only slightly less than that of the 5% AP-gel (Fig. 1c and f; i and l).

The intensities of some of the bands, particularly the slower migrating bands, were greater in RF-gels than in AP-gels. Similar increases in intensities of bands have also been observed previously with large pore AP-gels *(6)*. On the basis of recent studies on the quantitative aspects of disc electrophoresis *(4)*, the increased intensities of the bands may be explained as due to decreased resistance to migration of the high molecular weight lipoproteins which resulted in wide bands as opposed to sharp, narrow bands.

In order to emphasize that the results obtained were not peculiar to lipoproteins, experiments were also conducted with human and rat serum proteins. From these patterns (Fig. 1m–x), it was also evident that the 3.75% RF-gel was much larger in pore size than the corresponding AP-gel. The protein patterns in 5% RF gel was approximately the same as that in 3.75% AP-gel; however, these results and those with lipoproteins indicated that the pore size of the 5% RF-gel was somewhat larger than the 3.75% AP-gel. From subsequent experiments, it was established that the lipoprotein patterns in 6.0% RF-gel were very similar to the lipoprotein patterns in 3.75% AP-gel (Fig. 2a and d; e and h). Hence, it was concluded that the 6.0% RF-gel was equivalent to 3.75% AP-gel in terms of pore size. The protein patterns in 7.5% gels indicated also that the RF-gel was intermediate in pore size to the 5.0 and 7.5% AP-gels (Fig. 1o, q, r, u, w, x). Although the striking differences observed here in the pore sizes between RF-gels and AP-gels in the case of 3.75 and 5.0% acrylamide concentration have not been reported before, the present data is consistent with the early observations of Davis *(2)* and Steward and Barber *(11)* with respect to 7.5% gels which were either chemically polymerized or photopolymerized. Thus, these results demonstrated that the RF-gels have much larger pore sizes than the corresponding AP-gels and therefore gave rise to different lipoprotein and protein patterns.

Because of the difficulty encountered in the above experiments with respect to variability of the pore sizes of RF-gels and AP-gels at the same acrylamide concentrations, ammonium persulfate was removed from AP-gels by preliminary electrophoresis. As shown in Fig. 2i–p, no differences could be seen in either the human or rat serum lipoprotein


a b c d e f
g h i j k l
m n o p q r
s t u v w x

patterns at both 3.75% and 5.0% gel concentrations as a result of the removal of ammonium persulfate. It may therefore be conluded that residual ammonium persulfate in AP-gels does not cause artifacts in lipoprotein patterns.

In order to further confirm these results, isolated lipoprotein fractions were incubated with ammonium persulfate for 1.5 hours and electrophoresis was performed on the incubated and control samples. At a volume ratio of sample to ammonium persulfate solution of 1:1 it was rather surprising to note that no detectable changes were observed with either human or rat serum lipoproteins (Fig. 2q–x). Similarly, no significant differences were observed in the serum protein patterns as a result of incubation with ammonium persulfate (Fig. 2y, z) (left and right). The amount of ammonium persulfate used was about four times that would be present in the entire main gel (even, assuming that as much as 10% of the persulfate did not migrate during electrophoresis). Furthermore, this amount was sufficient to turn the blue Sudan black B solution into a brown solution within a few minutes. When the volume ratio of ammonium persulfate to sample was increased to 2, blurring of some of the bands and decreases in intensities of other bands were noted. However, in no case were new bands observed.

Thus, these experiments demonstrated that ammonium persulfate does not produce artifacts during disc electrophoresis of serum proteins and lipoproteins using the pH 9.5 system. The possibility of artifacts using the low pH disc system (pH 2.3) cannot, however, be ruled out because high concentrations of ammonium persulfate and temed are need-

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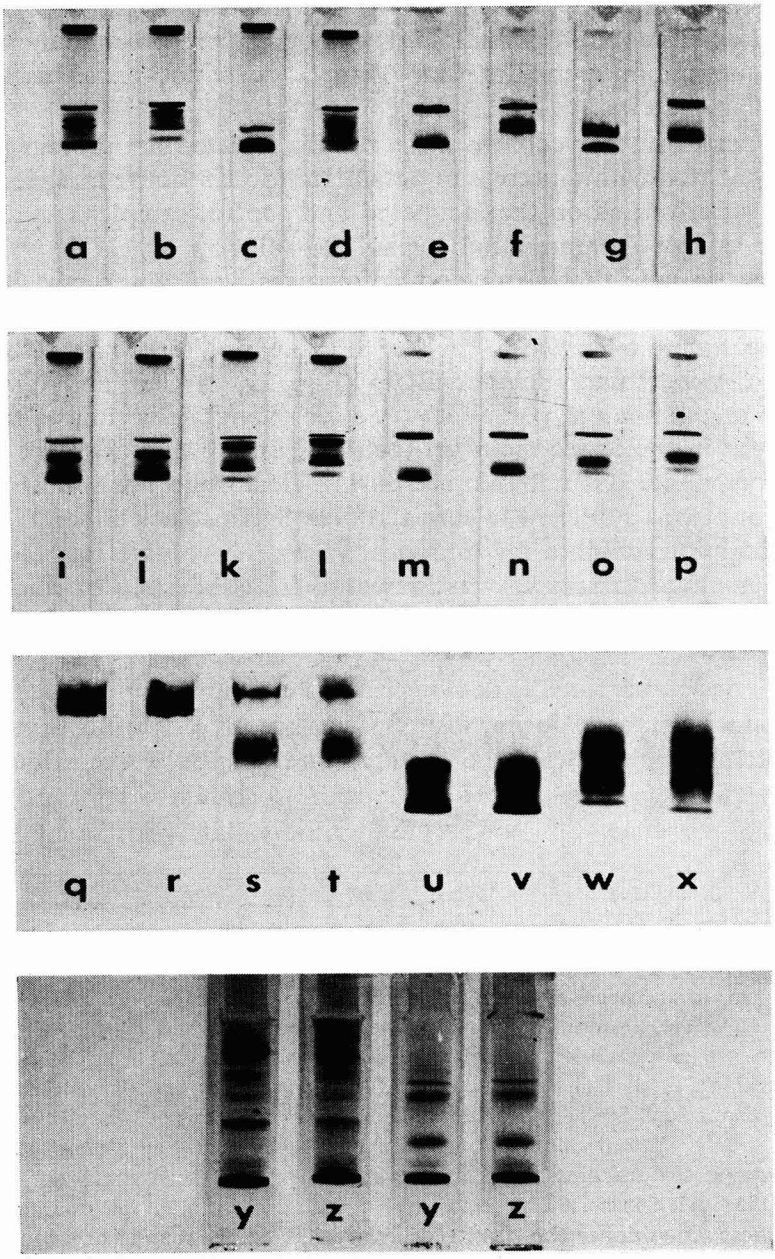
FIG. 1. Disc electrophoretic patterns of serum proteins and lipoproteins using ammonium persulfate and riboflavin catalyzed gels: (a–f and g–l), Sudan black B-prestained human and rat serum, respectively; (m–r and s–x), human and rat serum, respectively, stained with amido black 10B; a, c, e, g, i, k, m, o, q, s, u, w are ammonium persulfate catalyzed gels; and b, d, f, h, j, l, n, p, r, t, v, x are riboflavin catalyzed gels; a, b, g, h, m, n, s, t, 3.75% acrylamide concentration; c, d, i, j, o, p, u, v, 5.0% acrylamide concentration; and e, f, k, l, q, r, w, x, 7.5% acrylamide concentration. For Figs. 1 and 2 migration is from top to bottom and 2.5 mA/tube was used. When a protein stain was to be employed, the tracking dye was allowed to migrate to a fixed distance in all gels (1.125 inch for serum proteins and 1 inch for isolated serum lipoproteins). Under these conditions, the extent of migration of the bands are roughly indicative of the porosity of the gels.


a b c d e f g h
i j k l m n o p
q r s t u v w x
y z y z

ed to polymerize the gels and because the persulfate and proteins migrate in opposite directions in this pH system.

Since lipoproteins isolated from healthy human subjects or normal rats have significant amounts of antioxidants it is possible that they are not oxidized by small concentrations of ammonium persulfate that may persist in AP-gels. Certain sensitive proteins or enzymes such as insulin or enolase *(1, 3)* may be affected by ammonium persulfate even at low concentrations and may give rise to spurious bands. The further possibility that these proteins may be unstable in an oxygen-rich media in the total absence of reducing agents has been suggested by Ornstein (Disc Electrophoresis Newsletter, issue 9, page 8, August 1967, Canalco, Rockville, Md.). However, the results of this investigation have shown convincingly that, (i) the major serum proteins are unaffected by incubation with a small quantity of ammonium persulfate, and (ii) the differences in the serum protein patterns between the AP-gels and RF-gels are attributable to the differences in pore sizes of these gels. Therefore, future studies on the effect of ammonium persulfate on electrophoretic patterns in acrylamide gels should also take into consideration the large differences in the physical characteristics between AP-gels and RF-gels.

SUMMARY

Since the presence of residual ammonium persulfate in polyacrylamide gels has been reported to cause artifacts in electrophoretic patterns of certain proteins, extensive electrophoretic experiments were conducted with human and rat serum proteins and lipoproteins. These experiments included substitution of riboflavin

FIG. 2. The effect of ammonium persulfate on human and rat serum lipoprotein and protein patterns: (a–d), Sudan black B-prestained rat serum; (e–h), Sudan black B-prestained human serum lipoproteins; a, e, and b, f, are ammonium persulfate catalyzed gels of 3.75 and 6.0% acrylamide concentrations, respectively; c, g, and d, h, are riboflavin catalyzed gels of 3.75 and 6.0% acrylamide concentrations, respectively. The i–l and m–p are Sudan black B-prestained rat and human serum, respectively. The i, m, and k, o, are 3.75 and 5.0% gels, respectively, with ammonium persulfate removed by preliminary electrophoresis; j, n, and l, p, are 3.75 and 5.0% gels, respectively, without removal of ammonium persulfate. The q–z are 3.75% AP-gels stained with amido black 10B; q, r, and s, t, are human and rat serum low density lipoproteins, respectively; and u, v, and w, x, are human and rat serum high density lipoproteins, respectively; q, s, u, w, and r, t, v, x, are control and ammonium persulfate incubated samples, respectively. The y, z, (left) and y, z, (right) are human and rat serum, respectively; y, (left) and y, (right) are control samples; z, (left) and z, (right) are samples incubated with ammonium persulfate.

and light as a catalyst for ammonium persulfate at several gel concentrations, removal of ammonium persulfate from the gel by preliminary electrophoresis, and incubation of serum proteins and isolated lipoproteins with ammonium persulfate prior to electrophoresis. The riboflavin catalyzed gels were much softer and were of larger pore sizes than ammonium persulfate catalyzed gels of the same acrylamide concentration and, therefore, the elecerophoretic patterns in the two gel systems were different. However, differences were not observed when the ammonium persulfate was was removed by preliminary electrophoresis. Similarly, incubation of samples with ammonium persulfate did not produce spurious bands and, thus, established that ammonium persulfate does not produce artifacts during disc electrophoresis of serum lipoproteins and proteins using the pH 9.5 system.

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MICROCHEMICAL JOURNAL 14, 343–349 (1969)

Microdetermination of DL-Alanine. II. Microdetermination of L-Glutamic Acid, DL-Valine, and DL alanine in a Mixture in One Solution without Separating

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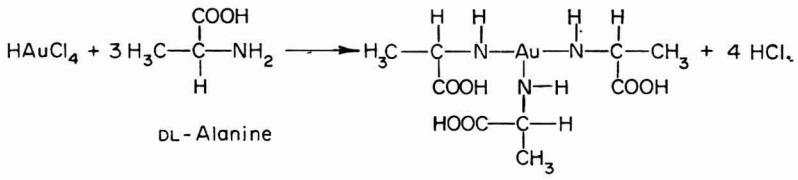
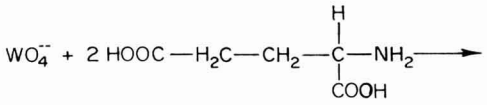
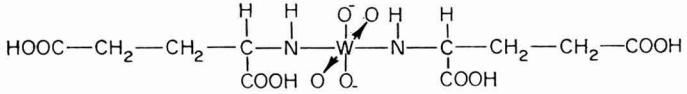
The literature is silent concerning the determination of L-glutamic acid, DL-valine and DL-alanine in the form of a mixture without separation. However, separately they have been determined by iodometric titration of their copper salts (*1*); the influence of cations on the ninhydrin reaction (*2*); the action of periodate (*3*); estimating 2,4-dinitrophenyl derivative (*4*); the application of Conway's method (*5*); oxidation with potassium permanganate (*6*); oxidyzing with ninhydrin to aldehyde (*7*); direct photometry (*8*); partition chromatography on silica gel (*9*); gas chromatography (*10*); by oxidation with chlorauric acid (*11*); paper chromatography (*12, 13*); and with carboxylic resins (*14*).

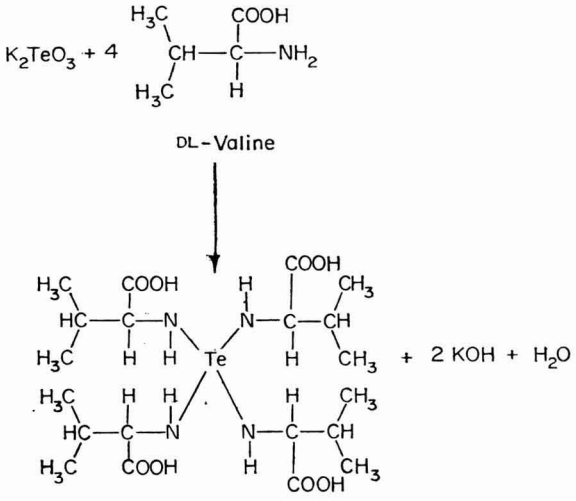
Present work deals with the determination of DL-alanine separately in micro amounts by titrating against gold chloride solution using xylenol orange as indicator. A mixture of DL-alanine, DL-valine and L-glutamic acid has, also, been determined titrimetrically in micro amounts without separating and in one solution. Potentiometric data and results of analysis show that complexes are formed between gold chloride and DL-alanine, potassium tellurite, and DL-valine, and sodium tungstate and L-glutamic acid in ratios of 1:3, and 1:2, respectively. Probably the following reactions take place:





L-Glutamic acid





EXPERIMENTAL METHODS

Reagents used. DL-alanine, DL-valine, L-glutamic acid, potassium tellurite, and sodium tungstate (E. Merck grade); gold chloride (PALMSTON's Indian grade); catechol violet, congo red and xylenol orange (B.D.H. grade).

Apparatus used. Micropipette and burette used had LC = 0.01.

Standard solutions of DL-alanine, DL-valine, L-glutamic acid, and sodium tungstate were prepared by dissolving the exactly weighed amounts in distilled water at room temperature. Gold chloride and potassium chloride solutions were prepared by dissolving the reagents in distilled water and then standardizing each (*15, 16*), respectively.

PROCEDURE

(a) Separate determination of DL-*alanine.* A known volume of a standard solution of DL-alanine is placed in a beaker, with a micropipette, and diluted to 30 ml. A few drops (2–3) of a solution of xylenol orange are then added and the whole solution becomes pink. Standard gold chloride solution is then run in the beaker, from a microburette, when at the end point the pink color changes sharply to very light rose and then light yellow after 1 minute.

(b) Determination of DL-*alanine,* DL-*valine and* L-*glutamic acid in the form of a mixture in one solution.* Known volumes of L-glutamic acid, DL-alanine and DL-valine solutions are placed in a beaker with a micropipette, and the volume is raised to 30 ml with distilled water. Thus, a mixture of these three amino acids is formed. A few drops of catechol violet solution are added and the whole solution becomes light-yellow; and L-glutamic acid is titrated against a standard solution of sodium tungstate run from a microburette. The end point is marked by a light rose color.

After titration of L-glutamic acid in the mixture, DL-alanine is titrated by adding a few drops (2–3) of congo red in the same beaker, and the whole solution becomes red. Standard solution of gold chloride is run into the beaker from a microburette. At the end point the red color changes sharply to light violet.

Lastly, DL-valine is titrated against a standard solution of potassium tellurite. In this case no more indicator is added to the light-violet colored solution mixture after titration of L-glutamic acid and DL-alamine; then a standard solution of potassium tellurite is run in the beaker. At the end point the light violet color changes to orange red. Thus, all three amino acids were titrated directly in one solution.

RESULTS AND DISCUSSION

Results are given in Tables 1 and 2. The ranges (mg/liter) in which L-glutamic acid, DL-alanine and DL-valine were estimated were 5.982×10^{-4} to 14.730×10^{-4}, 4.6602×10^{-4} to 18.6209×10^{-4}, and 11.6956×10^{-4} to 29.2390×10^{-4} respectively.

In Table 1, DL-alanine was estimated separately. Results show that in this set of experiments the maximum error, in the case or DL-alanine, is of 1.4%. Calculations were made in this case by multiplying the

TABLE 1

MICRODETERMINATION OF DL-ALANINE

DL-Alanine (0.04 *M*) (ml)	$HAuCl_4$ 0.0078 *M* (ml)	Amount of DL-Alanine ($\times 10^{-4}$ mg/liter)		Error (%)
		Taken	Found	
0.1	0.17	4–686	4.6602	0.5
0.2	0.34	4.372	9.3205	0.5
0.3	0.52	14.058	14.2550	1.4
0.4	0.70	18.744	18.6209	0.6

observed calculated values by 3, since the complex is formed between gold and DL-alanine in the ration of 1:3.

Table 2 shows the order of estimations of the three amino acids in the form of a mixture, without separation, in one solution. In these determinations only two indicators, catechol violet and congo red, were used. The L-glutamic acid and DL-valine were titrated in the ascending and descending order from the top, respectively. In these estimations if this order is not maintained then titration of these amino acids is not possible. In this mixture, calculations have been done for L-glutamic acid by multiplying the observed calculated values by 2 (since the ratio in which the complex is formed between sodium tungstate and L-glutamic acid is 1:2). In the cases of DL-alanine and DL-valine the observed calculated values are multiplied by 3 and 4, respectively, since the complex between gold and DL-alanine is formed in the ratio of 1:3, and between tellurium and DL-valine is formed in the ratio of 1:4. It is important to note that the volume in every set of experiments should be the same, whether it be separate estimation or in the form of a mixture. Results show that no error lies with the determination of L-glutamic acid; but DL-alanine and DL-valine show a maximum error of 1.4% and 0.16%, respectively.

ACKNOWLEDGMENT

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

TABLE 2

MICRODETERMINATION OF L-GLUTAMIC ACID, DL-ALANINE, AND DL-VALINE WITHOUT SEPARATION

I				II				III			
L-Glutamic acid 0.02 *M* (ml)	Na_2WO_4 0.01 *M* (ml)	L-Glutamic acid ($\times 10^{-4}$ mg/liter)		DL-Alanine 0.04 *M* (ml)	HAuCl 0.0078 *M* (ml)	DL-Alanine ($\times 10^{-4}$ mg/)liter		DL-Valine 0.01 *M* (ml)	K_2TeO_3 0.0104 *M* (ml)	DL-Valine ($\times 10^{-4}$ mg/liter)	
		Taken	Found			Taken	Found			Taken	Found
0.2	0.2	5.892		0.1	0.17	4.686	4.6602	2.5	2.5	29.2875	29.2390
0.3	0.3	8.838		0.2	0.34	9.372	9.3205	2.0	2.0	23.4300	23.2912
0.4	0.4	11.784		0.3	0.52	14.058	14.2550	1.5	1.5	17.5725	17.5434
0.5	0.5	14.730		0.4	0.70	18.744	18.6209	1.0	1.0	11.7150	11.6956

SUMMARY

DL-Alanine was determined in micro amounts separately and in a mixture of DL-valine and L-glutamic acid in one solution directly. Sodium tungstate, gold chloride, and potassium tellurite form complexes with L-glutamic acid, DL-alanine, and DL-valine in the ratios of 1:2, 1:3, and 1:4, respectively. Potentiometric data and results in analysis support the complex ratios. Maximum errors in determinations of L-glutamic acid, DL-alanine, and DL-valine are 0.0, 1.4, and 0.16%, respectively. Ranges mg/liter) in which L-glutamic acid DL-alanine, and DL-valine were estimated in a mixture were 5.892 $\times$ 10^{-4} to 14.730 $\times$ 10^{-4}; 4.6602 $\times$ 10^{-4} to 18.6209 $\times$ 10^{-4}; and 11.6956 $\times$ 10^{-4} to 29.2390 $\times$ 10^{-4} respectively.

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Book Reviews

Photoluminescence of Solutions with Applications to Photochemistry and Analytical Chemistry. By C. A. PARKER, Am. Elsevier, New York, 1968. xvi + 544 pp. $30.00.

This book is divided into five chapters which cover basic principles and definitions, kinetics of photoluminescence, apparatus and experimental methods, special topics and applications, and applications to analytical chemistry. In general the coverage of the subject matter is adequate but at times the author becomes engrossed in triva and in these places the reader will probably become bored rapidly. This is particularly true in the section on apparatus and experimental methods. As in most books of this length there are typographical errors as well as errors of fact. For the most part these errors will be of little consequence to the novice and will be easily recognized by the experienced investigator. The book is well indexed and the bibliography appears to be representative of the literature on photoluminescence. The list of mathematical symbols in the back of the book is very helpful but unfortunately the author did not conform to the nomenclature recommended by IUPAC. For example, he uses optical density in place of absorbance and molecular extinction coefficient rather than molecular absorptivity. In spite of these and some other minor shortcomings this book provides a useful and fairly comprehensive coverage of photoluminescence in solutions and will be very useful to anyone desiring a combination text and reference book.

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Electron Microscopy and Microanalysis of Metals. Edited by J. A. BELK AND A. L. DAVIES. Am. Elsevier, New York, 1968. ix + 254 pp. $17.50.

This book is a result of a series of lectures given at the Department of Metallurgy, of the University of Aston in Birmingham, England. Nine contributors cover the field, outlined by the title in logical sequence.

The title might be misleading to one accustomed to the use of terms in the United States. What is called an "electron probe" in this country is called a "microanalyzer" in this book. Thus the expression "microanalysis of metals" in the title connotes the use of the electron probe in the analysis of metals.

The basic principles of electron optics of the electron microscope is treated first. Details of construction are then discussed. The fundamental principles of electron diffraction is then taken up to the extent that the reader may understand the interpretation of simple diffraction patterns. A chapter on specimen preparation follows, with line drawings so that the reader would have no difficulty in following the techniques. Both the use of replicas and thin foil techniques are included.

The practical applications of electron microscopy and diffraction to metallurgical problems are then covered in four chapters. These include applications to deformation and defects in metal crystals, stacking faults and partial dislocations.

Applications to precipitates in metals is then detailed. In this connection, the determination of particle densities and size distribution are explained. The application of the dark field method to the determination of the magnitude and direction of the strain fields around precipitates in metal foils is then discussed. The principle behind this technique is also discussed.

The latter third of the book is devoted to the electron probe. The similarity of this technique to that used with the x-ray spectrometer is pointed out. Discussion of the curved crystal technique for diffracting and focusing the X-ray emission from the sample irradiated with the electron gun is clearly explained. The value of the technique in metal analysis is that a minute particle in the metal, of the order of 10^{-14}g, can be discriminated from its surroundings and its composition evaluated.

After the discussion of the principles behind the construction and utilization of the electron probe, two chapters are devoted to industrial applications. These include mineral analysis, analysis of precipitates in metals, and the distribution of metals across a diffusion bond.

The book is profusely illustrated and contains numerous line drawings and photographs which make the exposition readily comprehensible. The references given are of general interest and include texts on the various subjects so that the reader can further explore an area of particular interest.

The book accomplishes what it sets out to do. Namely, to design a text book for an introductory course in the use of the electron microscope and electron probe for examination and elemental analysis of metals. As such, it should serve a useful purpose at the university as a text for this purpose. It would also be useful for the analyst engaged in the field of metallurgy. Others who use the electron microscope for various purposes could also find this a useful source book for basic techniques and new ideas.

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Encyclopedia of Industrial Chemical Analysis. Volume 7. Edited by FOSTER DEE SNELL AND CLIFFORD L. HILTON. Wiley (Interscience), New York, 1968. xi + 712 pp. Each Volume $45.00 ($35.00 by subscription).

Like Ol' Man River, the Snell–Hilton project "just keeps rolling along"—a circumstance for which the analyst should be most thankful. This series is developing into a lot more than just an alphabetical listing of substances and their analytical procedures. It is a true encyclopedia. Volume 7, the latest to appear, starts with Benzene; then after a suitable number of subjects, there comes, between Bile Acids and Salts and Bismuth, a 32-page article on Bioassay. Not bioassay of any particular material but just bioassay, which turns out to be an excellent treatise on the subject in general—a text book in itself with 25 general and 53 specific references. The last subject in this volume (which does not get out of the B's) is a monograph on brewing—205 pages with over 300 references —which covers just about everything the control chemist in this industry needs to know.

The format of this issue is the same as in the previous volumes [for reviews see *Microchem. J.* **13,** 165 and 515 (1968)] and plates, charts, line drawings, etc. are all clear and well reproduced. As with the others, this volume is indexed individually. A cumulative index is planned at the end of the series. Previous standards have been maintained in all respects. Volume 7 can go on the book shelf with its predecessors while the analyst eagerly awaits the next.

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Advances in Chromatography. Volume 7. Edited by J. CALVIN GIDDINGS AND ROY A. KELLER. Marcel Dekker, New York, 1968. xviii + 313 pp. $15.75.

Volume 7 is a continuation of this series on the various aspects of chromatography. The book is subdivided into two sections on general chromatography and gas chromatography. Within the section on general chromatography are chapters on: Theory and Mechanics of Gel Permeation Chromatography by K. H. Altgelt; Thin-Layer Chromatography of Nucleic Acid Bases, Nucleosides, Nucleotides, and Related Compounds by G. Pataki; Review of Current and Future Trends in Paper Chromatography by V. C. Weaver and; Chromatography of Inorganic Ions by G. Nickless. The section on gas chromatography is divided into three chapters: Process Control by I. G. McWilliam; Pyrolysis Gas Chromatography of Involatile Substances by S. G. Perry; and Labeling by Exchange on Chromatographic Columns by H. Elias.

The book is surprisingly practical in its content. Only one chapter, the first, is devoted primarily to theoretical notions, and these are slanted toward the choice of ideal chromatographic conditions. The chapter on nucleic acid bases, etc., is quite thorough and should be most useful. The chapter on paper chromatography contains something of the history of the technique as well as a description of the present state of the art and trends for the future. The chapter on inorganic chromatography has been long overdue and should give an impetus to work in this field. The chapter on process control will be of great interest to industrial chemists and engineers. The last two chapters on pyrolysis gas chromatography and labeling will certainly aid in the development of these lesser known methods. The choice of topics and execution of the topics is superb. The book should be of great interest to a wide range of scientists, from biochemists to industrial and inorganic chemists. The literature coverage is approximately through 1966.

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Advances in Heterocyclic Chemistry

Edited by **A. R. Katritzky**
A. J. Boulton
School of Chemical Sciences
University of East Anglia, Norwich, England

Volume 9

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June 1968, 491 pp., $22.50

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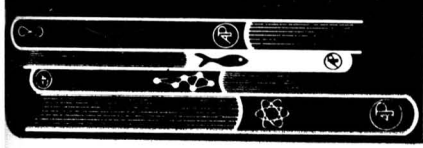

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Gas Chromatographic Determination of Hormonal Steroids

Edited by **Filippo Polvani**
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1968, 324 pp., $15.00

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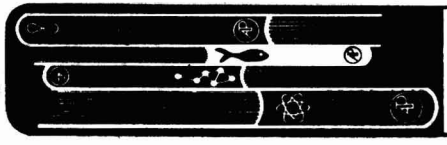

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